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Metabolism in corals from Antarctica, the deep-sea, and the shallow subtropics: contrasts in temperature, depth, and light

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Metabolism in Corals from Antarctica, the Deep-Sea, and the Shallow Subtropics: Contrasts in Temperature, Depth, and Light

by

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List of Acronyms

LDH	Lactate dehydrogenase
MDH	Malate dehydrogenase
CS	Citrate synthase
pCO_2	Partial pressure of carbon dioxide
PO_2	Oxygen partial pressure
P_c	Critical oxygen partial pressure
Q_{10}	Temperature coefficient
ANOVA	Analysis of variance
NOAA	National Oceanic and Atmospheric Association
ROV	Remotely operated vehicle
RVIB	Research vessel/icebreaker

Abstract

Coral habitats span the range from tropical to polar, extremely shallow to thousands of meters deep. The differences in light and temperature experienced in these varied habitats likely affect the metabolic rates of the corals residing there. The metabolism of three coral species from different habitats have been examined to elucidate the effects of these environmental parameters on metabolism, an under-studied aspect of coral biology. For all three species, measurements of oxygen uptake, ammonium excretion, and activity of the enzymes lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) were used to characterize their metabolism. Off Florida's Gulf coast, *Cladocora arbuscula* is known to be one of the species least damaged by bleaching events and is one of the quickest to recover, making it an ideal candidate for studying the effects of symbionts. The first set of experiments was designed to reveal the effect of disrupting the coral-algal symbiosis between this subtropical shallow-water coral and its dinoflagellate symbiont, *Symbiodinium*. The metabolic effects were described for "normal" *C. arbuscula* and those "bleached" by being held in total darkness for 4 months. Normal *C. arbuscula* had a relatively low rate of oxygen consumption at 21°C, averaging 2.43±0.65 μmol O_2 g_{wm}^{-1} h^{-1} (±S.E.), using tissue wet mass, while the bleached colonies had an average rate of 2.46±0.49 μmol O_2 g_{wm}^{-1} h^{-1}. Ammonium excretion averaged 0.07±0.02 and 0.10±0.03 μmol NH_4^+ g_{wm}^{-1} h^{-1} (±S.E.) for normal and bleached *C. arbuscula*, respectively. The activity values of the metabolic enzymes citrate synthase (CS) fell within the normal range expected for a cnidarian, averaging around 0.09±0.02 activity units (U) g_{wm}^{-1} for

both treatments, indicating normal aerobic ability. MDH was extremely high for the normal corals, compared to other cnidarians, averaging 2.5±0.4 U g_{wm}^{-1}, and a bit lower for the bleached corals, averaging 1.2±0.3 U g_{wm}^{-1}, indicating high MDH activity during both normoxia and hypoxia. LDH activity, also high, averaged 1.3±0.2 U g_{wm}^{-1} for both treatments, indicating anaerobic competence. These experiments show that *C. arbuscula* is adept at maintaining almost completely normal metabolic function when bleached, although the corals quickly become re-inoculated with symbionts upon return to normal light conditions in a tank with normal corals.

The second set of experiments served to characterize the metabolism of *Lophelia pertusa*, an azooxanthellate cold-water coral that thrives in water depths between 36 and 3383 m. *L. pertusa* is rather stenothermal, commonly found between 6-8°C, but in the Gulf of Mexico can be subjected to warm water incursions. This makes it an ideal candidate for the examination of the effects of temperature. *L. pertusa* exhibited a respiration rate of 1.14 μmol O_2 g_{wm}^{-1} h^{-1} at the control temperature of 8°C. Calculating the Q_{10} for bringing *L. pertusa* up to the environmental temperature of *C. arbuscula* results in a value of 1.8. The 11°C treatment group exhibited an 11% increase in respiration, while at 13°C, the corals showed a 23% rise from normal. The 5°C group showed a 32% decrease in respiration. The activity values of the metabolic enzyme citrate synthase (CS) fell into the normal range expected for a cnidarian, averaging 0.15, 0.20, 0.10, and 0.18 activity units (U) g_{wm}^{-1} for the 8°C, 11°C, 13°C, and 5°C treatments, respectively. Malate dehydrogenase (MDH) values were unexpectedly high, averaging 2.05, 1.48, 1.48, and 1.82U g_{wm}^{-1} for the 8°C, 11°C, 13°C, and 5°C treatments, respectively. Lactate dehydrogenase (LDH) was undetectable in this species, suggesting it has a different terminal glycolytic enzyme. Nonetheless, the other two enzymes indicate metabolic competence in both normoxic and

hypoxic conditions. *L. pertusa* is adaptable to temperatures within its range, although its respiration rate is lower than that of tropical corals.

The third set of experiments characterized the metabolism of the endemic Antarctic coral *Flabellum impensum*, one of the world's largest solitary corals. It resides at roughly the same depths as *L. pertusa*, but the water temperature in its habitat never strays far from 0°C. *F. impensum* had a low rate of oxygen consumption at 0°C, averaging 0.31 μmol O_2 g^{-1} h^{-1}, calculated using tissue wet mass. Calculating a Q_{10} for this species at *C. arbuscula*'s habitat temperature results in a value of 2.7. Ammonium excretion averaged 4.21 nmol NH_4^+ g_{wm}^{-1} h^{-1}. The activity values of the metabolic enzymes citrate synthase (CS), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH) fell within the normal range expected for a cnidarian, averaging 0.13, 1.01, and 0.42 activity units (U) g_{wm}^{-1}, respectively. A count of the skeletal growth bands on the calyx suggests that this species has a linear extension rate of approximately 1 mm per year. *F. impensum* is a long-lived, slow-growing coral, with a low metabolic rate.

Chapter One:

Introduction

History

Coral reefs have been evolving for 260 million years, having survived and transformed through major climatic changes and mass extinctions (Grigg, 1994; Brown, 1997). Scleractinia, or stony corals, first appeared in the fossil record sometime during the mid-Triassic and their diversity fluctuated throughout geologic time, with loss of a majority of taxa following the K-T mass extinction (Stanley and Swart, 1995; Stanley, 2003). Some late Triassic Scleractinia were zooxanthellate, although this symbiosis was probably not as efficient as it is in modern corals (Stanley and Swart, 1995; Leinfelder, 2001). It can be presumed that the symbiotic relationship between stony corals and zooxanthellae is what eventually allowed them to flourish and become an ecologically significant component of the reef framework. Scleractinians were a diverse and noteworthy presence on reefs throughout the Mesozoic era and their diversity rebounded during the Eocene; zooxanthellate Scleractinia did not become the dominant reef-builder until the Oligocene, coincident with declining atmospheric carbon dioxide (Pomar and Hallock, 2007).

The first deep-water reef frameworks formed by azooxanthellate corals started to appear in the Cretaceous period and although the K-T extinction also devastated these corals, it was to a lesser degree than experienced by zooxanthellate corals (Stanley and Cairns, 1988; Roberts et al., 2009). It took four million years for the diversity of zooxanthellate scleractinians to return to the level of diversity of the more extinction-resistant azooxanthellate corals (Rosen and Turnšek,

1989; Rosen, 2000; Kiessling and Baron-Szabo, 2004). Today, the family Caryophylliidae contains the highest diversity of azooxanthellate deep-water corals (Roberts et al., 2009), such as *Lophelia pertusa*, as well as a large number of zooxanthellate species, such as another coral of interest to this study, *Cladocora arbuscula.* Like *L. pertusa*, the third coral examined in this study, *Flabellum impensum* (family: Flabellidae), is also azooxanthellate.

Symbiosis in modern corals

Mutualism between an animal host and its algal symbionts evolved as a mechanism to maintain a competitive edge within an ecological niche, while faced with a low supply of food (Muscatine and Porter, 1977; Hallock and Schlager, 1986; Stanley and Swart, 1995; Muller-Parker and D'Elia, 1997). The general description of this symbiosis is that the autotrophic symbiont recycles inorganic nutrients provided by its heterotrophic host's metabolism, and in combination with photosynthesis, ends up largely supplementing the host's energy requirements (Hallock. 1981; Stanley and Swart, 1995; Yellowlees et al., 2008). The three clades (A, B, and C) of the genus *Symbiodinium* are the most common photosynthetic dinoflagellates existing in symbiosis with a coral host (LaJeunesse, 2002). A variety of studies report that these zooxanthellae provide anywhere from 32% to 92% of their hosts' daily metabolic needs (Beck, 1982), but in the tropics, shallow-water corals thrive in well-lit waters where the photosynthetic contributions of their symbionts can exceed their metabolic requirements (Piniak, 2002). Indeed, zooxanthellae allow corals access to orders of magnitude more carbon than would typically be available to a heterotroph (Beck, 1982; Hallock, 1981, 2001), so the success of zooxanthellate corals in nutrient-limited environments can be attributed to the energetic advantage bestowed upon the corals by their symbionts (Stanley and Swart, 1995; Stanley, 2003). In addition to utilizing carbon provided by their symbionts, zooxanthellate corals experience differences in

growth, metabolism, and ability to acclimate to environmental changes as a result of this mutualism (Reed, 1982; Gates and Edmunds, 1999; Muller-Parker and D'Elia, 1997; Hallock, 2001).

There is generally little knowledge about the intricacies of the physiological interactions between a symbiont and its scleractinian host (Gates and Edmunds, 1999) and there are no studies to this author's knowledge addressing these aspects in regard to the metabolism of *C. arbuscula*. The genus *Cladocora* is comprised of species whose associations with *Symbiodinium* span the range from obligately zooxanthellate to azooxanthellate. *C. arbuscula* is a zooxanthellate coral while a similar species, *C. debilis*, with which *C. arbuscula* is often confused, is azooxanthellate, resides at greater depth, and has a much larger geographic range (Cairns, 2000; Alvarez-Perez 2005). *C. arbuscula* is host to type B1 *Symbiodinium*, *S. pulchrorum/bermudense* (LaJeunesse, 2002), which have a high acclimatization capacity throughout a large range of irradiance levels (Iglesias-Prieto and Trench, 1997). Goreau (1959) observed calcification rates of *C. arbuscula* and found that these rates did not change much whether the corals were held in the light or dark, suggesting that this coral is not highly dependent upon its symbionts for skeletal formation. *C. arbuscula* can survive and reproduce after being held for over eighteen months in complete darkness in the laboratory, subsisting only on a weekly feeding of *Artemia franciscana* nauplii (personal observation), which is further evidence that the symbiosis is somewhat decoupled in this species and it is likely a facultative relationship.

Buddemeier and Fautin (1993) noted that the degree of specificity a host has for a certain symbiont type may be the determining factor of that host's capability to acclimate to long-term environmental changes. One way around this is that some corals have a more loose association

with their symbionts, with many temperate species being facultatively zooxanthellate and dissociating from the mutualistic relationship altogether as light levels become too low to support photosynthesis (Miller, 1995). In fact, Reed (1982) found that zooxanthellate *Oculina varicosa* living at 6 m depth actually have a lower growth rate than the azooxanthellate colonies living at 80 m depth, indicating that symbionts, despite their benefits, impose a metabolic cost to their hosts (Muller-Parker and D'Elia, 1997). In another species of *Oculina*, *O. arbuscula*, growth rates are maximal when the coral can benefit from a combination of heterotrophy and supplemental energy via zooxanthellae (Piniak, 2002). Another strategy that some corals may employ is to alter the constituents of their symbiont population, trading out some members of one type for those of another to optimize the combined physiological performance of the host and zooxanthellae for different environmental conditions (Gates and Edmunds, 1999). The ability of a coral to use any of these strategies and the degree to which the coral can control its trophic condition is likely unique to each coral-symbiont partnership.

Deep-sea and cold-water corals

Deep-sea and shallow-water corals share many similarities in that they occur in both colonial and solitary forms, exhibit a variety of colors, sizes and shapes, are hosts to many associated fauna, and include reef-building species (Jensen and Frederiksen, 1992; Mortensen et al., 1995; Roberts and Hirshfield, 2004). Although thousands of deep-sea corals have been described, researchers estimate that 800 stony species and their associated fauna have yet to be encountered (Roberts and Hirshfield, 2004). Twenty of the 703 known species of deep-sea stony corals form reefs (Roberts and Hirshfield, 2004). Deep-sea corals represent an important ecological niche in that they protect inhabitants from currents and predators, act as a nursery habitat, and provide feeding, breeding, and spawning ground for economically valuable fish and shellfish (Jensen and

Frederiksen, 1992; Mortensen et al., 1995; Roberts and Hirshfield, 2004). *L. pertusa* reefs exist worldwide, except at the poles (Zibrowius 1980; Cairns 1994) and some living reefs are estimated to be thousands of years old (based on the coral's slow growth rate compared to some shallow-water coral) (Freiwald et al., 2002).

Despite the evolutionary success conferred upon shallow-water corals by their symbiosis with photoautotrophic dinoflagellates, about 3300 (65%) documented species of corals are azooxanthellate and live in deep, cold waters, well below the photic zone, from 50-6000 m (Roberts and Hirshfield, 2004; Roberts et al., 2009; Etnoyer, 2010). There have not been many studies of deep-sea corals in their natural state, so their biology and ecology are still poorly understood. Although there is limited knowledge about the global distribution of deep-sea corals, most seem to occur on seamounts or the edges of continental margins, which are among the most diverse deep-sea habitats (Roberts and Hirshfield, 2004; Neulinger et al., 2008). One of the two dominant species of deep-living corals that occurs off the southeast coast of the United States is *L. pertusa*, which is pseudo-colonial and stenothermal, as well as one of the most widespread deep-sea corals (Rogers, 1999; Neulinger et al., 2008). The other dominant coral is the facultatively zooxanthellate *O. varicosa*, which occurs in both shallow and deep water (Reed, 2002). Seventeen species of scleractinian corals occur in Antarctic waters. As is true of most high-latitude corals, all Antarctic species, such as the solitary *F. impensum*, are azooxanthellate due to the low temperatures and seasonal swings in irradiance typical of the Antarctic system (Cairns, 1990).

Because *L. pertusa* and *F. impensum* are azooxanthellate, they depend upon the capture of plankton and detritus for nutrition. This, along with the fact that deep-sea corals experience lower temperatures and higher pressure, suggests that they might have a lower metabolic rate

than shallow-water corals. Because *C. arbuscula* seems to have a somewhat looser association with its zooxanthellae than other corals with which it co-occurs, it may also have a more flexible metabolism to accommodate varying symbiont populations.

Objectives

The purpose of this study was to explore the effects of light, temperature, and depth regime on the metabolic rates of corals. Techniques employed were measurements of respiration, ammonia excretion, and activity of the metabolic enzymes LDH, CS, and MDH. The zooxanthellate *C. arbuscula* was examined both with and without its symbionts to assess the effects of autotrophic contributions to host metabolism. Observations of the deep-sea, azooxanthellate *L. pertusa* at different temperatures also provided insight into the intricacies of coral metabolism. The azooxanthellate *F. impensum* was also included in the study to provide a polar representative for comparison. The literature contains a large range of linear extension estimates for *L. pertusa* and none exist for *C. arbuscula* or *F. impensum*. *C. arbuscula* probably has the highest metabolic rate due to its association with zooxanthellae; however, it seems less dependent on its symbionts for growth and survival compared to other tropical corals. Once the symbionts are expelled, these shallow-water corals may exhibit metabolic rates similar to that of deep-sea corals. Measuring these parameters allowed for a comparison of the metabolisms of these species and provided some insight as to what physiological effects a host coral experiences as a result of its symbiosis with *Symbiodinium* as well as over their temperature and depth distributions.

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Chapter Two:

The Effects of Symbiosis on Coral Metabolism

Abstract

Species of the scleractinian coral genus *Cladocora* span the range from azooxanthellate to obligately zooxanthellate in their associations with the genus *Symbiodinium*. The present study was designed to elucidate the effect of *Symbiodinium* symbiosis on the metabolism of a host coral, *C. arbuscula.* The effects of symbiosis were described for normal *C. arbuscula* and those bleached by being held in total darkness, with measurements of oxygen consumption rate and metabolic enzyme activity. *C. arbuscula* had a relatively low rate of oxygen consumption at 21°C, averaging 2.43±0.65 μmol O_2 g^{-1} h^{-1} (±S.E.), using tissue wet mass, while the bleached colonies had an average rate of 2.46±0.49 μmol O_2 g^{-1} h^{-1}. Ammonium excretion averaged 0.07±0.02 and 0.11±0.03 μmol NH_4^+ g^{-1} h^{-1} (±S.E.) for normal and bleached *C. arbuscula*, respectively, resulting in O:N ratios of about 55. The activity values of the metabolic enzymes citrate synthase (CS) fell within the normal range expected for a cnidarian, averaging about 0.09 activity units (U) g_{wm}^{-1} for both treatments. Malate dehydrogenase (MDH), and lactate dehydrogenase (LDH) activities were high compared to other cnidarians. MDH was extremely high for the normal corals, averaging 2.50 U g_{wm}^{-1}, and about half that for the bleached corals, averaging 1.20 U g_{wm}^{-1}. LDH activity averaged about 1.27 U g_{wm}^{-1} for both treatments. *C. arbuscula* is adept at maintaining almost completely normal metabolic function when bleached,

it quickly regains zooxanthellae upon being returned to normal light conditions in a tank with normal corals.

Introduction

Mutualism between an animal host and its algal symbionts evolved as a mechanism to maintain a competitive edge within an ecological niche characterized by a low supply of food (Muscatine and Porter, 1977; Hallock, 1981; Muller-Parker and D'Elia, 1997; Stoecker, 1998). The seven clades (A, B, and C) of the genus *Symbiodinium* are the most common photosynthetic dinoflagellates existing in symbiosis with a coral host (LaJeunesse, 2002). A variety of studies report that these zooxanthellae provide anywhere from 32% to 92% of their hosts' daily metabolic needs (Beck, 1982; Cook et al.,1988; Gaydos, 2006), but in the tropics, shallow-water corals thrive in well-lit waters where the photosynthetic contributions of their symbionts can exceed their metabolic requirements (Johannes et al., 1970; Muscatine, 1990; Piniak, 2002). Indeed, zooxanthellae allow corals access to orders of magnitude more carbon than would typically be available to a heterotroph (Hallock, 1981; 2001), so the success of zooxanthellate corals in nutrient-limited environments can be attributed to the energetic advantage bestowed upon the corals by their symbionts (Stanley and Swart, 1995; Stanley, 2003). In addition to utilizing energy provided by their symbionts, zooxanthellate corals experience differences in growth, metabolism, and ability to acclimate to environmental changes as a result of this mutualism (Reed, 1982; Gates and Edmunds, 1999; Muller-Parker and D'Elia, 1997).

In the Mediterranean, the most extensively studied *Cladocora* species, *C. caespitosa* is found in a variety of habitats and is host to a *Symbiodinium* from clade A that is prevalent throughout the Mediterranean Sea and also tolerates a large range of irradiance levels (Rodolfo-Metalpa et al., 2008b). *C. caespitosa* is the main endemic zooxanthellate coral in the Mediterranean and is

frequently found in turbid waters from 5-40 m (Rodolfo-Metalpa et al., 2006 and 2008a). In a series of studies, Rodolfo-Metalpa et al. (2006, 2008a, 2008b, 2010) exposed *C. caespitosa* to different levels of temperature, irradiance, and pCO_2, finding that both the coral and its zooxanthellae displayed extensive tolerance for short-term changes in environmental parameters. Buddemeier and Fautin (1993) noted that the degree of specificity a host has for a certain symbiont type may be the determining factor of that host's capability to acclimate to long-term environmental changes. One way around this is that some corals have a more loose association with their symbionts, with many temperate species being facultatively zooxanthellate and dissociating from the mutualistic relationship altogether as light levels become too low to support photosynthesis (Miller, 1995). In fact, Reed (1982) found that zooxanthellate *Oculina varicosa* living at 6 m depth actually have a lower growth rate than the azooxanthellate colonies living at 80 m depth, indicating that symbionts, despite their benefits, impose a metabolic cost to their hosts (Muller-Parker and D'Elia, 1997; Stoecker, 1998). In another species of *Oculina*, *O. arbuscula*, growth rates are maximal when the coral can benefit from a combination of heterotrophy and supplemental nutrition via zooxanthellae (Piniak, 2002). Another strategy that some corals may employ is to alter the constituents of their symbiont population, trading out some members of one type for those of another, to optimize the combined physiological performance of the coral holobiont depending upon environmental conditions (Gates and Edmunds, 1999). The ability of a coral to use any of these strategies and the degree to which the coral can control its trophic condition is likely unique to each coral-symbiont partnership.

There is generally little knowledge about the intricacies of the physiological interactions between a symbiont and its scleractinian host (Gates and Edmunds, 1999) and there are no studies to this author's knowledge addressing these aspects in regard to the metabolism of the

species examined in the present study, *C. arbuscula*. The genus *Cladocora* is comprised of species whose associations with *Symbiodinium* span the range from obligately zooxanthellate to azooxanthellate. *C. arbuscula* was the first species of *Cladocora* described and its existence has been recognized in the Caribbean for over 150 years (Lesuer, 1821; Baron-Szabo, 2005). *C. arbuscula* is unique compared to other species of *Cladocora* in that it is restricted to carbonate and soft bottoms and has a narrow geographic range, existing only off the coast of Florida, the Caribbean, and the Bahamas (Lesuer, 1821; Baron-Szabo, 2005). *C. arbuscula* is one of the most abundant species of endemic scleractinian corals off the west central coast of Florida, and a dominant, relatively fast-growing component of the patch reefs in the region (Rice and Hunter, 1992). *C. arbuscula* is a zooxanthellate coral found at a depth range of 0.5-27 m while a similar species, *C. debilis*, with which *C. arbuscula* is often confused, is azooxanthellate, occupies a deeper range of 24-480 m, and has a much larger geographic range (Cairns, 2000; Alvarez-Perez 2005).

The objective of this study was to distinguish the effects of zooxanthellae on the metabolic processes of *C. arbuscula* by bleaching individual colonies, then comparing rates of oxygen consumption, ammonium excretion, and the activities of the metabolic enzymes, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) of the normal and bleached colonies. If growth is maximal when a coral has both symbionts and another food source, it stands to reason that bleaching a coral would effectively reduce its growth rate by way of a reduced metabolic rate. Thus, I am testing the hypothesis that a possibly facultatively zooxanthellate coral provided with ample food can compensate for the lack of symbiont input.

Materials and Methods

Collection. Colonies of *Cladocora arbuscula* were collected off the coast of Tarpon Springs, Florida in September 2009 at depths between 3 and 4 m. Tennis ball-sized coral heads were hand-collected by divers using a hammer and chisel. Specimens were immediately placed into a cooler on board a boat provided by volunteers from the Tarpon Springs chapter of SCUBAnauts International. The corals were transported to the laboratory and immediately placed in a tank of recirculating seawater at 21°C. The *C. arbuscula* colonies were broken down into smaller chunks of 10-55 polyps each with a hammer and chisel. The chunks were briefly removed from the water (less than 30 sec each) and attached with polyps facing upward onto 1 in^2 tiles with parafilm-covered marine epoxy (to avoid permanent attachment). After the epoxy cured under water, the chunks were meticulously cleaned of any epibionts and placed into smaller tanks containing artificial seawater (Instant Ocean®, salinity: 35ppt). Half of the chunks from each colony were placed into a tank that was kept in complete darkness, while the others remained on a 12:12 h light:dark cycle. Both treatments were fed *Artemia franciscana* (Great Salt Lake, Utah, USA) nauplii, freshly-hatched in the lab once per week. It took approximately two months for the dark-adapted chunks to expel their symbionts. They were considered to be fully bleached when the polyps were a pale pink color when retracted and completely transparent when expanded (Figure 2.1; see page 27). Corals in both treatment groups were kept in their tanks for another two months before being used in the experiments.

Oxygen consumption experiments. The apparatus for measuring oxygen consumption consisted of an array of lucite chambers designed specifically for this type of experiment, as used by Torres et al. (1994). The chambers' construction allowed for water at 21°C, temperature-controlled by an electric bath, to continuously flow between the double-layered walls,

maintaining the seawater and experimental animals in the inner chamber at a constant temperature. Upon sealing a chamber, no air can enter or leave. Oxygen microelectrodes (Clark, 1956; Mickel et al., 1983) fabricated in-house were calibrated using air- and nitrogen-saturated water. Then they were inserted into the top of each chamber and oxygen levels of the water inside were measured every half-second while a stir bar under a perforated lucite false bottom constantly mixed the water.

The pieces of "bleached" *C. arbuscula* and "normal" *C. arbuscula*, were placed into chambers appropriate for their size with artificial seawater treated with 25 mg/L each of Streptomycin and Neomycin to minimize bacterial oxygen consumption (Torres et al., 1994). A control chamber with just the artificial seawater was included in each experimental run to confirm negligible bacterial respiration. Once the chambers were sealed, PO_2 of the water was measured for 24 hours in the dark. These measurements were made for 15 pieces of normal and 15 pieces of bleached *C. arbuscula*. At the end of all experimental runs, the polyps were alive and had their tentacles extended. The *C. arbuscula* samples were immediately prepared for enzyme analysis upon removal from the respiration chambers.

Before and after each run, a 20 ml sample of water was taken from each chamber to be analyzed for ammonia production (Oceanic Nutrient Laboratory, University of South Florida).

Enzyme analyses. The polyp tissues from each coral chunk were removed with a scalpel and forceps, weighed, and homogenized in a ground glass grinder with a 50 mM Imidazole/HCl buffer (pH 7.2 at 20 °C) solution that diluted the homogenate by 1:8. Each homogenate was spun down in a centrifuge for 10 minutes at 4750 x g and the supernatant was used in triplicate UV/visible spectrophotometric enzyme analyses at 10°C using the techniques of Torres and Somero (1988). Lactate dehydrogenase (LDH) activity was assayed at a wavelength of 340 nm

in a 0.2 M Imidazole/HCl buffer solution (pH 8.0 at 10 °C) with 0.15 mM NADH and 5.0 mM Na-pyruvate. Malate dehydrogenase (MDH) was measured at 340 nm in a 40 mM Imidazol/$MgCl_2$ buffer solution (pH 8.1 @ 10°C) with 0.15 mM NADH and 0.5 mM oxaloacetate. Citrate synthase (CS) activity was measured at 412 nm in a 50 mM Imidazol/HCl buffer solution (pH 8.0 @ 10°C) with 0.4 mM DTNB, 0.1 mM Acetyl-CoA, and 0.5 mM oxaloacetate. Any remaining coral tissue was removed from the skeleton using a Waterpik® filled with artificial seawater and the cleaned skeleton was then weighed to calculate total tissue wet mass for each sample via subtraction.

Results

Normal *Cladocora arbuscula* exhibited an average respiration rate of 2.43±0.65 μmol O_2 g_{wm}^{-1} h^{-1} (±S.E.), using tissue wet mass, while the bleached colonies had an average rate of 2.46±0.49 μmol O_2 g_{wm}^{-1} h^{-1}, an insignificant difference (ANOVA, F=0.001, df=1, p=0.97) (Table 2.1; see page 27). The oxygen consumption rates did not scale with wet mass for either treatment group (p>0.40 for both), which is atypical for most animal groups (Hemminsen, 1960). The reason for this is likely that the coral pieces were similar in size, so the rates measured were within the normal range for pieces of that particular size. The rates fall on the low end of the range for tropical/subtropical cnidarians (Davies, 1980; Schick, 1990) (Figure 2.2; see page 28).

Differences in ammonium excretion were also not significant (ANOVA, F=1.26, df=1, p=0.27), averaging 0.07±0.02 and 0.11±0.03 μmol NH_4^+ g_{wm}^{-1} h^{-1} (±S.E.) for normal and bleached *C. arbuscula*, respectively. Like the respiratory rates, those excretion rates did not scale with mass for either group (p>0.20 for both). The values result in an O:N atomic ratio of 63 and 46 (p=0.32) for the normal and bleached corals, respectively, indicating that both groups were catabolizing lipids, possibly indicating starvation. Even though the corals were fed weekly,

not all the *Artemia* were cleared over the course of the week, therefore, the corals had a constant food supply available. It is possible that the sudden lack of *Artemia* triggered a starvation response.

Activities of CS fell within the normal range expected for a cnidarian and were similar for both treatments, averaging 0.07±0.02 and 0.11±0.03 activity units (U) g_{wm}^{-1} (±S.E.) (ANOVA, F=1.74, df=1, p=0.21), for normal and bleached *C. arbuscula*, respectively, where activity units are micromoles of substrate converted to product per minute. MDH averaged 2.50±0.40 and 1.2±0.31U g_{wm}^{-1}, for normal and bleached corals, respectively, both of which are high values, considering the range reported for other cnidarians (Thuesen and Childress, 1994; Henry and Torres, 2013), with the normal corals having significantly higher values than the bleached corals (ANOVA, F=5.57, df=1, p=0.03). LDH activities were similarly very high for both groups, averaging 1.25±0.17 and 1.28±0.21U g_{wm}^{-1}, for normal and bleached corals, respectively (ANOVA, F=0.03, df=1, p=0.87).

Discussion

Although some studies have examined short-term growth rates of *Cladocora arbuscula* or *C. caespitosa* under experimental conditions (Rice and Hunter, 1992 and Rodolfo-Metalpa et al., 2008a, respectively), there are no records of *in situ* linear extension rates; anecdotal growth rates have been quoted to be about 5 cm y^{-1}, which may be unrealistically high. The Mediterranean *C. caespitosa* has been observed to grow at rates between 1.36-6.15 mm per year (Peirano et al., 1999; Kruzic and Pozar-Domac, 2002; Peirano et al., 2005; Kruzic and Benkovic, 2008), which may be similar to the rate at which *C. arbuscula* grows in the northern Gulf of Mexico. This is a slow growth rate compared to tropical corals, whose growth rates are typically measured on a scale of centimeters. Goreau (1959) observed calcification rates of *C. arbuscula* and found that

these rates did not change much whether the corals were held in the light or dark, suggesting that the species is not highly dependent upon its symbionts for skeletal formation.

At the conclusion of the present study, some of the bleached coral pieces that were not used in the experiments remained in the dark tank for long-term observation (the rest were returned to the lab's main coral tank to be re-inoculated with symbionts). I observed that *C. arbuscula* can survive, calcify, and asexually reproduce to increase the colony size after being held for over 18 months in complete darkness, subsisting only on a weekly feeding of *Artemia franciscana* nauplii, which is further evidence that the symbiosis is facultative in this species.

Two zooxanthellate coral species with similar oxygen consumption rates as *C. arbuscula* (Figure 2.2; see page 28), *Montastrea cavernosa* and *M. annularis*, have growth rates of 6 and <10 mm y^{-1}, respectively (Suggett et al., 2012; Foster et al., 2013). The relatively low respiration rate (2.43 μmol O_2 g_{wm}^{-1} h^{-1}) observed for *C. arbuscula* is consistent with the hypothesis that it may grow at a slow rate, similar to that of *C. caespitosa*. The fact that this rate does not significantly differ between the normal colonies and the bleached colonies further supports the idea that *C. arbuscula* may not be dependent on its symbionts for normal metabolic function.

Upon returning some of the coral pieces to a 12h:12h light/dark cycle in a large tank containing species of *Montastrea* and *Siderastrea*, signs of re-inoculation with symbionts were apparent after less than two weeks. The corals had reverted to their normal yellow-brown color after six weeks. It would have been interesting to determine if the same species and density of *Symbiodinium* repopulated the corals as were previously present.

The metabolic enzyme analyses demonstrate that *C. arbuscula* is both aerobically and anaerobically competent. The activities of the two Krebs cycle enzymes, citrate synthase (CS) and malate dehydrogenase (MDH) are indicators of aerobic function. MDH also has other

functions related to metabolism; it shuttles glycolytic reducing equivalents into the mitochondrion, as well as back into the cytosol for gluconeogenesis. The expected rate of CS activity combined with the elevated levels of MDH activity, especially in the normal treatment group, indicate that *C. arbuscula* is aerobically active and can also maintain redox balance during hypoxia. Bleaching this coral effectively diminishes its MDH activity, but to a level that is still impressive compared to that of other cnidarians (Thuesen and Childress, 1994; Henry and Torres, 2013). Dupont et al. (2010) found that compared to other co-occurring coral species, *C. arbuscula* is highly resistant to bleaching during a red tide event, and when it does bleach, will recover quickly, possibly due in part to its continued aerobic competency.

Cladocora arbuscula is host to type B1 *Symbiodinium*, *S. pulchrorum/bermudense* (LaJeunesse, 2002), which have a high acclimatization capacity throughout a large range of irradiance levels (Iglesias-Prieto and Trench, 1997). The tissue of *C. arbuscula* in the field contains a high density of zooxanthellae, possibly supporting as many as 17 layers of symbionts (Beck, 1982). The increased access to oxygen as a result of a high symbiont count seems to further increase the aerobic metabolic function of the coral.

The activity of the terminal enzyme in anaerobic glycolysis, lactate dehydrogenase (LDH), is an indicator of anaerobic capacity. The high LDH activity in both treatment groups demonstrates an increased anaerobic competence. The west coast of Florida is prone to outbreaks of *Karenia brevis*, the dinoflagellate responsible for red tides and the subsequent anoxic events. Rice and Hunter (1992) demonstrated that *C. arbuscula* displays resistance to burial by sediment, compared to other corals with which it co-occurs. The high anaerobic capacity of *C. arbuscula* is likely what allows it to survive red tide-induced anoxic events as well as sedimentation events. There are also regions of western Florida that are subject to riverine

outflow; the corals in the present study were collected not far from the mouth of the Alafia River. These regions experience intermittent levels of high sediment flow. The abundance of *C. arbuscula* off the Florida west coast can be partially attributed to its ability to thrive despite events that temporarily diminish water quality.

In summary, *C. arbuscula* is one of the most abundant species of scleractinian corals off the west central coast of Florida, and is a dominant component of the patch reefs in the region (Rice and Hunter, 1992). It thrives in a region subject to red-tides, intermittent high sedimentation, and the occasional hurricane. Despite local anecdotes, it appears to have a low growth rate that is similar to the Mediterranean *C. caespitosa*, since its metabolic rate is on the lower end of the spectrum for sub/tropical cnidarians. At the same time, its high levels of both aerobic and anaerobic function allow it to thrive in this environment, even when faced with conditions that cause widespread stress and mortality of benthic organisms. Although *C. arbuscula* can function quite well without zooxanthellae, it seems that its optimal state is to have endosymbionts included as members of the holobiont, as evidenced by the rapid re-browning observed both in the field and laboratory.

Acknowledgments

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Tables and Figures

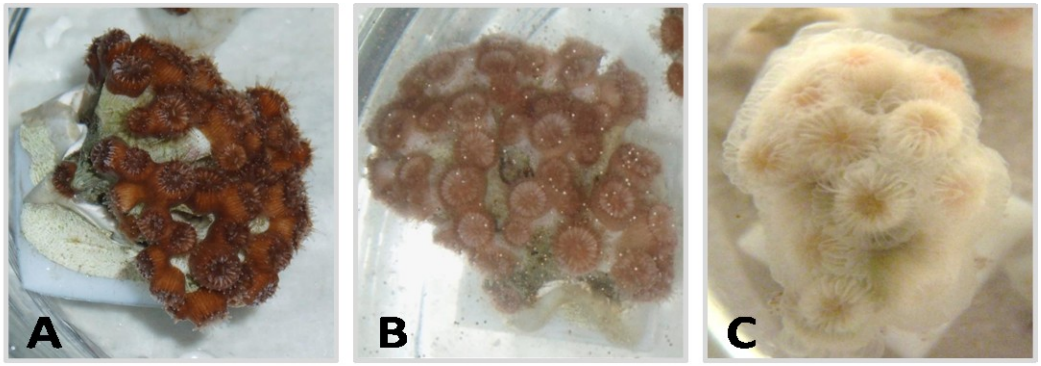

Figure 2.1. Bleaching over time. (A) A piece of *C. arbuscula* before being put into the dark. (B) The same piece (rotated) still showing some coloration after about a month in total darkness. The white specks are some leftover *Artemia* cysts. (C) A different piece, fully bleached and shown inflated after feeding to demonstrate complete loss of zooxanthellae.

Table 2.1. Oxygen respiration, nitrogen excretion, and enzyme activities for normal and bleached *C. arbuscula.*

	Respiration	Excretion	CS	MDH	LDH
	µmol O_2 g^{-1} h^{-1}	µmol NH_4^+ g^{-1} h^{-1}	U g_{wm}^{-1}	U g_{wm}^{-1}	U g_{wm}^{-1}
Normal	2.43±0.65	0.07±0.02	0.10±0.02	2.5±0.4	1.3±0.2
Bleached	2.46±0.49	0.1±0.03	0.07±0.01	1.2±0.3	1.3±0.2
F	0.001	1.26	1.74	5.57	0.03
p-value	0.97	0.3	0.2	0.03	0.9
df	1	1	1	1	1

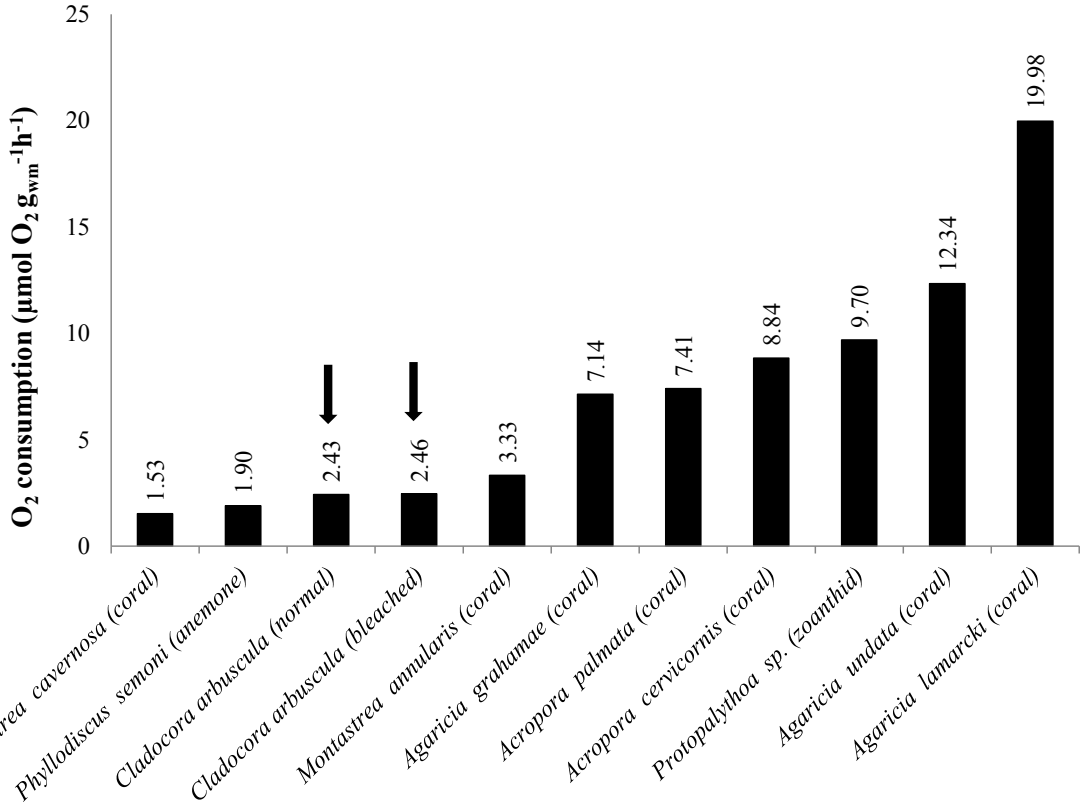

Figure 2.2. Metabolic rates of various cnidarians. *C. arbuscula* lies on the low end of the metabolic spectrum compared to some other cnidarians. Species listed in this graph include tropical corals (Davies, 1980), a zoanthid and an anemone (Shick, 1990).

Chapter Three:

The Effects of Temperature on Coral Metabolism

Abstract

Lophelia pertusa is a hermatypic cold-water coral that flourishes around the globe in water depths between 36 and 3383 m. The present study characterizes the metabolism of *L. pertusa* from Miami Terrace and the Gulf of Mexico with measurements of oxygen consumption and metabolic enzyme activity at four different temperatures, 5°C, 8 °C (control), 11°C, and 13°C. *L. pertusa* exhibited a respiration rate of 1.08 μmol O_2 $g_{wm}^{-1}h^{-1}$ at 8°C. The 5°C group showed a 32% decrease in respiration compared with the controls. The 11°C treatment group exhibited an 11% increase in respiration compared to the controls, while the 13°C corals showed a 23% increase. O:N atomic ratios averaged 31 for the Miami Terrace corals, indicating a predominantly lipid-based diet, and less than 8 for the Gulf corals, indicating a pure protein diet. The activity values of the metabolic enzyme citrate synthase (CS) fell into the normal range expected for a cnidarian, averaging 0.18, 0.15, 0.20, and 0.10 activity units (U) g_{wm}^{-1} for the 5°C, 8°C, 11°C, and 13°C and treatments, respectively. Malate dehydrogenase (MDH) values were unexpectedly high, averaging 1.82, 2.05, 1.48, and 1.48 U g_{wm}^{-1} for the 5°C, 8°C, 11°C, and 13°C treatments, respectively. Lactate dehydrogenase (LDH) was undetectable in this species. *L. pertusa* thickets serve as habitat to thousands of animal species, and with more research

focusing on deep-sea coral habitats as important deep-sea refugia, it is especially important to understand the basic biology of the reef-forming species.

Introduction

Despite the evolutionary success conferred upon shallow-water corals by their symbiosis with photoautotrophic dinoflagellates, about 3300 (65%) documented species of corals are azooxanthellate and live in deep, cold waters, well below the photic zone, from 50-6000 m (Roberts and Hirshfield, 2004; Roberts et al., 2009; Etnoyer, 2010). The existence of those deep-living corals has been recognized for over two hundred years (Jensen and Frederiksen, 1992; Roberts and Hirshfield, 2004), however, it wasn't until the development of submersible vehicles, that they became accessible to scientific research. There have been few studies of deep-sea corals in their natural state, so details about their biology and ecology are still in the process of being discovered. Although there is limited knowledge about the global distribution of deep-sea corals, most seem to occur on seamounts or the edges of continental margins, which are among the most diverse deep-sea habitats (Jensen and Frederiksen, 1992; Mortensen et al., 1995; Roberts and Hirshfield, 2004; Neulinger et al., 2008). One of the two dominant species of deep-living corals that occur off the southeast coast of the United States is *Lophelia pertusa*, which is pseudo-colonial and stenothermal, as well as one of the most widespread deep-sea corals (Rogers, 1999; Neulinger et al., 2008). *L. pertusa* occurs as either completely white or with colored polyps ranging from yellow to orange to pink. The other dominant coral is the facultatively zooxanthellate *Oculina varicosa*, which occurs in both shallow and deep water (Reed, 1982; 2002).

Twenty of the 703 known species of deep-sea stony corals form reefs (Roberts and Hirshfield, 2004). *L. pertusa* reefs exist worldwide, except at the poles (Zibrowius 1980; Cairns

1994) and some living reefs are estimated to be thousands of years old (estimated from the coral's slow growth rate compared to some shallow-water corals) (Freiwald *et al*., 2002). Linear extension rates for *L. pertusa* are estimated to be as low as 5 mm per year (Roberts, 2002) to at least 34 mm per year (Roberts et al., 2009) as observed from samples taken from man-made structures of known age, although Brooke and Young (2009) measured a much lower rate of 2.44-3.77 mm per year for transplanted pieces. *L. pertusa* has been recorded at depths between 36 and 3383 m and at latitudes between 71°N and 51°S (Roberts et al., 2009).

Atlantic *L. pertusa* reefs provide habitat to thousands of species of sponges, anemones, bryozoans, gorgonians, worms, fish, mollusks, and crustaceans (Reed et al., 1982; Reed and Mikkelsen, 1987; Roberts and Hirshfield, 2004; Etnoyer, 2010) and have three times the diversity of the surrounding soft bottom (Fosså et al., 2002). They represent oases of high diversity in a low-diversity environment (Fosså et al., 2002), both in terms of overall diversity and diversity within many taxonomic groups (Jensen and Frederiksen, 1992; Mortensen et al., 1995; Rogers, 1999). The high diversity within Lophelia reefs is believed to be possible because they occur in a stable environment with a predictable food supply (Rogers, 1999). Deep-sea corals represent an important ecological niche in that they protect inhabitants from currents and predators, act as a nursery habitat, and provide feeding, breeding, and spawning ground for economically valuable fish and shellfish (Jensen and Frederiksen, 1992; Roberts and Hirshfield, 2004). Although thousands of deep-sea corals have been described, researchers estimate that 800 stony species and their associated fauna have yet to be encountered (Roberts and Hirshfield, 2004).

Because *L. pertusa* is azooxanthellate, it must depend upon the capture of plankton and detritus for nutrition. *L. pertusa* have been observed in the field to feed "voraciously" on

zooplankton (Rogers, 1999), yet have survived seemingly unharmed in a laboratory for three months without supplemental nutrition (Dr. Sandra Brooke, pers. comm.). This, along with the fact that deep-sea corals experience lower temperatures, suggests that they might have a lower metabolic rate than shallow-water corals. *L. pertusa* is rather stenothermal, commonly found between 6-8°C, although they are reported to occur within the range of 4-12°C (Freiwald, 2002). A recent thermal tolerance study by Brooke et al. (2012) suggests that 15°C represents the LT_{25} for this species. Corals for this study were collected off the coast of Miami and in the northern Gulf of Mexico at sites that were ~8°C. Some *L. pertusa* reefs found on the oil/gas lease blocks in the Gulf are subject to temperature fluctuations due to internal waves as well as warm water intrusions from the Mississippi River (Davies et al., 2010) and Atlantic corals are subject to warm water meanders from the Gulf Stream (Bane et al., 2001). This indicates that *L. pertusa* are tolerant of sporadic warm water pulses that are several degrees higher than what they are accustomed to, which indicates that the species can accommodate short term stress. With Gulf temperatures expected to rise over 1°C within the century (Mendoza-Alfaro and P. Alvarez-Torres, 2012), one can hypothesize that *L. pertusa* will have enough metabolic flexibility to be able to adapt to limited temperature increases associated with climate change. This study aims to characterize the metabolic tolerance of *L. pertusa* to a range of temperatures that would normally be experienced in the field today, as well as what might become the norm within coming decades.

Materials and Methods

Collection. The initial collection and study took place in May/June 2007 off Miami Terrace aboard the R/V *Seward Johnson*. Corals were collected from 683 m depth via the manned submersible, *Johnson-Sea-Link II*. The second collection and set of experiments took

place in October 2010 in the Northern Gulf of Mexico aboard the NOAA ship *Ronald H. Brown*. Corals were collected off the Bureau of Ocean Energy Management lease blocks VK826 (489 m) and GC354 (528 m) with the ROV system *Jason/Medea*. Immediately following collection, *Lophelia pertusa* branches were broken down with a hammer and chisel to make pieces consisting of 1-11 polyps. For all experiments, these coral pieces were allowed a minimum of 12 hours of recovery and observation from collection/breakdown in a cold room close to the temperature at which they were found (5-8°C) before being used in any experiments. The criteria for suitability of the coral pieces for use in an experiment were that all polyps on the piece had to be extended, all polyps reacted to tactile stimulation, and the piece had been broken off the main branch in such a manner that there was no damage to any of the polyps (no tissue exposed on the bottom or sides of any pieces).

Oxygen consumption experiments. The oxygen consumption test apparatus was made up of an array of water-jacketed lucite chambers as described in Torres et al. (1994). Water that was temperature-controlled by a circulating water bath continuously flowed between the double-layered walls of each chamber, keeping the seawater and experimental animals in the inner chamber at a constant temperature. Once the chambers were sealed, the inner chamber was airtight. Oxygen microelectrodes (Clark, 1956; Mickel et al., 1983) manufactured in the lab were inserted into each chamber to measure oxygen levels of the water inside, while a magnetic stir bar situated beneath a perforated lucite false bottom kept the water well-mixed.

Individual *L. pertusa* pieces were placed into chambers appropriate for their size with 0.45 μm Millipore-filtered seawater instead of artificial seawater because *in situ* seawater was readily available. To determine the volume of seawater in the chamber accounting for the animal, at the end of each experiment, the coral pieces were placed in a graduated cylinder of

seawater and the volume of water displaced by each piece was subtracted from the previously measured water capacity of each chamber (25 replicates). The seawater was treated with 25 mg L^{-1} each of Streptomycin and Neomycin to minimize bacterial respiration and a control chamber was run for each experiment to ensure that any microbial consumption had a negligible effect on oxygen measurements (Torres et al., 1994). Upon sealing the chambers, the PO_2 of the water was measured every 30 seconds for 22 hours. To minimize effects of transferring the pieces to their chambers, no data prior to the 100 minute mark were used. This was the point by which all respiration rates had stabilized.

The Miami Terrace corals were run only at their habitat temperature of 8°C (18 replicates). There were 4 temperature treatment groups for the Gulf experiments, representing various points along this species' reported natural range. All specimens were collected at 8°C so the respiration rates measured in this treatment group (14 replicates) represent *L. pertusa's* normal rate. An 11°C treatment (8 replicates) and a 13°C treatment (8 replicates) were selected to determine the effects of bringing the corals up to the high end of their temperature tolerance. The 5°C treatment (8 replicates) represents the low end of the range and was also chosen because the temperature of the cold room on board the *Ronald H. Brown* ranged between 5°C and 7°C and tended to be closer to 5°C during hours when traffic in and out of the room were low. Except for the 5°C group, corals were taken from the cold room prior to the experiments and placed in a large unsealed chamber of seawater in the array that was increased from 5°C to whichever treatment temperature they were going to experience by increasing the temperature of the water bath 0.5°C every hour. The purpose of this was to avoid a heat shock response. At the end of each experimental run, coral pieces were inspected to see if they had their tentacles extended and reacted to tactile stimulation, were retracted but still responded to tactile

stimulation (by further retracting into the calyx), or were unresponsive (dead). Then they were promptly placed into a -80°C freezer for later analysis of metabolic enzymes.

Before and after each run, a 20 ml sample of water was taken from each chamber to be used for analysis of ammonium excretion via an auto-analyzer (Oceanic Nutrient Laboratory, University of South Florida).

Enzyme analyses. The frozen samples were stabilized at 4°C (the lower limit of the laboratory cold room) and weighed. Per the methods described in Chapter 2, after obtaining the wet mass of each whole piece, bits of tissue were removed from the skeletons with forceps, weighed, then homogenized in a glass grinder with an imidazole buffer solution that diluted the samples 1:8. The homogenate was spun down in a centrifuge for 10 minutes at 4750 x g and the supernatant was used in spectrophotometric analysis of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) activity levels at 8°C using the techniques of Torres and Somero (1988). Any remaining coral tissue was removed from the skeleton using a Waterpik® filled with artificial seawater at 4°C and the cleaned skeleton was then weighed again to determine total tissue wet mass for each sample.

Results

Oxygen consumption. At 8°C, there was no difference in oxygen consumption between the corals from Miami Terrace or the Northern Gulf of Mexico ($p=0.01$). At the control temperature, *Lophelia pertusa* exhibited a respiration rate of 1.16±0.18 µmol O_2 $g_{wm}^{-1}h^{-1}$ and 1.11±0.16 µmol O_2 $g_{wm}^{-1}h^{-1}$ (mean±S.E.), respectively. The 5°C treatment specimens exhibited respiration rates averaging 32% lower than that of the control group (Table 3.1; see page 47), which is an unexpectedly large drop for a temperature decrease of only 3°C. Corals in the 11°C treatment group exhibited an 11% increase in respiration from the control. At 13°C, the corals

showed a 23% rise in oxygen consumption, compared to the control, one quarter of the corals died, and all polyps were retracted into their calyces. The relationship between increasing respiration rates and increasing temperature was significant (ANOVA, $F=4.29$, $DF=3$, $p<0.01$). Tissue wet mass was not a causative factor in the difference between treatment groups (ANOVA, $F=0.31$, $df=3$, $p=0.8$). Dodds et al. (2007) found that *L. pertusa* lies midway between being an oxyconformer and an oxyregulator and calculated a critical oxygen partial pressure (P_c) of 9-10 kPa at 9°C, 9 kPa at 11°C, and 5-6 kPa at 6.5°C. No corals in the 5°C and 8°C treatments experienced conditions approaching a P_c, however some chambers did get low on oxygen in the 11°C and 13°C treatments, so a P_c of 9 kPa was assigned to the 11°C treatment and a P_c of 10 kPa was estimated for the 13°C treatment and only data from above those points were used in calculating respiration rates in order to capture "normal" respiration. In the few instances that the oxygen dropped to zero, the coral from that chamber was removed from the experiment early. Upon reviewing the data for the few corals that did seem to exhibit a P_c effect over the course of the experiment, it was discovered that these data aligned quite well with those of Dodds et al. (2007); the 11°C group showed a probable P_c around 8.7 kPa, while the 13°C group appeared to decline around 9.9 kPa. Only a few chambers were depleted enough to see a P_c effect, so these values are not definitive.

As temperature increased, the correlation between respiration rate and tissue wet mass became stronger. There was no significant correlation at 5°C ($p=0.1$) and 8°C ($p>0.05$), however, at 11°C ($p<0.001$) and 13°C ($p<0.001$) there was a clear trend of increased oxygen consumption with increasing wet mass (Figure 3.1; see page 47).

Metabolic enzymes. Temperature had no significant effect on CS (ANOVA, $F=1.44$, $df=3$, $p=0.3$) or MDH activity (ANOVA, $F=1.73$, $df=3$, $p=0.2$). The activity values of the

metabolic enzyme citrate synthase (CS) fell into the normal range expected for a cnidarian, averaging 0.18±0.02, 0.15±0.01, 0.20±0.02, and 0.10±0.01 activity units (U) g_{wm}^{-1} for the 5°C, 8°C, 11°C, and 13°C treatments, respectively, where activity units are micromoles of substrate converted to product per minute. Malate dehydrogenase (MDH) values were unexpectedly high, averaging 1.82±0.22, 2.05±0.14, 1.48±0.11, and 1.48±0.12 U g_{wm}^{-1} for the 5°C, 8°C, 11°C, and 13°C treatments, respectively. MDH activity in other cnidarians (medusae) is generally less than 1 U g_{wm}^{-1} (Thuesen and Childress, 1994). Lactate dehydrogenase (LDH) was undetectable in this species, suggesting it uses a different terminal glycolytic enzyme.

O:N ratios. Ammonium excretion was the only parameter that differed between the samples from Miami Terrace and the Gulf. The *in situ* O:N atomic ratio (oxygen respiration to ammonium excretion) for the Miami Terrace corals averaged 31, indicating that their diet consisted of more lipids than protein, while the Gulf corals averaged less than 8 for all treatments, indicating a diet of pure protein (Ikeda, 1974; Szmant et al., 1990; Torres et al. 1996).

Discussion

A fairly high rate of oxygen uptake was measured for *Lophelia pertusa* (an average of 1.14 μmol O_2 $g_{wm}^{-1}h^{-1}$ tissue wet mass for all corals at control temperature), as compared to other cold-water to temperate cnidarians. However, when making a Q_{10} correction for temperature (assuming Q_{10}=2) and comparing this rate to tropical corals, *L. pertusa* is on the lower half of the metabolic spectrum (Figure 3.2; see page 48). A Q_{10} represents the rate change of a biological/chemical reaction as a result of raising the temperature 10°C. Most biochemical reactions have a Q_{10} that lies between 2 and 3. Brooke and Young (2009) measured *in-situ* growth rates of *L. pertusa* transplants and found that the species has a linear extension rate of around 3 mm per year, which is a much slower rate than most tropical corals and strongly

reflects the lower metabolic rate. Naumann et al. (2013) studied the respiratory and calcification rates of *L.pertusa* and *Madrepora oculata* at 6°C, 9°C, and 12°C. Temperature did not affect the respiratory rate (reported in relation to surface area rather than tissue wet mass) of *L.pertusa*, but calcification decreased by 58% at the lowest temperature. The respiration rate of *M. oculata* decreased by 48% at the 6°C and 9°C degree treatments, while calcification decreased by 69% and 41%, respectively.

A wide range of growth rates have been reported, from 5 mm to over 3 cm per year, from observations in aquaria, stable isotope measurements, and discovery of colonies growing on man-made structures of known age (Roberts et al., 2009), although the growth rates are still well below that of many tropical corals. This may very well be a function of variability in substrate quality (zooplankton prey availability/unit time); when a substrate of opportunity arrives, *L. pertusa* have a higher growth rate that allows them to out-compete other would-be colonizers, while established colonies have settled into a slower growth rate due to competition for zooplankton resources. As colonies grow and age, the bioherm becomes host to many other animal species that then act as competion with the corals for food, reducing access to the nutrients necessary to support rapid growth.

Raising the temperature 3°C resulted in an 11% increase in oxygen consumption. The corals appeared unstressed by the increase, as all of the polyps were extended. However, this results in a Q_{10} of only 1.42. Raising the temperature by 5°C resulted in a 23% increase in oxygen consumption and the death of 25% of the corals. The surviving polyps had all retracted into their calyces, indicating stress. Still, this rise in metabolism only resulted in a Q_{10} of 1.51. These low Q_{10} values might be explained by the results of the "low temperature" treatment group at 5°C. For only a 3°C decrease in temperature, the 32% drop in oxygen consumption was

surprising. However, all of the control runs were performed first, so by the time the 11°C, 13°C, and 5°C experiments were begun, the corals had already been on board for several days in the 5°C-7°C cold room. Recalculating the Q_{10} values for the 11°C and 13°C treatments using the new "baseline" metabolism from the 5°C group results in a Q_{10} of 2.24 and 2.08, respectively. Therefore, even though the corals were slowly brought up to their experimental temperatures after being removed from the cold room, the observed oxygen consumption rates suggest that their metabolism was altered from a baseline closer to 5°C, rather than their *in-situ* rates. It is certainly interesting if this is indeed the case, as a few days seems like a rather short period of time for the mitochondrial densities in the coral tissue to change enough to alter baseline respiration (Pörtner, 2002). Perhaps 5°C isn't a difficult transition from 8°C because they experience slightly cooler water in the winter.

Acclimation to a temperature closer to 5°C would also help to explain the mortality observed at the highest temperature. 13°C is only one degree above their reported thermal range and two degrees below the 30% lethal temperature limit identified by Brooke et al. (2012). 25% of the corals didn't survive a 22-hour exposure to the highest treatment temperature, indicating that the reported upper tolerance of 12°C is accurate when adjusting for the lower baseline. Studies monitoring various environmental parameters of VK826, the site where most of the corals were collected, revealed that the temperature ranges from 6.5–11.6°C and the site can experience small fluctuations in temperature of around 0.5°C over 30 minutes to 0.8°C over 8 hours in 5-11 hour cycles (Davies et al., 2010; Mienis et al., 2012). Therefore, if the corals were acclimatized to 5°C, not only were they exposed to temperatures 8°C higher than what they had become used to, but they were also experiencing a temperature that was higher than anything they ever experience in the field. Brooke et al. (2012) exposed corals to 15°C for 24 hours, 7°C

above the ambient temperature. They all survived, but 30% died within the following week, indicating that 15°C is just above their LT_{25}. Some even survived a 20°C exposure, but all died within the week. Perhaps the combination of temperature stress and declining oxygen was too much for the corals to manage and that is why some of them were unable to handle a temperature increase that they normally would be able to survive for 22 hours (Pörtner, 2002). Some large fragments of *L. pertusa* were brought back to the laboratory after the Gulf cruise and set up in a cold room at 8°C. Unfortunately, the cold room failed sometime over the weekend and the temperature rose to 17°C. This killed all of the corals. It is unknown how long the corals remained at that temperature, but it was somewhere between 24 and 72 hours.

Metabolic enzyme activity correlates well with respiration (Hochachka and Somero, 2002), so CS, MDH, and LDH activities were measured. CS regulates the first step in the Krebs cycle. MDH also plays a part in this cycle, although it has several other roles related to metabolic function. The activities of these two Krebs cycle enzymes are indicators of aerobic function. Temperature did not significantly affect enzyme activity in these experiments. The CS activity values fell within the normal range expected for a cnidarian, however, the MDH values were rather high, coming in at 1.5-2 times the expected level (Thuesen and Childress, 1994). LDH, the terminal enzyme in anaerobic glycolysis, is an indicator of an animal's anaerobic capacity. This enzyme had an activity value too low to be detected; perhaps *L. pertusa* has an analogous enzyme to replace LDH. The coral colonies at VK826 live within an oxygen minimum zone, likely near their P_c (Davies et al., 2010). This region is also subject to high sediment flux due to Mississippi River outflow and algal blooms (Davies et al., 2010; Mienis et al., 2012). Additionally, Brooke et al. (2009) found that *L. pertusa* can tolerate fairly heavy sedimentation. These factors lead to the expectation that Lophelia would be anaerobically

competent and display a high level of LDH activity, considering that it is regularly exposed to low-oxygen conditions. Like some mollusks (Hochachka and Somero 2002), Lophelia may have another terminal enzyme for glycolysis, or the high MDH values may reflect an increase in activity of the malate shuttle to somehow "make up for" an apparent lack of a terminal glycolytic enzyme by being highly efficient in passing the reducing equivalents generated by glycolysis into the mitochondrion during bouts of hypoxia.

The O:N ratios obtained for the Gulf specimens all indicated a pure-protein diet, and this is consistent with environmental observations at VK-826. Both Davies et al. (2010) and Mienis et al. (2012) observed large diel vertical migrations of zooplankton over the reef that could serve as a consistent source of protein. As mentioned previously, *L. pertusa* has been observed to avidly feed on live prey in the field, seeming to prefer it. This does cause a bit of a mystery then, as to why *L. pertusa* from Miami Terrace exhibited an O:N ratio indicative of a lipid-rich diet. In this case, the high lipid catabolism is not very likely to indicate starvation, as this species survives well in laboratory conditions without food. Lee and Mooers (1977) observed migration of zooplankton as deep as 700m on Miami Terrace, so the corals there do have live prey available, although their particular prey spectrum appears to be more lipid-rich than that of the Gulf corals, possibly due to the currents near that particular collection site providing an influx of more lipid-rich species.

In summary, at *in-situ* temperature, *L. pertusa* has a rather high metabolic rate, as compared to cold-water and temperate cnidarians. However, as its slower growth rate would indicate, it has a low metabolic rate compared to tropical corals. This species seems to adapt quickly to temperatures within its normal range. However, when experiencing declining oxygen levels, it struggles to survive at temperatures outside its thermal window. Many *L. pertusa* in the

Gulf of Mexico live in an oxygen minimum zone and near their P_c. With water temperature on the rise, this may have implications for how this species will survive when faced with increased thermal stress. *L. pertusa* does exhibit high aerobic capability, however, which may mean that it is metabolically efficient. With the predicted rise in Gulf temperature being a gradual one, this coral may be in a position to adapt to this change after all.

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Tables and Figures

Table 3.1. Summary of data from all treatment groups.

Treatment group	Respiration (μmol O_2 $g_{wm}^{-1}h^{-1}$± S.E.)	CS (U g_{wm}^{-1})	MDH (U g_{wm}^{-1})	O:N
8°C Miami	1.16±0.18	0.17±0.01	2.24±0.19	34
8°C Gulf	0.11±0.16	0.13±0.01	1.79±0.02	7
11°C	1.20±0.08	0.20±0.02	1.48±0.11	5
13°C	1.33±0.11	0.10±0.01	1.48±0.12	3
5°C	0.74±0.15	0.18±0.02	1.82±0.22	2

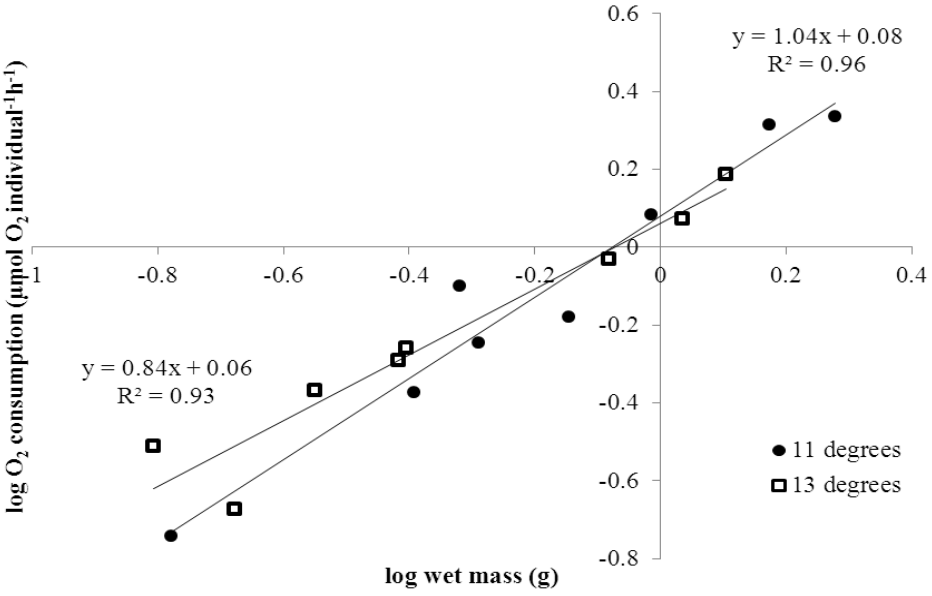

Figure 3.1. Wet mass versus oxygen consumption at 11 and 13°C.

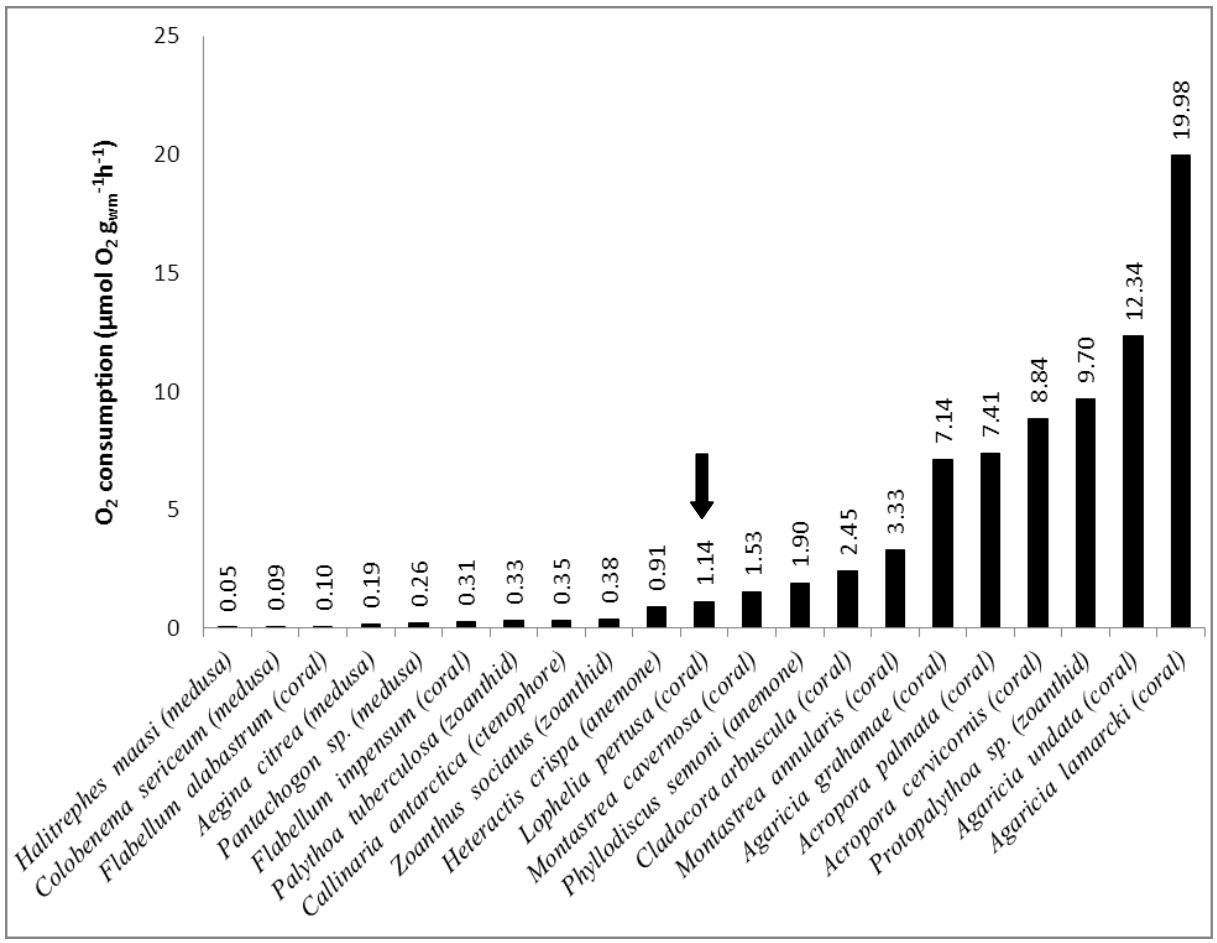

Figure 3.2. *Lophelia* within the metabolic spectrum of cnidarians. *L. pertusa* lies on the high end of the metabolic spectrum compared to some other cnidarians, however, compared to tropical corals, it lies on the lower end, even when assuming a Q_{10} of 2 and adjusting for a temperature of 28°C. Species listed in this graph include medusae (Thuesen and Childress, 1994), zoanthids and anemones (Shick, 1990), a ctenophore (Scolardi et al., 2006), and corals (Buhl-Mortensen et al., 2007; Davies, 1980; Henry and Torres, 2013).

Chapter Four:

Metabolism of an Antarctic solitary coral, *Flabellum impensum*

A Note to Reader

This work has been previously published in the Journal of Experimental Biology and Ecology. Permission to reproduce this work can be found in Appendix I. The author contributed >95% of the work involved in this publication.

Abstract

Few physiological or behavioral studies have been undertaken on the genus *Flabellum*, particularly on Antarctic species. The present study characterizes the metabolism of the endemic Antarctic coral *F. impensum*, one of the world's largest solitary corals, with measurements of oxygen consumption rate and metabolic enzyme activity. *F. impensum* had a low rate of oxygen consumption at 0°C, ranging from 0.06-0.64 μmol O_2 g_{wm}^{-1} h^{-1} and averaging 0.31 μmol O_2 g_{wm}^{-1} h^{-1}, calculated using tissue wet mass. Ammonium excretion averaged 4.2 nmol NH_4^+ g_{wm}^{-1} h^{-1} (range: 0.5-14 nmol NH_4^+ g_{wm}^{-1} h^{-1}). The activity values of the metabolic enzymes citrate synthase (CS), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH) fell within the normal range expected for a cnidarian, averaging 0.13 (range: 0.04-0.32), 1.0 (range: 0-3.5), and 0.42 (range: 0.18-0.99) activity units (U) g_{wm}^{-1}, respectively. Skeletal density averaged 22% more than the density of pure aragonite and a count of the growth bands on the calyx suggests

that this species has a linear extension rate of approximately 1 mm per year. *F. impensum* is a long-lived, slow-growing coral, with a low metabolic rate.

Introduction

Seventeen species of scleractinian corals occur in Antarctic waters. As is true of most high-latitude corals, all Antarctic species are azooxanthellate due to the low temperatures and seasonal swings in irradiance typical of the Antarctic system (Cairns, 1990). The genus *Flabellum* is one of two flabellid genera known to occur in the Antarctic region (Cairns, 1990). Corals within the family Flabellidae are exclusively solitary and the family exhibits a cosmopolitan distribution (Cairns, 1990).

Flabellum impensum is endemic to Antarctica and has a circumpolar distribution at depths of 46-2200 m. It is most commonly found from 100-1000 m (Cairns, 1990, 1982). *F. impensum* is one of the largest flabellate corals and also one of the largest solitary Scleractinia, achieving a height of up to 8 cm. It exhibits quite a variable range of morphologies (Cairns, 1990), causing smaller specimens to be mistaken for other species of *Flabellum*. Habitat information for Antarctic species of *Flabellum* is sparse. However, in the North Atlantic, corals belonging to the genus are abundant on the soft bottom of the continental slope, in abyssal areas, and on the mid-ocean ridge (Buhl-Mortensen et al., 2007; Hamel et al., 2010; Mercier et al., 2011).

Waller et al. (2008) found that *Flabellum impensum* is gonochoric and females brood several stages of planulae year-round. Studies exist for just a handful of other species of *Flabellum* from various sites around the world. Reproductive strategies have been described for *F. curvatum* (Squires, 1962; Waller et al., 2008), *F. thouarsii* (Waller et al., 2008), *F. alabastrum*

(Waller and Tyler, 2011), and *F. angulare* (Mercier et al., 2011; Waller and Tyler, 2011). Those studies revealed that Southern Ocean species of *Flabellum* (*F. curvatum* and *F. thouarsii*) are gonochoric brooders, while North Atlantic species (*F. alabastrum* and *F. angulare*) are gonochoric spawners, producing lecithotrophic larvae. Buhl-Mortensen et al. (2007) studied field/laboratory behavior and respiration in *F. alabastrum* and found that the species is slightly mobile and has a low metabolic rate. Seasonal growth rates described by Hamel et al. (2010) indicated that *F. alabastrum* is also long-lived and slow-growing. Other than what has been ascertained by those few studies, little else is known about the basic biology of corals in the genus *Flabellum*, or that of cold-water corals in general.

Many polar marine species are stenothermal and, with some exceptions, adaptation to their environment includes lower rates of growth and metabolism (Clarke, 1980; Clarke, 1998; Peck, 2002), compared to their non-polar relatives. The present study analyzed the rates of oxygen consumption, nitrogen excretion, and metabolic enzyme activity in order to characterize the metabolism of *F. impensum*.

Materials and Methods

Collection. Corals were collected with a small (10 m) otter trawl by the RVIB *Nathaniel B. Palmer.* Trawls were conducted on the western Antarctic Peninsula during March of 2010 off Anvers and Charcot Islands at 600 m and 200 m depth, respectively. Corals were carefully examined after collection for signs of trauma. Individuals that showed no signs of damage were allowed to recover in pre-chilled seawater in a 0°C incubator for 12-22 hours before being used in any experiments. After the recovery period, only those that were fully extended and that reacted quickly to tactile stimulation were selected for the experiment.

Oxygen consumption experiments. The apparatus for measuring oxygen consumption consisted of an array of water-jacketed lucite chambers as described in Torres et al. (1994). The chambers' construction allowed for water (temperature-controlled by a circulating refrigerated water bath) to continuously flow between their double-layered walls, keeping the seawater and experimental animals in the inner chamber at a constant temperature (0°C). Once sealed, no air can enter or leave the inner chamber. Oxygen microelectrodes (Clark, 1956; Mickel et al., 1983) fabricated in-house were inserted into each chamber to measure oxygen levels of the water inside every 30 seconds, while a magnetic stir bar under a perforated lucite false bottom kept the water well-mixed.

Individual *F. impensum* were placed into chambers appropriate for their size with 0.45 μm Millipore-filtered seawater. To determine the volume of seawater in the chamber, accounting for the animal, the volume of each coral was calculated as an elliptical cone and subtracted from the previously measured water capacity of each chamber (25 replicates). The water was treated with 25 mg L^{-1} each of Streptomycin and Neomycin to minimize bacterial growth and a control chamber was run for each experiment to ensure that any microbial consumption had a negligible effect on respiration measurements (Torres et al., 1994). Once the chambers were sealed, the PO_2 of the water was measured continuously for 24 hours. To minimize effects of animal transfer, no data were used until 100 minutes had elapsed. This was the point by which each coral's respiration rate had stabilized. Measurements were taken on a total of 9 specimens; the small sample size was due to the difficulty of obtaining intact individuals. At the end of the experimental runs, all corals appeared healthy, had their tentacles extended, and reacted to tactile stimulation. They were promptly placed into a -80°C freezer for later analysis of metabolic enzymes.

Before and after each run, a 20 ml sample of water was taken from each chamber to be analyzed for ammonium excretion via an auto-analyzer (Oceanic Nutrient Laboratory, University of South Florida).

Enzyme analyses. The frozen samples from the respiration experiments were stabilized at 4°C (the lower limit of the cold room) and weighed using an adaptation of the buoyant weight technique described by Davies (1989). After obtaining the buoyant weight of each sample, the coral tissue was removed from the skeleton using a Waterpik® filled with artificial seawater at 4°C. Per the procedure described in Chapter 2, pieces of tissue were separated from the mucus and excess water, weighed, and homogenized in a glass grinder with an imidazole buffer solution that diluted the samples 1:8. The homogenate was spun down in a centrifuge for 10 minutes at 4500 rpm and the supernatant was used in spectrophotometric analysis of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) activity levels at 0°C using the techniques of Torres and Somero (1988).

Skeletal observations. After all the tissue had been cleaned off each calyx, they were soaked in a 10% solution of commercial bleach in seawater for 24 hours to remove any remaining bits of tissue, rinsed three times in artificial seawater, and buoyantly weighed again (all at 4°C) to obtain a value for the percentage buoyant weight of tissue versus skeleton. The skeletons were then rinsed three times with milli-Q water and allowed to soak for another 24 hours to remove the salt before being put into a 60°C drying oven until they reached a constant weight (approximately 3 weeks). The dry weight of each calyx was then measured to calculate skeletal density. The growth bands were also counted for each specimen. The bands in this species are obvious enough to count under a dissecting microscope with a light illuminating the

calyx, without the aid of stains (Figure 4.1; see page 64) that are necessary for smaller, thicker-walled species (e.g. Goffredo et al., 2004; Hamel et al., 2010).

Results

The corals exhibited a low rate of oxygen consumption, ranging from 0.06-0.64 μmol O_2 g^{-1} h^{-1} and averaging 0.31±0.07 μmol O_2 g^{-1} h^{-1} (±S.E.), using tissue wet mass. Total oxygen consumption increased with increasing wet mass (ANOVA, F=6.23, df=1, p=0.02). However, as is typical of most animal groups (Hemmingsen, 1960), the respiration rate per gram of tissue was lower in the larger corals (Figure 4.2; see page 64).

Ammonium excretion fell between 0.5-14 nmol NH_4^+ g^{-1} h^{-1} and averaged 4.2±1.5 nmol NH_4^+ g^{-1} h^{-1} (±S.E.), resulting in an O:N atomic ratio (oxygen respiration to ammonium excretion) that ranged from 11-91 and averaged 39. This indicates that individual corals had different prey spectra, although most were catabolizing lipids to some degree (Ikeda, 1974; Mayzaud and Conover, 1988; Szmant et al., 1990). Nitrogen excretion per gram of tissue was lower than expected for the larger specimens, however, excretion did show a general trend of slowly increasing with wet mass (ANOVA, F=5.64, df=1, p=0.03) (Figure 4.2; see page 64).

Activity of CS and MDH fell within the range previously reported for cnidarians, averaging 0.13±0.03 (range: 0.04-0.32) and 1.0±0.3 (range: 0-3.5) activity units (U) g_{wm}^{-1} (±S.E.), respectively, where activity units are micromoles of substrate converted to product per minute. LDH activity was on the higher end of the range for a cnidarian, averaging 0.42±0.08 U g_{wm}^{-1} (range: 0.18-0.99). Mass-specific enzyme activities did not change with increasing mass (all p-values were greater than 0.3).

Tissue weight averaged 20% of total buoyant weight and skeletal density averaged 3.58 g cc^{-1}, which is above the density of pure aragonite (2.94 g cc^{-1}), a value that is sometimes assumed in studies where the density of the coral skeleton is used in a calculation. The difference indicates that other substances are being incorporated into the skeletal matrix. Smaller corals (<5 g tissue wet mass) had skeletons whose densities averaged 3.13 g cc^{-1}, which is fairly close to the density of aragonite, while the larger corals (>25 g tissue wet mass) averaged 4.02 g cc^{-1}. Analysis of two "clean" pieces of two different skeletons with a scanning electron microscope (Electron Microscopy Laboratory, University of South Florida) showed that the only element heavier than calcium that was detected at a notable concentration was, as expected, strontium, which averaged about 2% by weight. The high density of the whole skeletons is likely due to the visible sediment incorporated into the skeleton on the "dirty" side of the calyx (Figure 4.3; see page 65), rather than the skeletal matrix taking up heavier elements as it grows.

The growth bands nearest the bottom of the calyx were the thickest (~3-4 mm) and progressively decrease in thickness from the bottom to the top. If these bands are laid down annually, as in other solitary corals (e.g. Goffredo et al., 2004; Hamel et al., 2010), comparing the number of bands to the height of the calyx yields an average linear extension rate of 1.11 mm per year for these specimens.

Discussion

A fairly low rate of oxygen uptake was measured for *Flabellum impensum* (an average of 0.31±0.07 µmol O_2 g^{-1} h^{-1} tissue wet mass), placing it in the lower half of the metabolic spectrum when compared to other scleractinians for which there are such data, even with a Q_{10} correction for temperature (assuming Q_{10}=2) (Table 4.1; see page 66).

As sessile species, most corals have low activity costs and are therefore likely to have a low metabolism, particularly in polar or other cold-water environments. Most species of *Flabellum*, however, are more active than other corals; they are free-living individuals as adults and are capable of movement. Captive individuals of *F. alabastrum* have been observed leaving tracks in the sediment at a rate of 3.2 cm mo^{-1}. Moreover, the polyp can inflate to more than 10x its normal, relaxed (not retracted) size, which would give it the ability to use currents as a means of transportation (Buhl-Mortensen et al., 2007). At the end of some experimental runs, the larger coral polyps had expanded within the respiration chamber. Presumably, as the oxygen concentration in the chamber decreased, the polyps expanded to facilitate oxygen uptake over a greater surface area. Alternately, it may have been an attempt to "escape" the declining oxygen in the chamber. Despite the behavioral response, the PO_2 within the chambers never dipped below 35 mm Hg and respiratory rate remained constant over the course of the experiment, suggesting that the corals remained well above their $P_{c.}$

Buhl-Mortensen et al. (2007) found that *F. alabastrum* had a respiratory rate (2.2 µl O_2 g^{-1} h^{-1} tissue wet mass= 0.10 µmol O_2 g^{-1} h^{-1}) lower than that of *F. impensum*. Further, Hamel et al. (2010) reported that *F. alabastrum* was a slow growing coral, reaching its maximum height of about 43 mm at about 45 years of age. Goffredo et al. (2004) studied a Mediterranean solitary coral, *Balanophyllia europaea*, and determined its maximum size to be about 20 mm after as many years. The maximum size of specimens used in the present study was 62 mm, and with a metabolic rate in the same range as that of *F. alabastrum*, it is likely that it has a similar growth rate. Cairns (1990) noted a maximum size of 80 mm for *F. impensum*, meaning that the species may live to be around 80 years of age. The specimens examined in the present study appeared to have annual growth bands similar to those described by Goffredo et al. (2004) and Hamel et al.

(2010), with the thickest bands occurring before the age of 5 years and gradually thinning as the coral aged, indicating greater linear extension in earlier years. This result correlates well with the oxygen consumption data, as more energy is needed for growth in the early years, accounting for the large differences in metabolic rate among the smaller specimens. As the corals age, the metabolic rate still increases, as the calyx is becoming wider and the coral has a larger body to support. However, less linear extension is occurring in the calyx, so the rise in metabolic rate decreases. This may also explain why the smaller corals had skeletal densities closer to that of pure aragonite as compared to their larger counterparts.

Young corals, which grow quickly and live upright, attached to a substrate, may be exposed to fewer particles that could become incorporated into their skeletal matrix than that of older, slower-growing and mobile individuals that are in constant contact with the sediment. Buhl-Mortensen et al. (2007) noted that in addition to lateral movement across the sediment, *F. alabastrum* was also capable of rotating and righting itself if placed upside-down. Cairns (1990) postulated that *F. impensum*, after reaching a size at which it becomes dislodged from the substrate on which it settled as a planula, remains in an upright position, possibly by partially burying itself in the sediment. This was based on his observation of the placement of epibionts on the skeleton. All specimens collected for this study had a worn pedicel, which indicates they had long been detached from their substrate. The larger *F. impensum* appeared to have been lying in a prone position, as one side of the skeleton appeared "dirtier" than the other, with more bits of debris embedded in the skeleton. This also suggests a "preferred" side upon which to rest, which would be possible if they have the same ability as *F. alabastrum* to rotate. This was less apparent in the smaller specimens, which had no noticeable epibionts or skeletal inclusions.

Oxygen consumption isn't the only variable that can differ between a polar coral and its temperate and tropical relatives. The enzyme-mediated metabolic reactions of a polar marine invertebrate can be 10-30 times slower than that of its tropical counterpart (Clarke, 1998). Decreases in environmental temperature reduce the number of enzyme molecules available with enough energy to catalyze their respective reactions. Animals that compensate for such environmental differences exhibit 1) an increase in the number of enzyme molecules present, 2) a different type of enzyme used to catalyze a specific reaction, or 3) a modified activity rate of the enzymes (Hochachka and Somero, 1973). With no observable compensation in oxygen uptake, it is unlikely that *F. impensum* has evolved any change in the activity of its metabolic enzymes, as metabolic enzyme activity tends to correlate with respiration.

Lactate dehydrogenase (LDH) is the terminal enzyme in anaerobic glycolysis and its activity in an organism is an indicator of the individual's anaerobic capacity. Citrate synthase (CS) regulates the first step in the Krebs cycle, and malate dehydrogenase (MDH) also plays a role in this cycle, although it has several other functions related to metabolism. The activities of the two Krebs cycle enzymes are indicators of aerobic function. Activities of CS and MDH (0.13±0.03 and 1.0±0.3 U g^{-1}, respectively) extracted from *F. impensum* fell within the ranges measured for medusae (Table 4.1; see page 66), indicating that it is similarly aerobically poised. The LDH activity (0.42±0.08 U g^{-1}), however, was on the higher end of the range reported for other cnidarians, indicating a moderate anaerobic potential as well. Perhaps if sediment becomes disturbed (glacial activity, movement of another animal) near an individual and it has the capacity to inflate enough to use currents as a means of transportation, as Buhl-Mortensen et al. (2007) have suggested, it may need to temporarily rely on anaerobic respiration to dislodge itself and move to another location.

F. impensum excreted 4.2±1.5 nmol NH_4^+ g^{-1} h^{-1}. The O:N atomic ratio ranged from 11-91 and averaged 39, indicating that lipids are an important component in this coral's diet (Ikeda, 1974; Youngbluth et al., 1988). Many planktonic Antarctic organisms experience a drop in their metabolism during the winter and go into a state of reduced activity. To prepare for this, they store lipids. The lipid-rich animals form part of the coral's diet and contribute to its lipid stores. Lipids then become a significant metabolite when the corals are not feeding.

In summary, the endemic Antarctic coral, *F. impensum*, like other related solitary corals, is slow-growing and has a low metabolic rate. It is one of the largest solitary corals, displays reasonable aerobic and anaerobic capacity, has a lipid-rich diet, and is quite long-lived, possibly capable of reaching 80 years in age. Polar corals are not a well-studied group; this study adds much needed information to the body of knowledge concerning them, as well as contributing new physiological insights pertaining to the genus, *Flabellum*.

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Tables and Figures

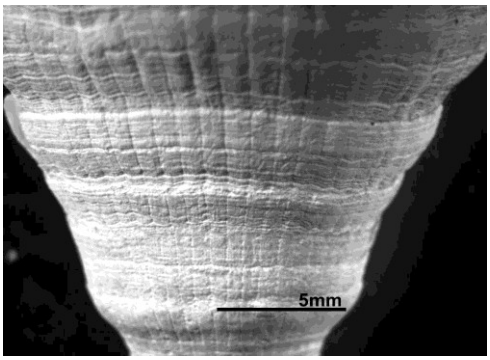

Figure 4.1. A close-up of a specimen of *F. impensum*. Growth bands are clearly visible on the calyx surface.

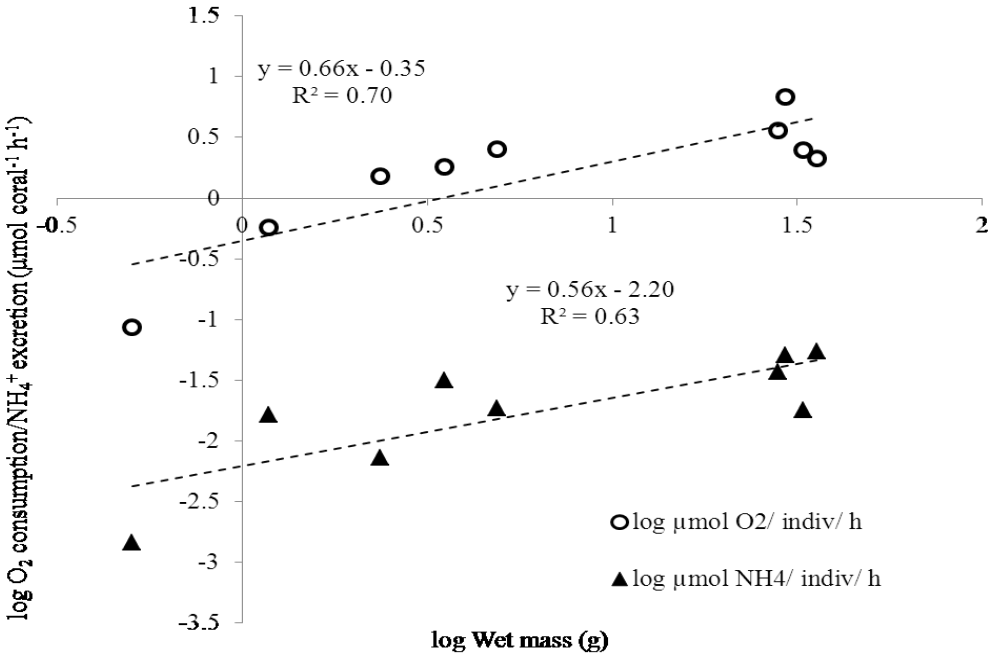

Figure 4.2. *F. impensum* respiration and nitrogen excretion rates in relation to wet mass. Increasing body size results in an increased respiratory rate (p=0.005), but as the corals near their maximum size, this increase slows. Nitrogen excretion is variable, but does show some increase with mass in smaller specimens (p=0.01).

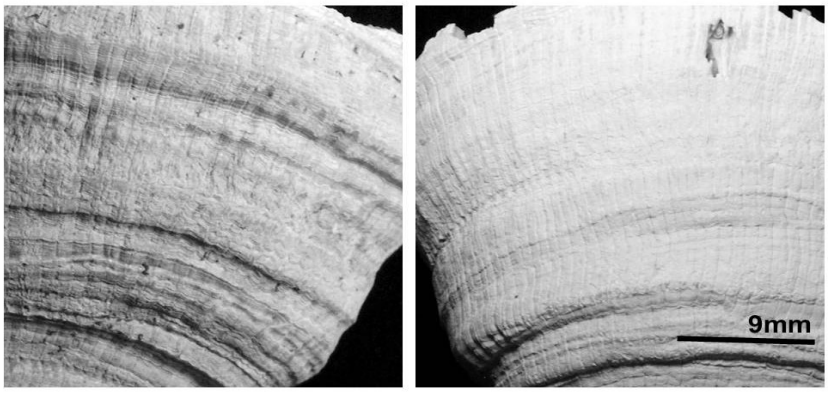

Figure 4.3. The “dirty” versus “clean” side of the calyx of an *F. impensum* specimen.

Table 4.1. Respiration and enzymes in comparable cnidarians. A 95% water content was assumed for values expressed relative to dry weight in order to convert to µmol O_2 g_{ww}^{-1} h^{-1}. ("na"= not measured , "nd"= not detected)

animal	species	O_2 consumption (mean± S.E.) (µmol O_2 g_{ww}^{-1} h^{-1})	T_{resp} (°C)	CS activity (units g^{-1} ± S.E.)	LDH activity (units g^{-1} ± S.E.)	MDH activity (units g^{-1} ± S.E.)	$T_{enzymes}$ (°C)	source
scleractinian	*Flabellum impensum*	0.313±0.074	0	0.126±0.029	0.423±0.083	1.008±0.344	0	this study
scleractinian	*Flabellum alabastrum*	0.10	7.7	na	na	na	na	Buhl-Mortensen et al., 2007
scleractinian	*Montastrea annularis*	3.33	28	na	na	na	na	Davies, 1980
scleractinian	*Acropora palmata*	7.41	28	na	na	na	na	Davies, 1980
scleractinian	*Acropora cervicornis*	8.84	28	na	na	na	na	Davies, 1980
scleractinian	*Montastrea cavernosa*	1.53	28	na	na	na	na	Davies, 1980
scleractinian	*Agaricia lamarcki*	19.98	28	na	na	na	na	Davies, 1980
scleractinian	*Agaricia undata*	12.34	28	na	na	na	na	Davies, 1980
scleractinian	*Agaricia grahamae*	7.14	28	na	na	na	na	Davies, 1980
alcyonacean	*Gersemia rubiformis*	1.0	3-5	na	na	na	na	Hargrave et al., 2004
alcyonacean	*Acanella arbuscula*	1.4	3-5	na	na	na	na	Hargrave et al., 2004
zoanthid	*Protopalythoa sp.*	9.7	30	na	na	na	na	Shick, 1990
zoanthid	*Zoanthus sociatus*	0.38	25	na	na	na	na	Shick, 1990
anemone	*Phyllodiscus semoni*	1.9	30	na	na	na	na	Shick, 1990
anemone	*Heteractis crispa*	0.91	30	na	na	na	na	Shick, 1990
medusa	*Polyorchis penicillatus*	na	na	0.238±0.029	0.172±0.016	0.939±0.019	20	Thuesen and Childress, 1994
medusa	*Haliscera bigelowi*	0.128±0.030	5	nd	0.028±0.009	na	20	Thuesen and Childress, 1994
medusa	*Halitrephes maasi*	0.046±0.006	5	0.004±0.001	0.017±0.005	na	20	Thuesen and Childress, 1994
medusa	*Crossota rufobrunnea*	0.154±0.024	5	0.147±0.013	0.011±0.007	0.578±0.432	20	Thuesen and Childress, 1994
medusa	*Vallentinia adherens*	1.932±0.478	15	3.563±0.860	0.057±0.008	na	20	Thuesen and Childress, 1994
medusa	*Aegina citrea*	0.185±0.037	5	0.043±0.007	0.085±0.015	0.624±0.084	20	Thuesen and Childress, 1994
medusa	*Atolla wyvillei*	0.134±0.044	5	nd	0.243±0.055	0.768±0.208	20	Thuesen and Childress, 1994
medusa	*Paraphyllina ransoni*	0.333±0.104	5	0.124±0.044	0.195±0.088	na	20	Thuesen and Childress, 1994
medusa	*Periphylla periphylla*	0.094±0.017	5	0.017±0.003	1.711±0.552	0.669±0.144	20	Thuesen and Childress, 1994
ctenophore	*Callianira antarctica*	0.35±0.18	0.5	na	na	na	na	Scolardi et al., 2006
ctenophore	*Beroe sp.*	0.044	-1.5	na	na	na	na	Ikeda and Bruce, 1986
ctenophore	*Mertensiidae sp.*	0.237	-1.6	na	na	na	na	Ikeda and Bruce, 1986

Chapter Five:

Concluding Remarks

Symbiosis between corals and their algal symbionts evolved as a mechanism to maintain a competitive edge within shallow oligotrophic waters; however, lack of symbiosis has not impeded the success of corals in the deep. Despite the fact that shallow water corals are susceptible to bleaching events that disrupt their symbiosis, many species can eventually recover and some do not appear to suffer much damage from the process. From the experiments conducted on three different types of corals from different light, temperature, and depth regimes, it can be concluded that:

- *Cladocora arbuscula*, one of the most abundant species of endemic scleractinian corals off the west central coast of Florida, thrives in a region subject to red-tides, intermittent high sedimentation, and the occasional hurricane. Data from the experiments described here show that its metabolic rate is on the lower end of the spectrum for sub/tropical cnidarians, suggesting that it has a low growth rate that is similar to the Mediterranean *C. caespitosa*. In spite of this, its high levels of both aerobic and anaerobic enzyme function allow it to thrive in this environment, even when faced with conditions that cause widespread coral bleaching. Although these experiments have shown that *C. arbuscula* can function well without zooxanthellae, it seems to function most optimally with endosymbionts as members of the holobiont.

- The deep-sea coral, *Lophelia pertusa*, has a high metabolic rate compared to cold-water and temperate cnidarians at *in-situ* temperature, which, expectedly, is low compared to tropical corals. Calculating the Q_{10} for bringing *L. pertusa* up to the environmental temperature of *Cladocora arbuscula* results in a value of 1.8. This species seems to adapt quickly to temperatures within its described normal range, however, declining oxygen levels threaten its survival at temperatures higher than it normally experiences. Many *L. pertusa* in the Gulf of Mexico live in an oxygen minimum zone and near their P_c. With water temperature on the rise, this may have implications for how this species will survive when faced with increased thermal stress. *L. pertusa* does exhibit high aerobic capability, however, which may mean that it is metabolically more efficient. It is likely anaerobically efficient as well, despite the apparent lack of LDH. With the predicted rise in Gulf temperature being a gradual one, this coral may be in a position to adapt to this change.

- Polar corals are not a well-studied group. The endemic Antarctic coral, *Flabellum impensum*, like other related solitary corals, is slow-growing and has a low metabolic rate. Calculating a Q_{10} for this species at *Cladocora arbuscula*'s habitat temperature results in a value of 2.7. It is one of the largest solitary corals, displays reasonable aerobic and anaerobic capacity, has a lipid-rich diet, and is quite long-lived, possibly capable of reaching 80 years in age. This study adds much needed information to the body of knowledge concerning them, as well as contributing new physiological insights pertaining to the genus, *Flabellum*.

In the past, only shallow water corals such as *C. arbuscula* were given the honor of being thought of as great habitat builders that house and protect many other animal species. It is now known that many corals in the mid- to deep-sea can serve this same function. Gulf and Atlantic *L. pertusa* reefs provide habitat to thousands of species from a wide range of phyla and can have

several times the diversity of the surrounding soft bottom. They create patches of high diversity in low-diversity environments, which is possible because they occur in a relatively stable environment with a predictable food supply. Deep-sea corals represent an important ecological niche in that they can block currents and predators, act as a nursery habitat, and provide feeding, breeding, and spawning ground for economically valuable species. Although thousands of deep-sea corals have been described, researchers estimate that 800 stony species and their associates have yet to be encountered.

Exploitation of deep-sea reefs by humans has been rapidly increasing despite the lack of biological and ecological information. It is already known that shallow-water corals recover slowly after an acute trauma, and it is likely that deep-sea reefs are even more sensitive to destruction. Natural and anthropogenic causes are to blame for the state of decline both shallow-water and deep-sea reefs are in a today, as compared to a century ago. It is important to characterize the biology of corals so that resource managers can have the information necessary to allow them to appropriately regulate anthropogenic activities in and around reef environments. It is also necessary to have a better understanding of coral physiology so that we may have a sense of how corals are going to respond to changes in climate, natural disasters, or human-mediated environmental catastrophes.

Appendix I

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About the Author

Lara Henry was born and raised in San Francisco, California. She graduated from the University of California at Davis in 2002 with a B.S. in Neurobiology, Physiology, and Behavior in preparation for medical school. After an intensive marine science course at the Bodega Marine Laboratory in Bodega Bay, CA and a marine science teaching internship at the Marine Resources Development Foundation Marine Lab in Key Largo, FL, she decided to instead pursue oceanography. She received a Howard Hughes Medical Institute scholarship for a marine science apprenticeship at the Friday Harbor Marine Laboratory in Friday Harbor, WA where she gained valuable field and laboratory experience in comparative invertebrate physiology. In 2005, Lara was admitted into the graduate program at the College of Marine Science at the University of South Florida. She joined the Physiology Laboratory in 2007 to begin studying coral physiology under the guidance of her wise and patient advisor, Dr. Joseph J. Torres. For her own research purposes, as well as those of other Physiology Lab members, Lara has spent nearly 2000 hours at sea. Her research has allowed her to travel all over the Gulf of Mexico, dive to the cold depths of the Atlantic Ocean in a manned submersible, and to traverse the frozen seas of Antarctica (for which she was awarded the Antarctica Service Medal and Certificate from the National Science Foundation in 2010).