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Growth and Reproduction in Gulf of Mexico Black Corals (Antipatharians) in Field and Laboratory Studies

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GROWTH AND REPRODUCTION IN GULF OF MEXICO BLACK CORALS (ANTIPATHARIANS) IN FIELD AND LABORATORY STUDIES

A Thesis

by

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GROWTH AND REPRODUCTION IN GULF OF MEXICO BLACK CORALS (ANTIPATHARIANS) IN FIELD AND LABORATORY STUDIES IN SOUTH TEXAS

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ABSTRACT

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Black corals provide an important ecosystem of marine life and are found throughout all the oceans of the world at depths between 2 and 8,600 m. However, little is understood about their life history and the factors that control the distribution of black corals, particularly in the Gulf of Mexico (GoM). Given the impacts of the Deepwater Horizon oil spill on soft corals (e.g., black corals and octocorals) in the GoM, studies of their growth and reproductive biology in both natural and aquaculture environments are crucial for restoration efforts. The objectives of this study were to examine reproductive processes, compare growth rates *in situ* and in aquaculture, and provide an aquaculture guide for two species of black corals (*Stichopathes luetkeni* and *Antipathes atlantica)* in the GoM. The data collected during this study will provide vital information for the protection and management of black corals at mesophotic depths in the GoM.

DEDICATION

The completion of my master's degree would not have been possible without the love and support of my family and friends. To my mother, Diana, my father, Luis, my sister, Adriana, my brother Luis, and the rest of my family - thank you for always supporting me and believing in me. To my husband, David - thank you for both encouraging me and grounding me through this journey. To my in-laws I want to thank you both for supporting me in more ways than I could have imagined. To my friends that have helped me throughout my master's degree, thank you for all your help, support, and words of encouragement.

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CHAPTER I

INTRODUCTION

Corals can be found throughout the oceans from the surface down to 8,600 m(Wagner *et al.*, 2011). Corals provide essential habitats and resources for numerous sessile and mobile organisms (Cole *et al.*, 2008). For example, they provide food, and spawning, and nursery grounds for a range of organisms, including commercially important fish species (Carreiro-Silva *et al.*, 2013). Corals are also economically important for coastal communities, providing beach renourishment for tourist beaches, attracting tourist divers and snorkelers, and protecting the shorelines from storms damages (Burke *et al.*, 2011).

Corals are marine invertebrates within the ten orders of sub-classes Hexacorallia and Octocorallia of the class Anthozoa (phylum Cnidaria). Although corals appear to be single large organisms, many are in fact a colony of potentially thousands of genetically identical polyps living together. Each individual polyp is a soft-bodied organism ranging in diameter from about 1 mm to more than 20 cm in some species (Goreau, 1979) with a set of tentacles surrounding the mouth. Each polyp is connected to one another via the coenosarc, forming a colony of individuals that act as a single organism (Kvitt *et al.*, 2015). Corals can be assigned to one of two groups, hermatypic (reef-building corals) or ahermatypic (non-reef-building corals) based on their growth form (Nybakken, 2001). There are approximately 5,600 species of hermatypic and

ahermatypic corals, 65% (~3,640) of which occur in water deeper than 50 m (Roberts *et al.*, 2009).

Hermatypic corals or "reef-building corals," are predominately of the order Scleractinia, which produce exoskeletons of aragonite, a crystallographic form of calcium carbonate, that forms the framework of large 3D structures known as coral reefs (Wainwright, 1964). Hermatypic corals are the foundation species of shallow-water coral reef ecosystems, forming the predominant structural habitat, and are the foremost contributors to reef development and growth (Cole *et al.*, 2008). There are over 800 species of reef-building corals described to date (Burke *et al.*, 2011) that are generally restricted to shallow and warm, low-nutrient waters within coastal areas of the Pacific, Indian, and Atlantic oceans (30°S to 30°N) (Hoegh-Guldberg *et al.*, 2017). Coral reefs cover 250,000 km^2 of the ocean floor and serve as habitat for approximately 4,000 coral-reef-associated fish species (Burke *et al.*, 2011). Hermatypic corals are also important land builders, forming entire chains of islands and altering the shoreline of continents (Knowlton and Jackson, 2013). Many reef-forming corals contain intracellular dinophycean symbionts in their tissues known as zooxanthellae (Wainwright, 1964). Zooxanthellae photosynthesize organic compounds from sunlight and pass along the bulk of their food (up to 98%) to their coral hosts (Davidson, 1998), which requires this energy for growth by calcification (Angélica and Ramírez, 2013). However, there are corals that are non-reef forming corals (ahermatypic) that have zooxanthellae and some hermatypic corals that lack them (Schuhmacher and Zibrowius, 1985a).

Ahermatypic corals do not construct massive carbonate reef structures. Zoanthidea, Antipatharia, and Octocorallia are a few examples of ahermatypic corals (Cairns, 2007). Although, they lack the ability to build massive carbonate reefs, they can still be present in reef

ecosystems forming 3D structures of colonies or solitary polyps. Ahermatypic corals can develop dense assemblages that form important structural habitats for invertebrates, vertebrates, and other sea life (Roberts *et al.*, 2006). They provide a habitat for food, shelter, nursery, and a solid surface for other fish and invertebrates. Ahermatypic corals are not restricted geographically nor bathymetrically because most species lack zooxanthellae and can therefore be widely distributed (Cairns and Stanley, 1981) across a range of conditions such as depth, temperature, and light (Schuhmacher and Zibrowius, 1985). Although some species of ahermatypic corals contain zooxanthellae, most species rely on heterotrophic feeding with prey capture using their tentacles or mucus. Ahermatypic species represent a greater portion of total coral species (Baker *et al.*, 2016) and are the predominant corals in deeper parts of the ocean (Riegl and Dodge, 2019).

Hermatypic corals are generally located in shallow waters (< 70 m; rarely over 100 m) (Cairns and Stanley, 1981 and Wells, 1966) in part because their zooxanthellae need light for photosynthesis and the corals and their symbionts require a minimum of about 16°C for reproduction and have an upper limit of temperature tolerance of 40°C (Wells, 1966). In contrast, ahermatypic corals are widely distributed at depths ranging from 1-2 m (or surface at low tides) to 6,000 m (Knowlton and Jackson, 2013) because they generally do not require light for photosymbionts and are found at temperatures ranging from 0.5°C to over 27°C (Wells, 1966). Ahermatypic corals that live in shallow waters are generally found in caves or other shaded niches or are heavily pigmented due to light exposure (Wells, 1966). Light intensity decreases generally with a depth leading to an extension of coral reef ecosystems known as mesophotic coral ecosystems (MCEs) from approximately 30-60 m (Knowlton and Jackson, 2013), where the coral species transition from hermatypic to ahermatypic (Riegl and Dodge, 2019). This

transitional area can vary between locations due to water clarity, temperature, substrate, and light levels (Baker *et al.*, 2016).

Most research on coral biology and ecology has been focused on shallow, tropical, reef-dwelling corals, whereas corals living in cold and deep-sea habitats are understudied due to the challenges in accessing these environments (Demopoulos *et al.*, 2017). Exploration of deep-sea (>50 m) corals, including those of MCEs, is relatively new, in part because of the limitations of conducting research at the depths they inhabit. Typically, these species are found below the depth limits (~40 m) of recreational SCUBA diving. MCEs have been known since the nineteenth century, but focused scientific exploration only began ~50 years ago and has widely been adopted by the scientific community since 2008 (Riegl and Dodge, 2019). These knowledge gaps hinder the ability to assess the resilience and functionality of MCEs and to understand ecosystem-scale connectivity (Riegl and Dodge, 2019). With recent advancements involving underwater remote sampling methods such as remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), baited remote underwater video (BRUVs), and submersibles (Wagner *et al*., 2011), sampling has increased in the deep sea and on MCEs.

Mesophotic coral ecosystems are characterized by the presence of light-dependent corals and associated communities that are typically found at depths ranging from 30-40 m and extending to over 150 m in tropical and subtropical regions (Baker *et al.*, 2016). The "Deep reef refugia" hypothesis by Bongaerts *et al.* (2010) states that potentially threatened species, mainly hermatypic corals, from shallow reefs will be less vulnerable to potential threats at mesophotic depths and may serve as a possible source of propagules to replenish shallow species (Bongaerts *et al.*, 2010). However, little is understood of the degree to which environmental factors (nutrient

levels, currents, and competition) control the distribution and community structure of MCEs (Baker *et al.*, 2016).

Communities that provide structural habitats for fauna on MCEs include corals, sponges, and algae. Most conspicuous habitat-forming organisms are among the Octocorallia (particularly gorgonian corals), Antipatharia (black corals), algae, rhodoliths (free-living coralline red algal nodules), and sponges (Lesser *et al*., 2009, Riegl and Dodge, 2019). These light-limited ecosystems exhibit an increase of heterotrophic coral species that lack zooxanthellae, such as black corals. Black corals, in addition to octocorals, can be the predominant structure-forming fauna on some MCE and in the deep sea, forming "coral forests," where they provide habitat for numerous marine species (Morgan, 2003). However, little is known about antipatharian life history and reproduction, yet this information is crucial for management and conservation and for determining the vulnerability of these organisms to climate change and anthropogenic stressors.

Close to 240 species are recognized in seven families of Antipatharia: Antipathidae, Schizopathidae, Cladopathidae, Leiopathidae, Myriopathidae, Aphanipathidae, and Stylopathidae (Smithosonian, 2016). Antipatharian corals are typically found in deeper waters, with over 75% of known species being restricted to depths deeper than 50 m (Etnoyer *et al.*, 2018). Habitats for these corals include inland fjords, continental shelves, slopes, offshore banks, and seamounts (Roberts *et al.*, 2009) where they can become the dominant structure-forming fauna for numerous marine species. Black coral colonies increase in diversity and abundance with depth because of favorable environmental factors enhancing their settlement (Riegl and Dodge, 2019). Black corals have been reported to have low densities of symbiont cells (zooxanthellae) within their tissues, however, antipatharians do not require symbiont-based

carbon to survive (Wagner *et al*., 2011). Therefore, antipatharian corals are not restricted to shallow-water habitats. Black corals are colonial species that form a noncalcareous, spiny axial skeleton, called a corallum, constructed of concentric lamellae of protein and chitin deposited around a hollow, central canal (Riegl and Dodge, 2019). Black corals have a variety of morphologies, including trees, bushes, fans, whips, and grass forms. The tissues of antipatharians have no structural protection against abrasive forces, and their muscular systems are poorly developed such that tentacles can only contract slightly but not retract into a calyx like other anthozoans (e.g., sea anemones) (Wagner *et al.* 2011). Despite their prevalence as habitat-forming taxa at depth, little is known about their basic biology and ecology, such as larval biology, reproductive seasonality, reproductive age, and growth rates.

Determination of antipatharian growth rates can be challenging due to difficulty with access, their slow growth, and challenges with recreating *in situ* conditions for laboratory culture (Roberts *et al.*, 2009). Several methodologies have been used to estimate growth rates and longevities of antipatharians, including time-series measurements of colony fragments in aquaria, time-series measurements of tagged colonies in the field, measurements of colonies on artificial structures of known age, growth ring counts, analysis of size-frequency distributions, and radioisotope-dating techniques (Wagner *et al*., 2011). Despite these challenges, research has shown many black corals are among the slowest growing deep-sea corals with lower growth rates ranging from 8-22 µm per year (Prouty *et al.*, 2011) (Table 1). However, some species have considerably higher growth rates on the scale of 10s of centimeters per year. For example, *Stichopathes luetkeni,* an unbranched coral reported from the Gulf of Mexico (GoM) and Caribbean, can average growth rate of 4.5 cm/mo or 76 cm/yr (Bo *et al.*, 2009a). However,

Antipathes and *Leiopathes*, branched black corals were reported from the Gulf of Mexico and Caribbean, typically having an average growth rate of 10 -100 µm/yr (Hitt *et al.*, 2020).

Table 1**.** Summary of previously published information on population densities, growth rate, and the sexual reproduction of antipatharians (G = gonochoric; G* = gonochoric based on the study of a limited number of specimens collected over a short time span; Seq. H = sequential hermaphrodite; PTM = primary transverse mesenteries; T = tentacle; - = information unknown).

Species	Family	Collection location	Depth (m)	Vertical growth rate (cm/yr)	Sex	Gonad location	Oocyte size (µm)	Spermato-cyst size (µm)
Antipathes atlantica	Antipathidae	Caribbean coast off	15-50	-	-	-	-	-
Antipathes cf. atlantica	Antipathidae	Providencia Island,	30-50	-	-	-	-	-
Antipathes caribbeana	Antipathidae	Cozumel, Mexico	20-75	-	-	-	-	-
Antipathes caribbeana	Antipathidae	Banco Chinchorro, Mexico	20-75	-	-	-	-	-
Antipathes dichotoma	Antipathidae	Mediterranean	201-256	-	G*	PTM	250	100
Antipathes cf. dichotoma	Antipathidae	Palau	6-75	4.52-9.32	-	-	-	-
Antipathes grandis	Antipathidae	Hawai'i	28-111	-	G*	PTM	13-125	-
Antipathes grandis	Antipathidae	Hawai'i	40-146	2.92	-	-	-	-
Antipathes griggi	Antipathidae	Hawai'i	40-70	6.42	-	-	-	-
Antipathes griggi	Antipathidae	Hawai'i	23-99	-	G*	PTM, T	16-131	-
Antipathes spp.	Antipathidae	Indo-Pacific	-	-	G	PTM, T	-	-
Cirrhipathes anguina	Antipathidae	Indonesia	36	-	G*	PTM		
Cirrhipathes cf. sp.	Antipathidae	Indonesia, Hawai'i	10-35	-	G*	PTM	12-137	≤120
Cirrhipathes sp.	Antipathidae	Indonesia	40		Seq. H	PTM	40-200	-
Cirrhipathes sp.	Antipathidae	Indo-Pacific	-	-	G	PTM, T	-	-
Plumapathes pennacea	Myriopathidae	Cozumel, Mexico	20-75	-	-	-	-	-
Plumapathes pennacea	Myriopathidae	Banco Chinchorro, Mexico	20-75	-	-	-	-	-
Stichopathes gracilis	Antipathidae	Curacao	>10	46.8-84.76	-	-	-	-
Stichopathes luetkeni	Antipathidae	Cozumel, Mexico	20-75	-	-	-	-	-
Stichopathes luetkeni	Antipathidae	Banco Chinchorro, Mexico	20-75	-	-	-	-	-
Stichopathes luetkeni	Antipathidae	Jamaica	18	76.65	-	-	-	-
Stichopathes spiessi	Antipathidae	Eastern North Pacific seamounts	550-1150	-	-	-	-	-
Stichopathes spp.	Antipathidae	Caribbean coast off Columbia	15-50	-	-	-	-	-
Stichopathes spp.	Antipathidae	Providencia Island, Caribbean	30-50	-	-	-	-	-
Stichopathes sp.	Antipathidae	Hawai'i	10-59	-	G*	PTM	7-132	-

With exception of a few studies on shallow-water species (<50 m), most information on the sexual reproduction of antipatharians is derived from the anatomy of reproductive tissues (Wagner *et al*., 2011). Black corals, like most corals, can reproduce asexually and sexually (Figure 1). Asexual reproduction can occur via budding, fragmentation, and the production of asexual larvae in aquaculture (Wagner *et al*., 2011). Budding occurs when a coral polyp reaches a certain size and divides producing a genetically identical new polyp and continues as part of the existing coral colony. Fragmentation takes place when a piece of a coral colony is either intentionally or unintentionally (e.g., storms, human disturbance, etc.) broken (Sultan, 2014) resulting in the fragment resettling and forming a new colony. One form of sexual reproduction involves the production of eggs and sperm that are subsequently released in a broadcast spawning event (mass spawning) (Neely *et al.*, 2018). Corals will release gametes in synchrony into the water column and fertilize externally. Another form of sexual reproduction is brooding, which occurs internally (Rakka *et al.*, 2017) after a male coral has released his sperm into the water and it has been taken in by a female coral and has fertilized oocytes. The developing planula larvae will eventually be released and settle in new areas to grow. However, there is no evidence of internal fertilization within the Antipatharia to date (Wagner *et al*., 2011).

Available data on black corals suggest each sexually mature colony takes 10-14 days to spawn completely, and maximum egg release may be associated with lunar cycles (Miller, 1998; Parker *et al*., 1997; Wagner *et al*., 2011) and seasonal temperature changes (Neely *et al.*, 2018). A study on *Antipathes griggi* found that during the reproductive season, colonies < 40 cm in height did not contain any gametes and were considered sexually immature (Wagner *et al*., 2011). The state of reproduction percentage of sexually mature colonies increased to ~80% for colonies measuring 60-69 cm in height and 100% for colonies > 130 cm (Wagner *et al.*, 2011).

However, a previous spawning event was recorded on September 2, 1999, seven days after the full moon on August 26, 1999, at approximately 18 m in depth at the Texas Flower Garden Banks, Gulf of Mexico (Vize, 2006).

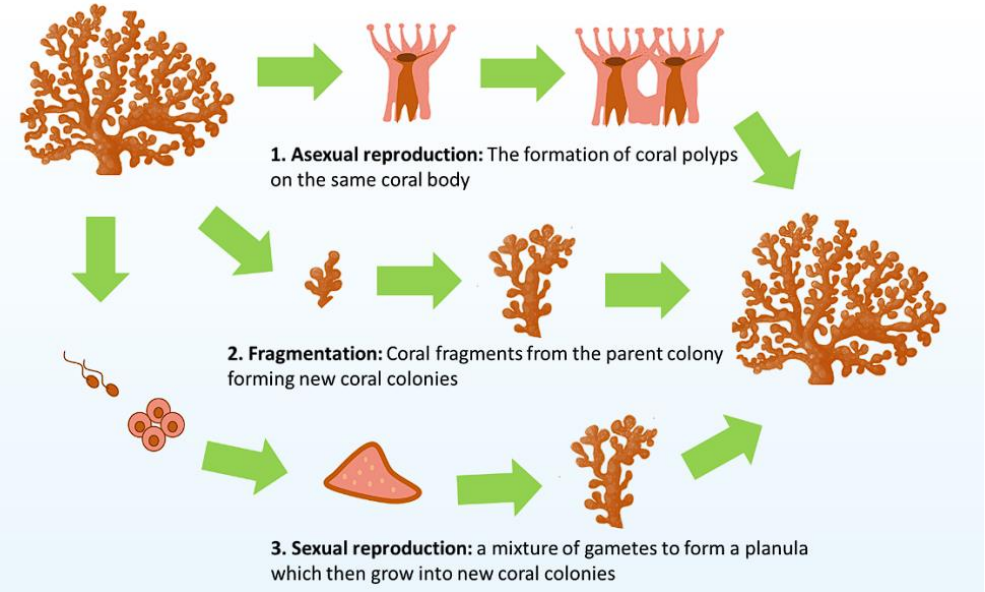


Figure 1. Coral Reproduction Diagram showing (1) asexual reproduction by coral polyps splitting or budding within the same colony, (2) coral fragmentation originating from breakage of the parent colony, and (3) sexual reproduction consisting of a mixture of male and female gametes forming planules that disperse via currents to produce new coral colonies. (Image taken from Khaulah Mujahidah, 2020)

Two predominant species of black corals on Gulf of Mexico (GoM) mesophotic reefs are *Antipathes atlantica* and *Stichopathes luetkeni*. Both these species are accessible within recreational diving limits at the USTS Texas Clipper artificial reef, so they can be collected at dive depths (< 40 m). Nevertheless, little is known about the reproductive biology and growth of these species, which provide essential habitat for a variety of fauna on GoM and Caribbean coral ecosystems. Although shallow coral reefs are not abundant in the GoM as in other areas such as the Caribbean, GoM coral ecosystems are still considered among the Large Marine Ecosystems in the World (Gil-Agudelo *et al.*, 2020). The GoM contains >1.5 million km² of coral reefs, of which only 2,640 km² (<0.2%) are shallow reefs, whereas the rest are located in the mesophotic

zone or deeper (Gil-Agudelo *et al.*, 2020). A combination of factors such as low frequency recruitment events, delayed first reproduction, limited larval dispersal, longevity, and slow growth rates suggest that it could take centuries for these species to recover from disturbance events (Prouty *et al.*, 2011). Knowledge of reproductive biology and the associated processes of dispersal and recruitment are essential prerequisites for ecological studies of corals and for the conservation of coral populations and communities. Because little is known about the ecology and biology of *Antipathes atlantica* and *Stichopathes luetkeni*, their study will contribute to increasing knowledge of black coral biology and reproduction, and thus our understanding of the differentiation in life-history characteristics of black corals in MCEs, in particular within the GoM (Table 1).

These knowledge gaps can pose difficulties when addressing anthropogenic environmental disasters such as the Deepwater Horizon (DWH) oil spill. On April 20, 2010, the DWH mobile drilling unit exploded at the Macondo Prospect in the GoM resulting in a massive release of oil causing loss of life and extensive damage to natural resources (Ocean Trustee Implementation Group, 2019). Oil began to spread from Macondo Prospect at 1522 m depth, in the east Mississippi Canyon area (N 28.73667 W 88.38694) in the northern GoM, covering >112,000 km^2 (Beyer *et al.*, 2016). According to the US v. BP trial (2015), 3.19 million barrels (~5000,000 m^3) of oil were released into the ocean, spreading laterally in deep waters (>1000 m depth) (Beyer, Trannum, Bakke, P. V. Hodson, *et al.*, 2016). This event impacted antipatharians octocorals, and branching corals at sites near the spill (Nuttall *et al.*, 2022).

In 2010, 11 sites were studied to determine the level of impact on individual deep-water coral communities. It was concluded that there was a widespread sign of stress, including tissue loss, sclerite enlargement, excess mucous production, bleached commensal ophiuroids (brittle

stars), and covering by brown flocculent material (floc) (White *et al.*, 2012). Of 43 corals photographed, 46% presented evidence of impact on more than half of the colony, and a quarter of all the corals showed impact to >90% of the colony (White *et al.*, 2012).

In 2015, further observations were done to study injuries mesophotic reefs attained in the GoM. *Antipathes atlantica* and *Stichopathes* spp. were among the corals that exhibited injuries to their skeletons. For *S. luetkeni*, it was occasionally seen to have discolored soft tissue and polyps lacking attachment to holdfast (anchor) (Silva *et al.*, 2016). Some *A. atlantica* showed small patches of green biofilm covering branches and sediment covering the anchor portion of the coral (Silva *et al.*, 2016).

Several studies (White *et al*., 2012; Beyer *et al*., 2016; Silva *et al*., 2016; Ocean Trustee Implementation Group, 2019; Nuttall *et al*., 2022) provided insight into the extent of the environmental impacts the DWH had on the GoM habitats and specific marine organisms that lead to the Deepwater Horizon Oil Spill Open Ocean Trustee Implementation Group Draft Restoration Plan 2/ Environmental Assessment: Fish, Sea Turtles, Marine Mammals, and Mesophotic and Deep Benthic Communities (MDBC) (Ocean Trustee Implementation Group, 2019). The project restoration approaches focused on the protection and management of MDBC, placing hard ground substrate and the transplantation of coral, and monitoring and adaptive management activities to improve understanding of MDBC to inform better management and ensure resiliency (Ocean Trustee Implementation Group, 2019).

Because we cannot currently predict spawning, recruitment patterns, growth, capacity for recovery, or population connectivity pathways for most black coral species, making informed management decisions, and planning restoration efforts for these injured species is difficult. To

begin filling this knowledge gap and inform management and restoration decisions, this study examined two morphologically distinct species, *Antipathes atlantica* and *Stichopathes luetkeni,* for the timing of reproductive activities (gamete formation and recruitment) and growth.

Objectives

The specific objectives of this study were (1) to determine and compare colony growth rates of two antipatharian species (*Antipathes atlantica* and *Stichopathes luetkeni) in situ* (USTS Texas Clipper artificial reef) and in a coral aquaculture system, (2) to document the reproductive activity (reproductive strategy (gonochoric or hermaphroditism), reproductive cycle, and mode of reproduction (spawners or brooders)) of both antipatharian species colonies *in situ* seasonally using histological techniques and field observations, (3) to determine the sex ratio of black corals *in situ,* and (4) to estimate female oocyte size-frequency distributions and spermatocyte production throughout the study period.

Because these species had not been reared in aquaculture system, an additional goal was to build a successful coral husbandry for both *A. atlantica* and *S. luetkeni* and to compare growth rates in aquaculture with those taken *in situ*. In addition, this experiment was aimed to (1) determine water quality parameters required to sustain growth, (2) determine the feed and feeding frequency required to sustain life, (3) determine maintenance required (e.g., water changes, filter maintenance, etc.) to keep the system running optimally to maintain coral health, and (4) determine and monitor signs of sick or distressed corals.

Hypotheses

Because of previous studies (Table 1) (Wagner *et al.*, 2011) conducted on antipatharian corals, I hypothesize that *S. luetkeni* and *A. atlantica* will have similar growth rates. (1)

Stichopathes luetkeni was predicted to have an average growth rate of 13 cm/mo or 76 cm/yr (Wagner *et al*., 2011) and (b) *A. atlantica* was predicted to have an average growth rate of 0.17-0.33 cm/mo or 1-2 cm/yr (Hitt *et al.*, 2020). A spawning event was documented on September 2, 1999, at the Texas Flower Garden Banks involving both shallow and deep water corals (Vize, 2006), (2) I hypothesize that both species will be gonochoric, broadcast spawners, and will likely reproduce10-14 days after a full moon in August or September, (3) sex ratio is hypothesized to be 1:1 as previous documented on antipatharians (Wagner *et al.*, 2012), and (4) female oocyte size-frequency distributions and spermatocyte production will be highest during the Summer when spawning is expected.

Study Taxa

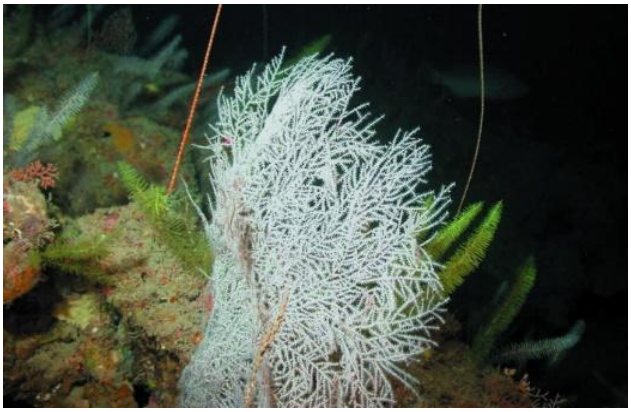


Figure 2. *Antipathes atlantica* (60-100 m depth at the Flower Garden Banks, Gulf of Mexico). Photograph courtesy of NOAA-FGBNMS/UNCW-UVP. Identifications by D. Opresko.

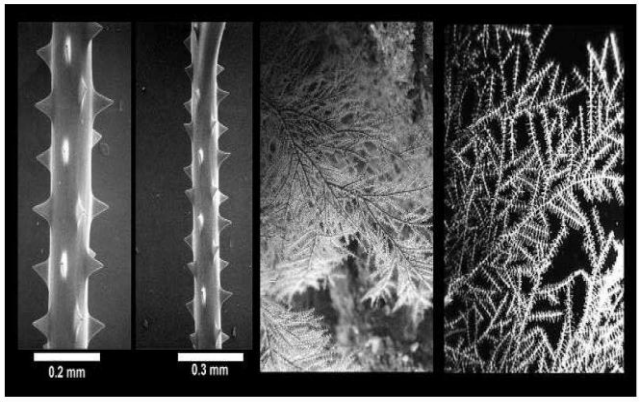


Figure 3. *Antipathes atlantica* (Bahamas, Cat, 20-30m). Scanning electron microscope (SEM) of the axis (0.2 mm and 0.3 mm) and living colonies. (*In situ* of Dennis M. Opresko and Juan Armando Sanchez, 2005).

Antipathes atlantica, commonly known as the grey sea fan, form large colonies, growing to 0.5 m or more in height with a reported apical growth rate of 0.325 cm/mo (Grange and Singleton, 1988) (Figure 2 & 3). They are described as densely branched, fan-shaped colonies, which may be without distinguishable primary branches. Adjacent branches are sometimes fused together to form a net-like pattern. The skeletal spines are short, triangular, smooth, 0.05-0.07 mm tall, and equally as wide at the base (Figure 3)(Warner, 1981). The spines are arranged in seven to eight rows with 3.5-5.0 spines/cm in each row (Warner, 1981). The polyps are arranged on one side of the corallum (entire skeleton), are generally 0.5-1.1 mm in transverse diameter,

and are spaced 1.1-1.7 mm apart (Table 2) (Opresko *et al.*, 2016). Living colonies are grayish white or greenish in color and have been reported from the Caribbean, GoM, and Northwest Atlantic at depths ranging between 15-100 m (Gray *et al.*, 2005).

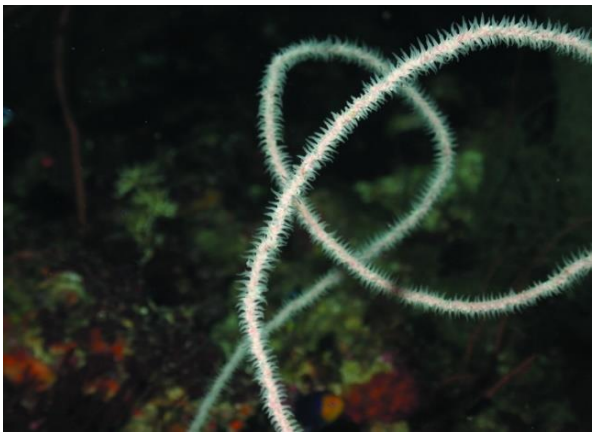


Figure 4. *Stichopathes luetkeni* (50-75 m depth at the Flower Garden Banks, Gulf of Mexico). Photograph courtesy of NOAA-FGBNMS/UNCW-UVP. Identifications by D. Opresko.

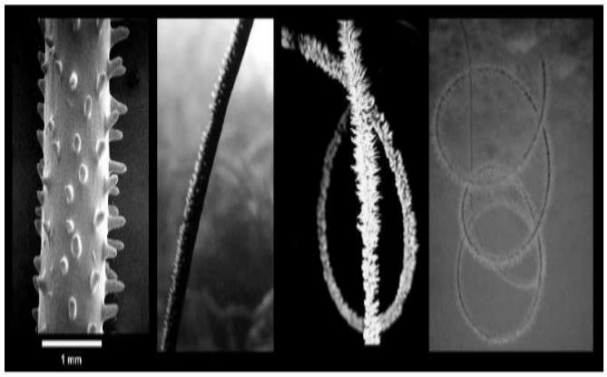


Figure 5. *Stichopathes luetkeni* (25 m depth at the Bocas del Toro, Panama). Scanning electron microscope (SEM) of the axis (1 mm) and living colonies. (*In situ* of Dennis M. Opresko and Juan Armando Sanchez, 2005).

Stichopathes luetkeni (Figure 4 & 5) has an unbranched and whip-like corallum, can reach 1 m or more in height and up to ~3 mm in diameter at the base, and has a growth rate of approximately 1.3 cm/mo (Bo *et al.*, 2009b). The lower part of the corallum is usually straight or slightly curved, whereas the upper part may form wide spirals 10 cm or more in diameter (Opresko *et al.*, 2016). Polyps are up to 0.8-1.7 mm in transverse diameter, arranged in a single row on one side of the corallum (Table 2) (Wagner and Shuler, 2017). They are widely

distributed geographically and range in depths from 60 to 90 m but are moderately abundant from 50 to 75 m (Opresko *et al.*, 2016). They can be found in both reef and soft-bottom habitats and in a variety of colors (orange, beige, reddish-brown) with translucent tentacles (Wagner *et al.*, 2011).

Table 2. Morphometric comparison of antipatharian species from previously published data (- = values not reported).

	Antipathes griggi (Verrill, 1928)	***Antipathes atlantica* (Pallas, 1776)**	*Stichopathes echinulata* (Brook, 1889)	***Stichopathes luetkeni* (Brook, 1889)**
Colony:				
Branching pattern	Bushy	Bushy	Unbranched	Unbranched
Max. height (m)	3	≥ 0.5	1	≥1
Terminal branch diameter with tissue at midpoint (mm)	0.87 (0.46-1.66)	-	1.00 (0.74-1.22)	3
Polyps:				
Transverse diameter (mm)	1.12 (0.58-1.75)	0.5-1.1	0.98 (0.51-1.35)	1.4
Polyp spacing (mm)	1.43 (0.57-2.83)	1.1-1.7	1.33 (0.81-1.93)	-
Density (polyps/cm)	7 (5-10)	-	8 (6-10)	-
Spines:				
Forks or apical knobs present	Yes	Yes	No	Yes
Secondary spines present	Yes	-	No	-
Polypar spine height (µm)	181 (105-382)	30-70	139 (81-190)	360
Abpolypar spine height (µm)	127 (68-243)	-	89 (54-147)	250
Spine spacing (µm)	379 (209-654)	~90-350	367 (187-670)	-
Habitat:				
Depth range (m)	10-99	15-100	129-183	20-100
References:	(Wagner *et al*., 2011)	(Warner, 1981)	(Wagner *et al.*, 2011)	(Opresko *et al.*, 2016)

CHAPTER II

METHODOLOGY

Study Site

In situ growth studies and specimen collections occurred at the Texas Clipper Reef site (Figure 6), located 31 km (17 nmi) northeast of South Padre Island, Texas (26°11´21´´ N, 96°51´33´´ W). Texas Clipper Reef is part of the Artificial Reef Program managed by the Texas Parks & Wildlife Department (TPWD) with the goal of promoting, maintaining, monitoring, and enhancing the artificial reef potential of Texas offshore waters (Dianne *et al*., n.d). The main structure at the reef site is the USTS Texas Clipper (hereafter Texas Clipper), a 144 m long ship. The Texas Clipper is unique as an artificial reef because of its large compartments that create shaded habitats that mimic and provide mesophotic light conditions at shallower than mesophotic depths. The Texas Clipper was sunk on 17 November 2007 and lays on its port side at 40 m depth, making it accessible for recreational scuba diving. The Texas Clipper represents hard-bottom habitat that mimics natural hard-bottom habitats that occur sporadically throughout the unconsolidated seafloor of the continental shelf in the GoM (Hicks *et al.*, 2015). This location is widely recognized as a biodiversity hotspot by anglers, divers, and scientists in the area.

Seawater temperature measurements were collected on the Texas Clipper at approximately 25 m in depth using several HOBBO water level data loggers from September 2015- July 2016 and July 2021-September 2021. Although data was not collected for the entire

2021 year, data was collected from 2015-2016 from the same location. Except for during the 2021 freeze, temperatures at the Texas Clipper were similar throughout the years.

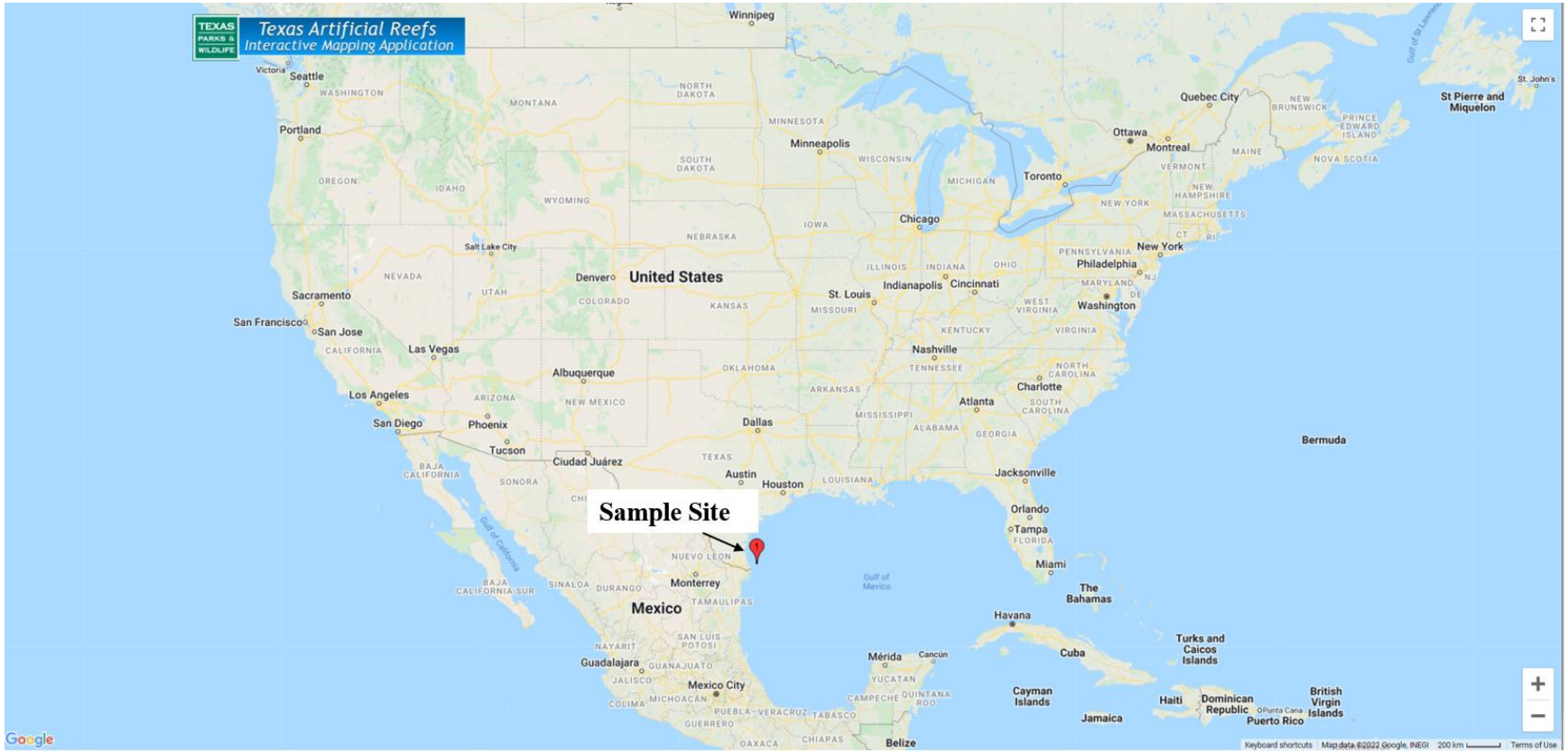
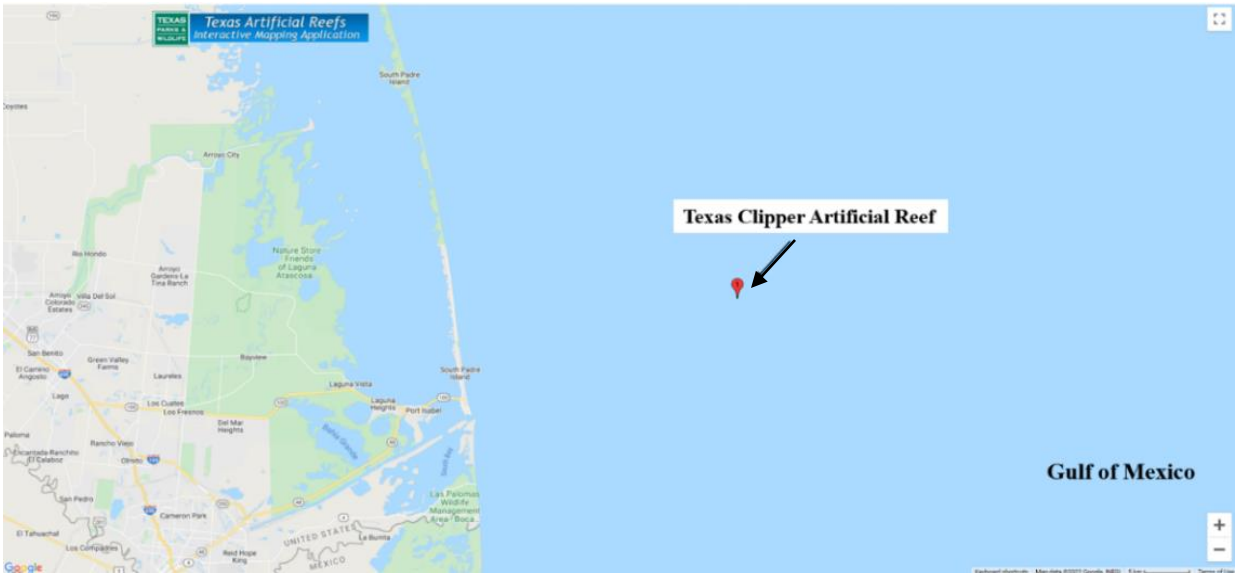


Figure 6. Sampling site in the Gulf of Mexico where Anitpatharian corals were collected.

(A)



(B)

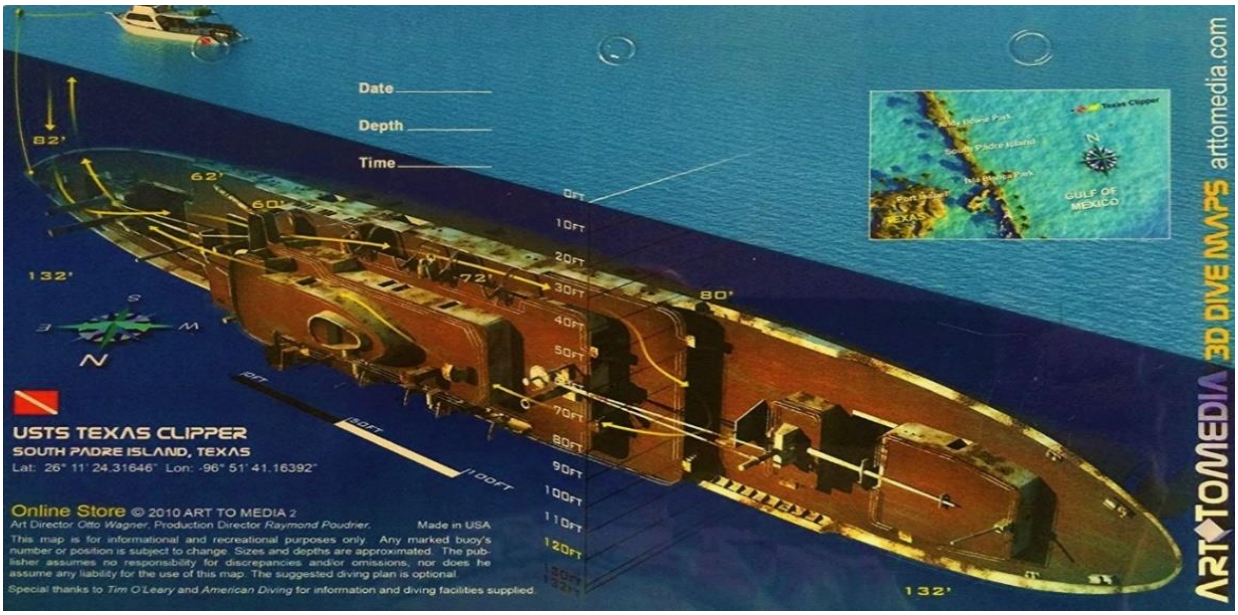


Figure 7. Texas Clipper (location of sampling site: 26° 11´ 21´´ N, 96° 51´ 33´´ W) in southern Texas coast of the Gulf of Mexico. (A) South Padre Island, ~17 nm northeast from the Texas Clipper artificial reef. (B) Texas Clipper is 144 m long and lays portside at 40m in depth.

In situ Growth Measurements

To determine antipatharian growth rates at the Texas Clipper, 20 specimens (10 *A. atlantica* and 10 *S. luetkeni)* > 10 cm in height were chosen haphazardly at 20-35 m depth and tagged with numbered cattle tags. Cattle tags were attached a few centimeters above their anchorage using plastic zip ties. Tagged colonies were measured periodically (Appendix A and Appendix B) for 5 months from January 30, 2020 - May 30, 2020 and for 4 months from June 2021 - September 2022 with a Keson™ flexible measurement tape (± 1.0 cm). Measurements were recorded in pencil on Duracopy™ waterproof paper attached to a plastic slate. To reduce human error, the same volunteer divers were used to gather growth measurements throughout this project. Due to the COVID-19 pandemic, sampling trips were put on hold from May 2020 to June 2021. Unfortunately, some tags were lost or were not found during two field work trips over a five-month period.

Stichopathes luetkeni has a spiral whip-like morphology that required scuba divers to carefully extend each colony to its maximum height for measurement. Two to three scuba divers were assigned to locate and gather measurements of tagged colonies. One diver would oversee holding the flexible measuring tape at the base of the coral colony while the other diver carefully extended the coral alongside the measuring tape. Once the measurement was closely examined the second or third diver would document the measurement.

Antipathes atlantica has a bush-like morphology that allowed divers to measure the height and width of each colony. Divers were reminded to carefully select the longest branchlets when measuring height and width. Typically, one diver would take the measurements and the second diver would document the data.

Aquaculture Setup

A 90-gallon Starfire™ Tank (48 × 18 × 24 in) was selected for this experiment to prevent sudden and rapid changes in water quality often associated with smaller systems. A custom-made wooden stand (49 x 18 x 26 in) was built to hold the aquaculture (Figure 8). A custom PVC stand was built to hold a sheer plastic cover to reduce coral light exposure (Figure 9). To produce flow inside the tank, an EcoTech MP40 Pump™ was attached to the glass magnetically on the right side of the tank approximately 12 inches from the surface. It has a maximum flow of 4,500 gph specifically for a tank ranging from 50-500 gallons; flow was kept constant at 1% throughout the day. A sump tank with a Trigger System Triton44 V2 Sump™ was connected to the main aquaculture by a PVC pipe to accommodate water filtration devices to increase water flow, improve filtration, increase oxygen levels, and water volume. The sump tank has three main sections: (1) a large section fed by dual inputs that hold 4" filter socks with 10 μm mesh and flows through an adjustable overflow wall into (2) the biological filtration or refugium that was connected to (3) the section containing Aquamaxx Carbon Filter Media Reactor™ that removes general contaminants, dissolved organics, and toxins that corals and algae may produce (Figure 10). The first section was equipped with a Finnex 300-watt aquarium heater™ set at a constant 23ºC, dry rock substrate, and a Reef Octopus Protein Skimmer™. Live rock is used as substrate in the sump to support a broad range of microfauna and microbes such as copepods and amphipods that serve as main nutrient exporters to the main tank. The protein skimmer is located between the first and second sections to extract dissolved organics from the water column as it passes into the refugium that can grow specifically cultured macroalgae that helps consume excess nutrients to prevent nuisance algae growth. This refugium contained a nutrient substrate for corals (Miracle Mud™), approximately 40 lb. (18 kg) of Macro Rocks Saver Premium

Rock™ (dry rock) made up of all-natural calcium carbonate rock (pest and organic free), and an LED (Light Emitting Diode) light fixture Al Prime 16 Fuge Refugium Light™ to provide optimal light for growing macro-algae (cultured Chaetomorpha Linum). After passing through the last section, water was returned to the main aquarium with a USA Eflux 6010 DC Flow Pump™. In summary, water drained from the main tank into the first sump section, was filtered, and pumped back to the main tank, creating a recirculating aquaculture system.

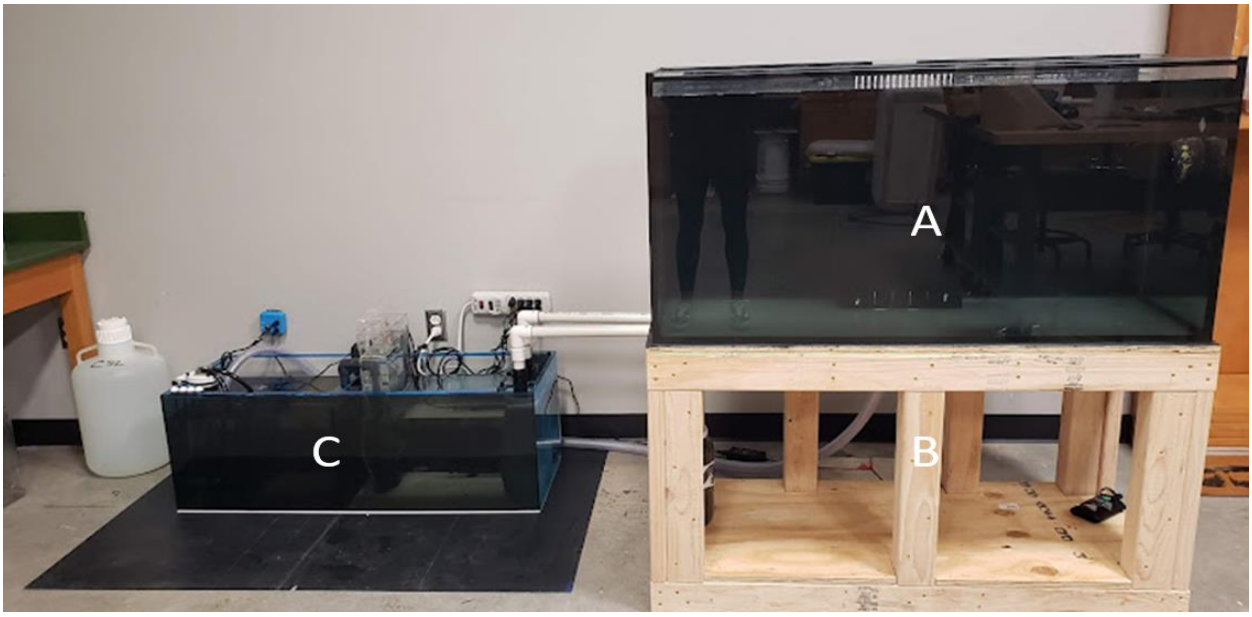


Figure 8. Aquaculture built for antipatharian fragments (A) 90-gallon tank, (B) Wooden stand, and (C) Sump.

Figure 9. Aquaculture cover (A) cover and (B) PVC stand.

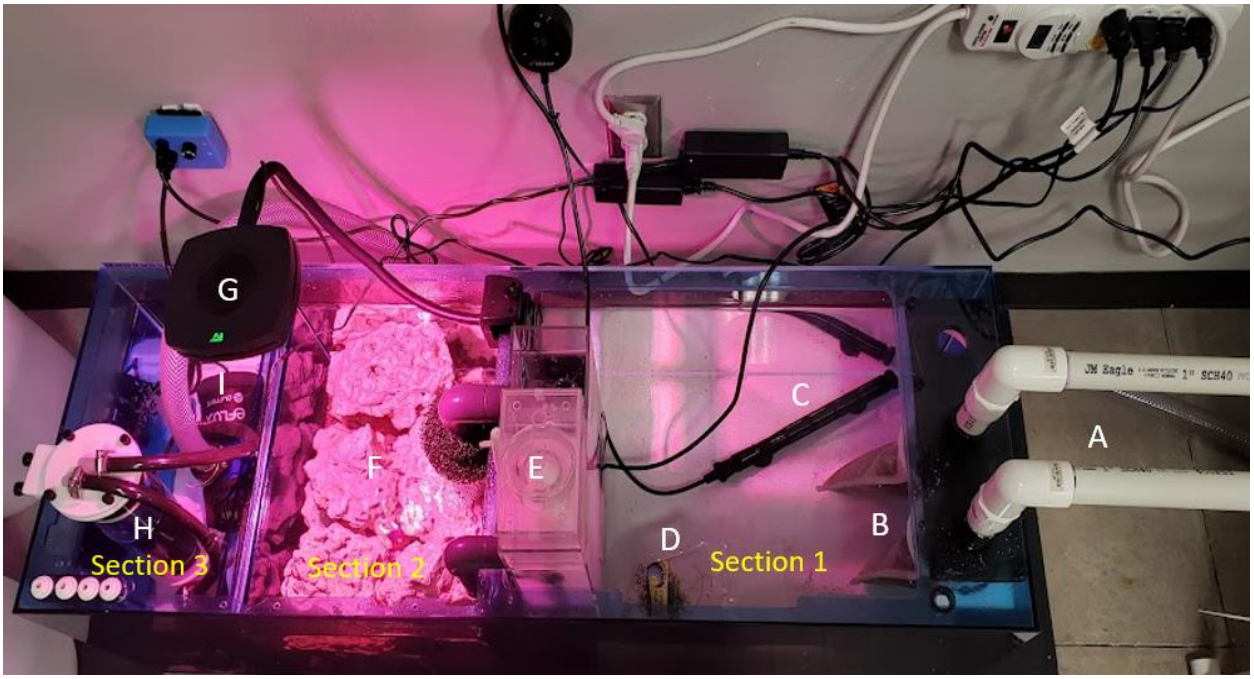


Figure 10. Sump, Section 1: (A) pvc pipes connected to aquaculture, (B) bio-socks, and (C) heater. Section 2: (D) Chaetomorpha macroalgae, (E) protein skimmer, (F) live rock, and (G) refugium light. Section 3: (H) carbon filter and (I) return pump.

Aquiculture: Water-Quality Parameters

Temperature (Marine Salinity Tester-HI98319), salinity (Marine Salinity Tester-HI98319), and pH (Hanna Checker® Plus pH-HI98100 and HI98129) were tested daily using Hanna instrument testers. Ammonia (Seachem® MultiTest™ Ammonia [free & total]), nitrite (Nitrite Colorimeter-Checker®HC-HI764), nitrate (HACH Pocket Colorimeter II 04/2014, Edition 1 [DOC022.97.80451]), calcium (Hanna Marine Calcium Checker® HC-HI758U), alkalinity (Hanna Checker® HI772, Alkalinity DKH Colorimeter), magnesium (Red Sea Reef Foundation Pro Multi Test Kit, Mg), and phosphate (HACH Pocket Colorimeter II 04/2014, Edition 1 [DOC022.97.80451[) were tested 3–4 times per week using Hanna instruments, HACH Pocket Colorimeter, and Red Sea Reef Test Kit Pro.

Aquiculture: Collection of Corals

Black coral specimens were collected from the Texas Clipper on 28 June and 1 July 2022. Four large coral fragments of each species were cut from haphazardly selected colonies using dissection scissors and placed in resealable plastic bags with ambient seawater. *Antipathes atlantica* fragments (10-15 × 5-10 cm; H × W) were clipped from the colonies branchlets. *Stichopathes luetkeni* fragments (15-20 cm in height) were clipped from the top to the middle portion of the colony. Samples were placed in an ice chest filled with seawater and ice to mimic temperatures at the depths collected during transportation to the UTRGV Port Isabel laboratory, where samples were removed from sample bags and zip tied to coral racks inside the aquarium. Coral fragments were allowed to recover until at least 5 July 2022 prior to further manipulation.

Aquaculture: Feeding

All coral fragments were fed twice a day during the week and once on weekends. Before feeding, the return pump and protein skimmer were turned off for 2 hours while the flow generator was set to “feed” mode (higher flow rate). Two Little Fishes: Marine snow plankton diet (TopDawg Pet Supply, Item model number: 4162, ASIN: B00025K166, Fort Lauderdale, FL 33309) was administered by pouring 25 mL of food into a cup to dilute with ~300 ml of tank water and target feeding to corals. This formula, which has suspended microscopic particles ranging in size from < 0.2 µm-150 µm, was chosen as it met the special needs of cold-water corals that feed on particulate and dissolved organic such as phytoplankton and zooplankton. Target feeding was accomplished by gently squeezing a 30 ml turkey baster ~ 2-5 cm from each coral fragment.

Aquaculture: Maintenance

Every other week, 20% (~25 gallons) water changes were performed in May 2021 with filtered seawater to increase the establishment of nutrient cycling bacteria in the aquaculture system. As nitrogen cycling bacteria and refugium algae began to increase so did nitrate levels. The addition of food also caused an increase in detritus at the bottom of the tank. Weekly water changes (20%) began in July 2021, along with siphoning out detritus that would accumulate. The protein skimmer was cleaned weekly for the first two months but was changed to cleaning every two days. Bio socks that receive the input flow from the tank were cleaned biweekly and the carbon reactor was emptied, cleaned, and new carbon was added every month.

Aquiculture: Placement of Coral Fragments

Initial fragments were cut using dissection scissors and glued (Seachem Laboratories, reef glue, item model number:67131150, USA) to ceramic coral plugs (Ocean Wonders LLC,

ceramic coralline purple coral frag plugs, ASIN: B07BM9ZSNK). *Antipathes atlantica* fragments were cut into 15 single branched fragments (0.7-1.8 cm) and 15 multi-branched fragments (1.5-6.9 × 1.5-3.1 cm; H × W) and *S. luetkeni* fragments were cut into 15 fragments (2.8-6.0 cm). Fragments were then placed in magnetic racks (Ocean Wonders LLC, N52 magnetic coral frag rack, ASIN: B06W59DD9H) attached to the front glass of the aquarium (Figure 11). Fragments were initially placed haphazardly throughout the aquarium but after it was observed that a *S. luetkeni* colony was presenting signs of tissue damage potentially caused by an adjacent *A. atlantica* colony, species were separated. Parent colonies were placed in separate racks on the left aquarium glass and at the bottom rack (Figure12).

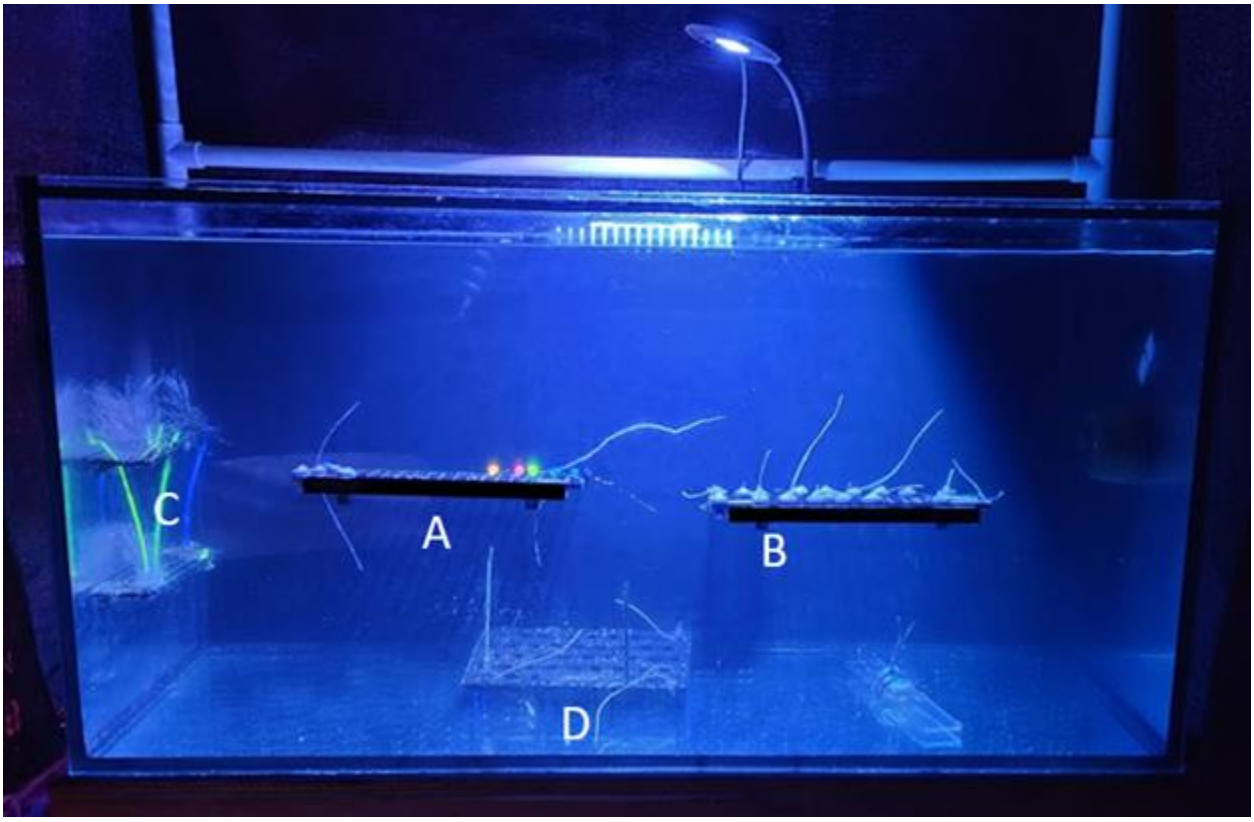


Figure 11. Magnetic Racks Setup, (A) Left, (B) Right, (C) Left sided parent colonies, and (D) Bottom parent colony.

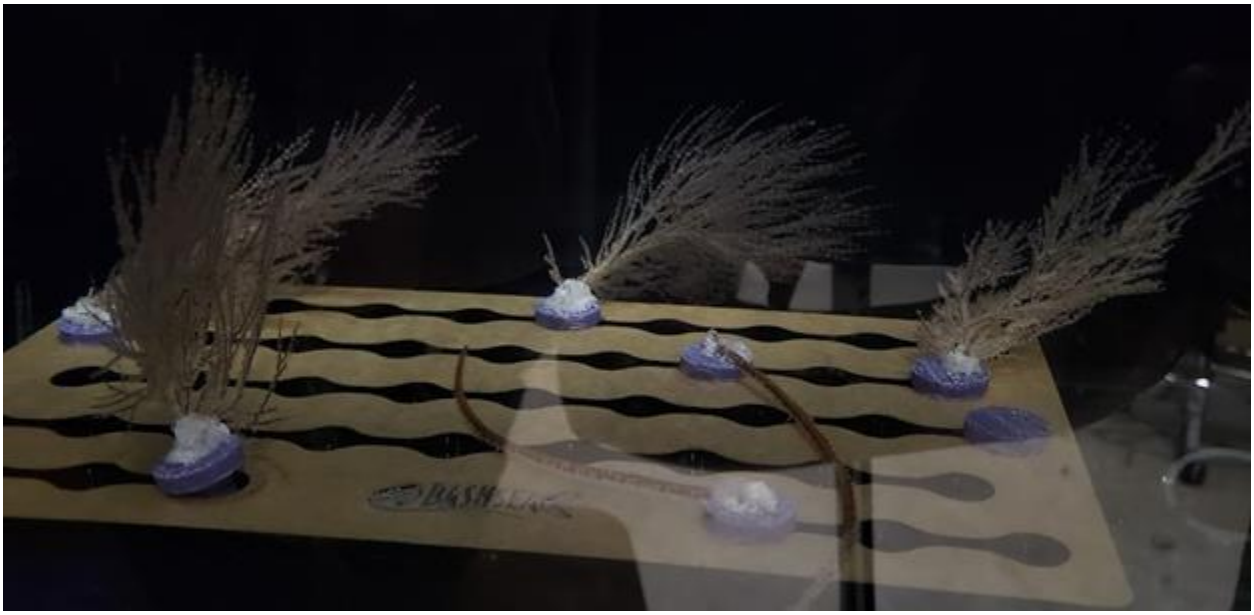


Figure 12. Parent colonies placed in bottom rack.

Aquaculture: Growth Measurements

Antipathes atlantica fragments were cut into single branched and branched fragments to observe differences in recovery and/or growth (Figure13). Five single branched and five branched fragments were cut, glued, and placed in a right rack on 7 July 2021. Ten more single and branched fragments were added on 21 July 2021, after losing fragments to detachment or breakage. Single branched fragments initial size ranged from 0.7-1.8 cm. Branched fragments initial size ranged from (1.5-6.9 × 1.5-3.1 cm; H × W). *Stichopathes luetkeni* colonies were cut into seven fragments on 6 July 2021, and six more frags were added on 12 July 2021. The fragments initial sizes ranged from 2.8-6 cm in height. Each fragment was removed individually from the rack but kept underwater while measured with a flexible plastic ruler every 2 w. *Stichopathes luetkeni* and *A. atlantica* single branched fragments were measured for height and *A. atlantica* branched fragments were measured for height and width.

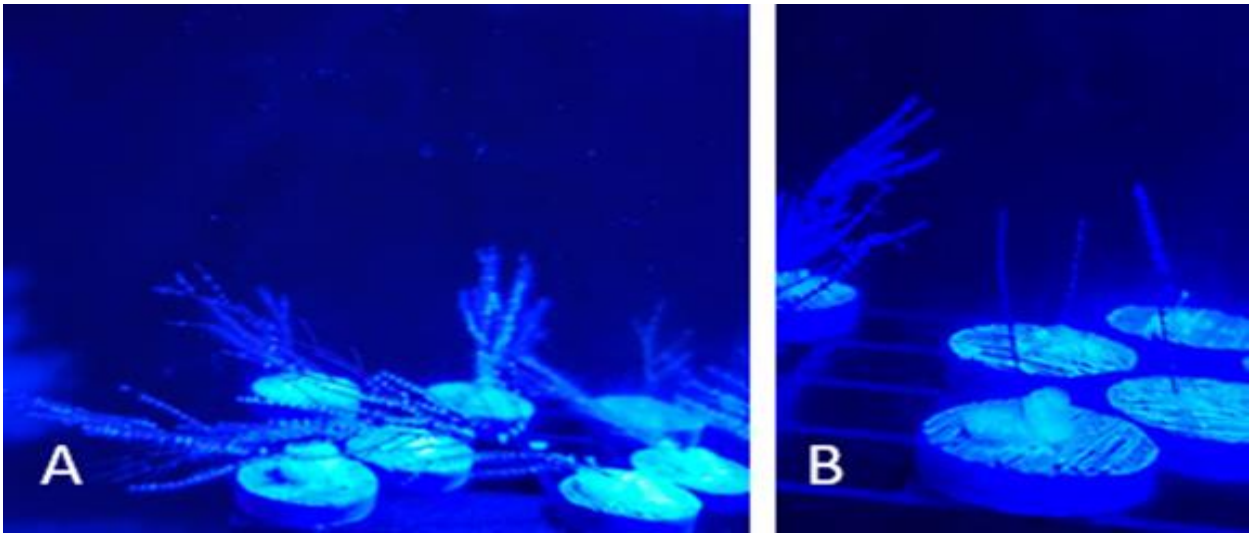


Figure 13. *Antipathes atlantica* (A) branched and (B) single branched fragments placed in left rack.

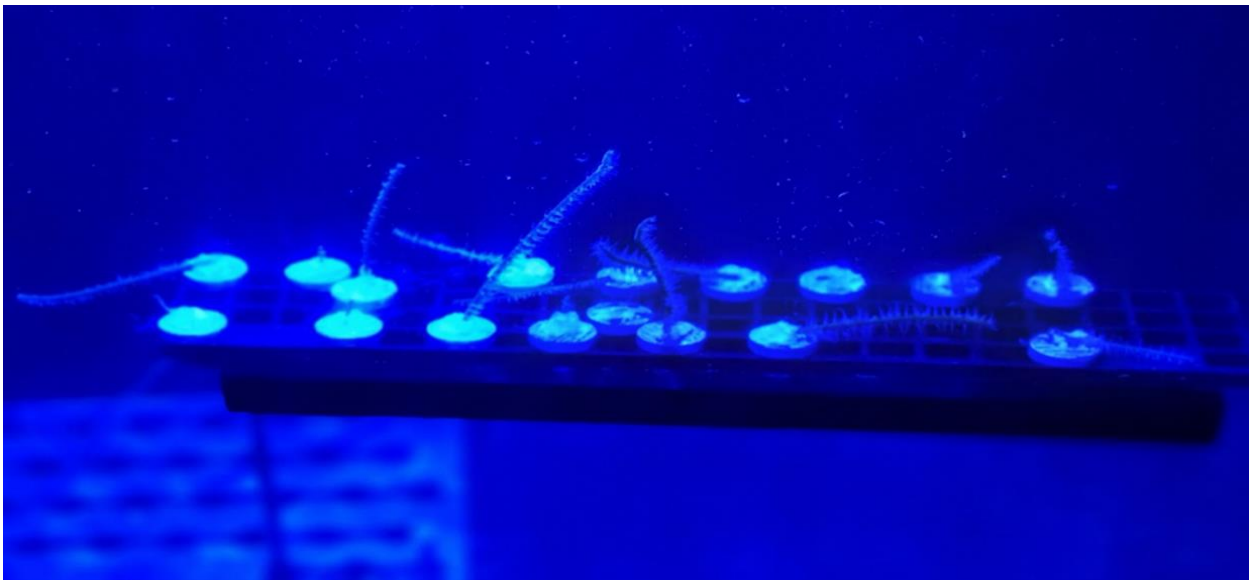


Figure 14. *Stichopathes luetkeni* fragments placed in right rack.

In situ Histological Sample Collection

Reproduction status for each species (*A. atlantica* and *S. luetkeni*) was tracked seasonally (Fall, Winter, Spring, and Summer) October 2019, January 2020, May 2020, June 2021, July 2021, August 2021, September 2021 at the Texas Clipper.

Samples used for this study were selected haphazardly from various locations on the Texas Clipper at 20-40 m. Volunteer scuba divers collected tissue samples from six different

colonies per species each dive. Approximately 5-15 cm of coral tissue was clipped from the branchlets near the top of each colony using dissection scissors. Samples were stored in 50 ml vials containing seawater from the sample location. On the surface, sample vials were kept in an ice chest filled with sea water and ice. Samples were then transported to the Port Isabel Laboratory, where each sample was placed in 100% ethanol and stored at -20°C.

Histological Preparation

Histological methods were based on previous studies on antipatharian black corals *A. fiordensis* (Parker *et al*., 1997) and *A. griggi* (Wagner *et al*., 2011). Each sample was removed from the 100% ethanol and clipped into 1-5 cm fragments for easier manipulation. The fragments from each sample that presented the largest or most protruded polyps were chosen for dissection. Chosen fragments were placed into a plastic Petri dish with 100% ethanol and were manually dissected under a stereo microscope (7X-45X Dissecting Circuit 144-LED Zoom Stereo Microscope with 8MP Digital Camera, SKU: SM745TP) using 10x, 20x, and 40x magnification. For each fragment, one or two polyps were removed from the skeleton using dissection needles and fine tipped forceps. Dissection needles were used to remove (scrape) tissue from one side of the skeleton and in between polyps while forceps held the fragment in place. As each polyp was removed it was immediately placed into a plastic cell culture plate with 100% ethanol for a total of twenty polyps (per sampling period). Dissected polyps were placed into individual 1.5 ml polypropylene microcentrifuge tubes with 1 ml of 4% Paraformaldehyde solution and stored at 4°C overnight for sample fixation and transportation to the UTRGV Brownsville campus.

The histology process for *S. luetkeni* and *A. atlantica* polyps consisted of dehydration, paraffin blocks, microtome, and staining over four days as follows. Vials containing *S. luetkeni* polyps were drained carefully. Polyps were then dehydrated in sequential submersions into a series of ethanol dilutions (50%, 75%, 95%, and 2 ×100%) with each vial being filled with 480 µl of diluted ethanol using a pipette (Dagon Lab 100-1,000 µl), placed on a VWR® mini shaker (VWR, TROEMNER LLC, Thorofare, NJ 08086-0087) at 150 speeds for 30 minutes, and drained of ethanol using a pipette. *Antipathes atlantica* polyps had to stained because of their small size and lack of color, so they were not dehydrated with an ethanol series. Instead, vials were drained of 4% Paraformaldehyde and filled with 480 µl of eosin solution (Catalog no.1170811000, CAS: 15086-94-9, CI: 45380, MilliporeSigma, Burlington, MA, USA) for 2 hours to stain the polyps for easier identification. After completing the ethanol series or eosin staining, the vials were twice filled with 480 µl of xylene, left for 15 minutes then, and drained of xylene. Paraffin wax (Leica-Paraplast, Leica, Buffalo, IL, USA) was melted using a gravity convection oven (Thermo Scientific Lindberg/Blue M, Model Number: GO1390SA-1, General-Purpose Gravity Convection Oven with stainless steel exterior, 120 VAC) at 58ºC. Vials were then incubated in a 1:1 xylene/paraffin mixture at room temperature for approximately 18 hours. On the second day, polyps were infiltrated with Paraffin wax and poured into block molds. Vials containing the 1:1 xylene/paraffin mixture were placed in the oven at 58°C until melted (~1 hour). Once melted each vial was drained carefully, refilled with new melted paraffin wax, and placed in the oven for 1 hour; this process was repeated three times. Once the series of paraffin was completed, wax from each vial was drained into a labeled disposable base mold on foil paper. A Bunsen burner was used to heat up dissection needle tips to maneuver polyps into the center of the mold. Base molds were then filled completely with extra melted paraffin wax and a

labeled blue tissue cassette (Epredia™ 1000961) was added to the top of the base mold, filled with more melted paraffin wax, and left for approximately 18 hours at room temperature.

The following day paraffin blocks were removed from base molds and placed under ice for at least 20 min (max 3 h), and excess wax was cut off with razor blades. Serial histological cross-sections were cut at 3-5 µm using a rotary microtome machine (Leica, Buffalo, IL, USA). Approximately, 4-5 cuts were made before removing the wax strip from the microtome using forceps and a dissection needle. The paraffin waxed strip that was mostly intact was chosen and placed in a beaker of 1L water heated to 45-55 ℃ using a hotplate. The paraffin waxed strip was then mounted (scooped up) onto a labeled glass slide (Catalog no. 12-550-15, Super frost™) that was then set on in a slide warmer (Fisher Scientific 77) at 43 ºC for 5 hours.

Lastly, sample slides were rinsed in xylene three times (different container each time) for 5 min each. Slides were carefully blotted with paper towels to remove xylene to avoid any chemical reaction. Tissue sections were rehydrated in series: (1) 100% ethanol for 5 min (2) 100% ethanol for 5 min, (3) 95% ethanol for 5 min, (4) 75% ethanol for 5 min, (5) 50% ethanol for 5 min, and (6) deionized (DI) water three times for 10 min each. Hematoxylin solution (100 µl) was applied using a pipette. After 10 min the slide was washed in DI water for 4 min. Eosin was applied using a pipette and left for 1 hour before washing in DI water for 1 mi. The slide was placed in the warmer for 10-30 min. Tissue section was rehydrated twice in 100% ethanol for 5 min each time and slides were carefully blotted using paper towels. Sections were cleared in a xylene series (3 min, 6 min, 6 min), and 1 drop of xylene-based glue XYL (Catalog no. 8310-4, Cytoseal XYL, Thermo Scientific, Waltham, WA, USA) was added before placing a glass coverslip (Fisherbrand™, Superslip Cover Slips) on top of the sample. Forceps were used to carefully press bubbles out from under the coverslip for clear visualization.

Histological Analyses

Histological observations were completed using the Zeiss Microscope Axioscope 5 (Zeiss, Microscope Axioscope 5/7 KMAT, Product ID: 490041-9880-010) at 10x, 20x, and 40x lenses (Zeiss, N-Achroplan, Product IDs: 5x-420930-9901-000, 10x-420940-9901-000, 20x-421250-99001-000, 40x-421260-9906-000). Digital images were obtained using the Axiocam 208 color cable camera (Zeiss, Microscopy Camera Axiocam 208 color, 8.3 M pixels).

Individual polyps were documented as either containing or lacking gametes and recorded as the percentage of polyps per colony containing gametes. For polyps that contained gametes, sex were determined by identifying oocytes or spermatocytes in each specimen and reproductive (maturity) stages were determined based on the overall appearance of the gonads and the state of the development as described by Parker *et al*. (1997). Gamete production (number of oocytes or spermatocytes per polyp per colony) was also calculated.

Diameters of every oocyte were calculated for a total of 13 polyps, which was measured using Zeiss Zein microscopy image-analysis software. The average was taken from all the oocytes within each polyp for every female colony sample collected throughout the sampling period/season.

Maturity Stages

Stages of maturity as described by Parker *et al*. (1997) included stages of oogenesis (six stages) and spermatogenesis (five stages). For oogenesis, *stage 0* (unsexable stage: Figure 30 A) there was no evidence of gametocytes in the primary transverse mesenteries making the sexing impossible. For *stage 1* (early stage: Figure 30 B), a few small, scattered oocytes in the gastrodermis of the primary mesentery and an ooplasm surrounding the nucleus of the oocytes (germinal vesicle) can be found. For *stage 2* (growing stage: Figure 30 C), oocytes are larger in

size with smaller oocytes found throughout the gastrodermis. Larger oocytes may begin to appear vacuolated (bound within cells) in structure at this stage. In *stage 3* (maturing stage: Figure 30 D), oocytes are present in larger quantities and are larger in size within the mesenteries. Oocytes are more uniform in structure at this stage. In *stage 4* (mature stage: Figure 30 E), a large number of oocytes are tightly packed in the mesenteries and appear to be at or near full size. For *stage 5* (spent stage: Figure 30 F), the mesenteries are largely spent of oocytes and those few relict oocytes are smaller in size and not uniformed in shape.

For spermatogenesis, *stage 0* (unsexable stage: Figure 30 A) is no evidence of gametocytes which makes determining sex impossible. For *stage 1* (early stage: Figure 31 A), small spherical clusters of spermatocytes are present in the gastrodermis of the primary transverse mesentery. In *stage 2* (maturing stage: Figure 31 B), a thick layer of spermatocytes forms in a chamber where the heads and tails of the spermatozoa are mostly oriented in the same direction. In *stage 3* (mature stage: Figure 31 C), spherical spermatocyte clusters are now mature and tightly packed within the mesenteries. At *stage 4* (spent stage: Figure 31 D), the mesenteries are largely spent with a few relict spermatozoa present.

Statistical Analysis

Overall growth was calculated by subtracting final and initial growth measurements. Growth rates were determined by dividing growth by the elapsed time (days) between final and initial growth measurements. Species growth rates were averaged and standard errors calculated and compared between *in situ* and aquaculture settings using a t-test with JMP statistical software.

Oocyte diameters were compared across sampling month and season using a one-way ANOVA. Gamete production (number of oocytes or spermatocytes per polyp per colony) was

compared across seasons using a one-way ANOVA. Gamete production between males and females was compared using a t-test. P-values of <0.05 were considered statistically significant. Gametogenesis (spawning period) data sets were examined for possible correlation to environmental cues.

CHAPTER III

RESULTS

Growth of *In Situ* Corals

Stichopathes luetkeni in situ mean growth rates of fifteen colonies were 0.79 cm/wk (± 0.90 SE). Five colonies (33%; colonies #002, #027, #033, #050, and #059) experienced breakage between initial and final measurements resulting in negative growth rate (Figure 15 Appendix A). However, nine colonies (60%; colonies #003, #007, #022, #025, #030, #037, #038, #055, and #057) all had a positive growth rate ranging from 0.17-7.44 cm/wk (8.86-386 cm/yr) across the sampling period.

Temporal height measurements across the sampling periods in two colonies of *S. luetkeni* exhibited increases in height from January 2020-May 2020 (colonies #003 and #007, Figure 16). Unfortunately, neither of the colonies could be relocated during the 2020 Summer sampling season. Five colonies showed increases in height from June 2021-September 2021 (33%; colonies #022, #030, #037, #038, and #055); one colony did not show any change in height from July 2021-September 2021 (7%; colony #51); seven colonies decreased in height (33%; colonies #002, #027, #033, #050, #059) (Appendix A). The longest surveyed colony *in situ*, #002 displayed evidence of several breakages in May 2020, July 2021, and September 2021and only one period of height increase in July 2021 (Figure 16).

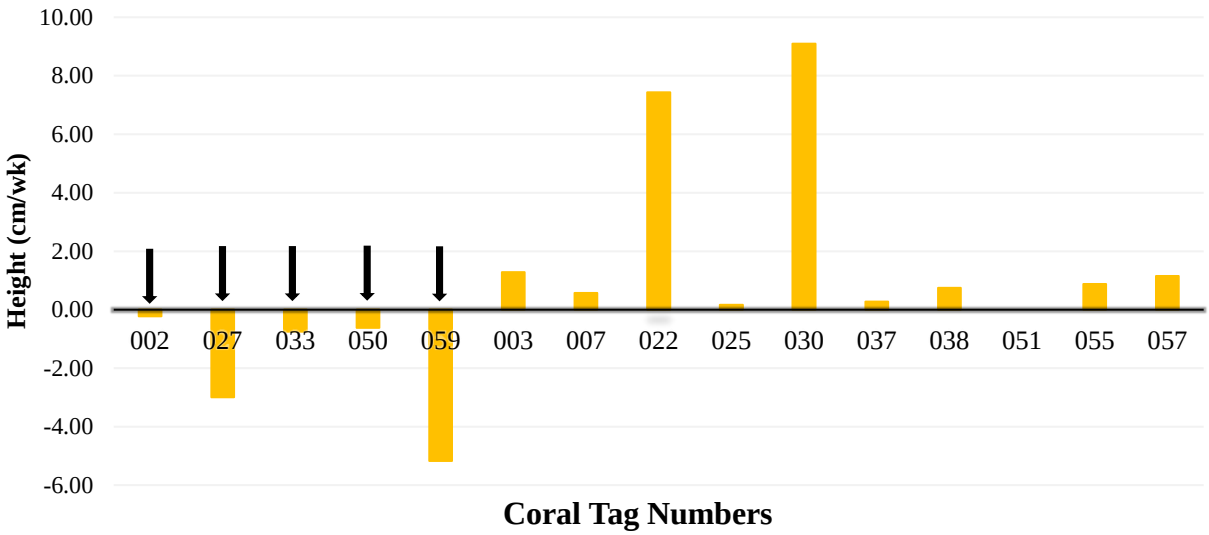


Figure 15. Growth rates of *Stichopathes luetkeni* at the Texas Clipper artificial reef according to the coral tag numbers. Black arrows indicate negative growth rates.

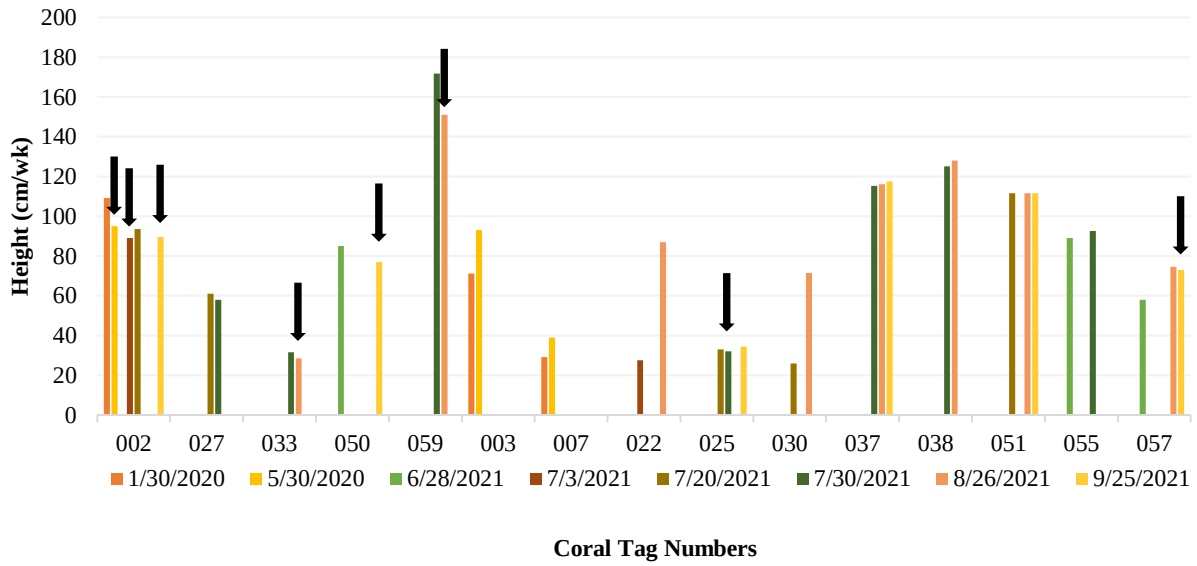


Figure 16. Height measurements of *Stichopathes luetkeni* at the Texas Clipper artificial reef depicting increases of height during monitored months, which included: January 2020-May 2020, June 2021-September 2021. Black arrows indicate likely apical fracture of the colony.

Overall mean growth rates for eleven *in situ A. atlantica* colonies were 0.26 cm/wk (± 0.10 SE) in height and 0.28 cm/wk (± 0.12 SE) in width (13.52 × 14.56; H × W). Individual colonies had varying height and width growth rate responses from initial to final measurements. Colony #016 exhibited negative growth rate in both height and width. Colony #017 exhibited negative height growth rate and positive width growth rate. Colony #043 showed positive height growth rate and negative width growth rate (Figure 17). Five colonies showed a greater width growth rate than height (45%; colonies #018, #042, #044, #045, and #049) and two colonies showed greater height growth rate than width growth rate (18%; colonies #046 and #048) (Figure 17).

Temporal height and width measurements also varied across the sampling period. Colony #018 showed an increase in height from January 2020-May 2020. From June 2021-September 2021 most tagged colonies showed an increase in height (63%; colonies #040, #042, #043, #044, #046, #048, and #049). However, two colonies showed a decrease in height from January 2020-May 2020 (18%; colonies #016 and #017). In addition, colony #045 showed a decrease in height from June 2021-September 2021 from 25 cm to 21 cm (Figure 18).

When analyzing width growth measurements two colonies showed an increase in width growth from January 2020-May 2020 (#017 and #018). However, one colony had a decrease in width growth from January 2020-May 2020 (#16). Eight of them showed a gradual increase in width (72%; colonies #017, #018, #042, #044, #045, #046, #048, and #049) while only one colony did not show any changes in growth from June 2021-September 2021 (#40). In addition, colony #043 showed a decrease in width from July 2021-September 2021 (Figure 19).

Analysis of the data recorded over 1-91 weeks of measurements taken *in situ* of both *S. luetkeni* and *A. atlantica in situ* did not show a significant difference in height growth rates between species (t = 0.58, P = 0.72, n = 25) (Figure 20).

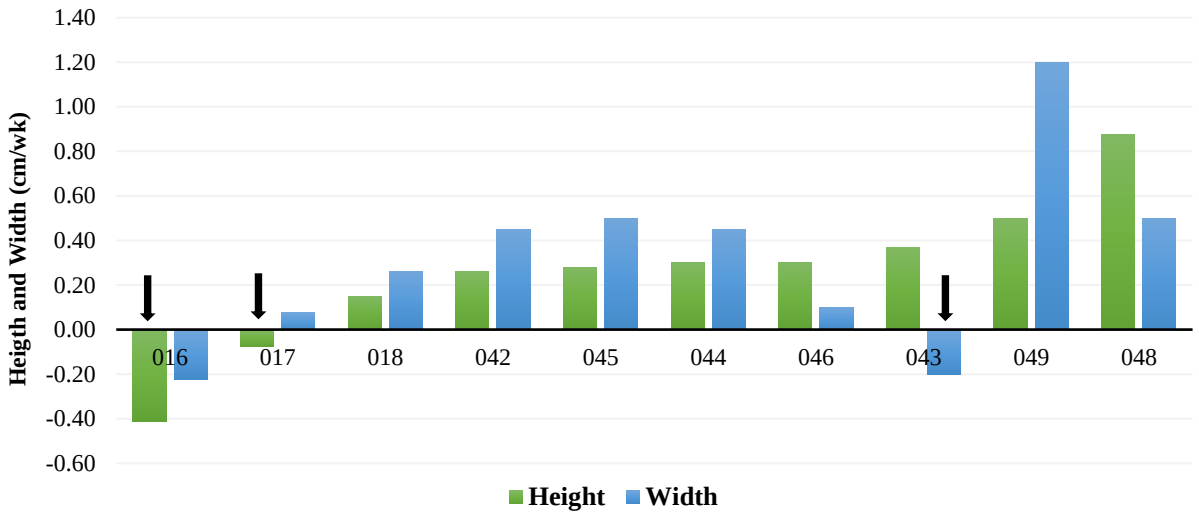


Figure 17. Height and width growth rates of *Antipathes atlantica* at the Texas Clipper artificial reef. Black arrows indicate negative growth rates of the undisturbed colonies.

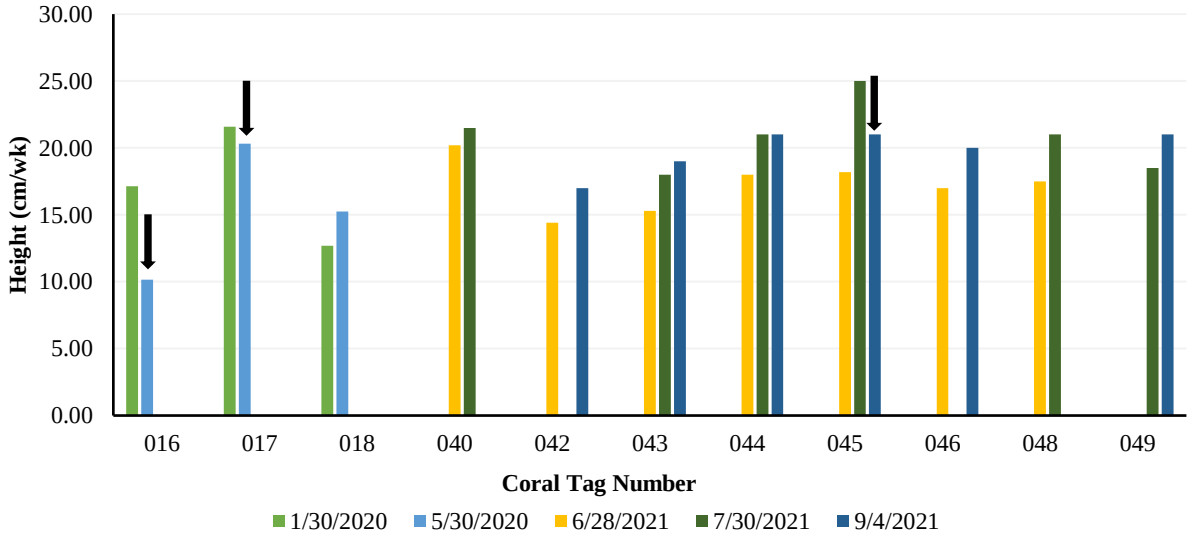


Figure 18. Growth measurements of *Antipathes atlantica* at the Texas Clipper artificial reef. Increases in height measurements occurred in January 2020-May 2020, June 2021-September 2021. Black arrows on the graph indicate a decrease in height.

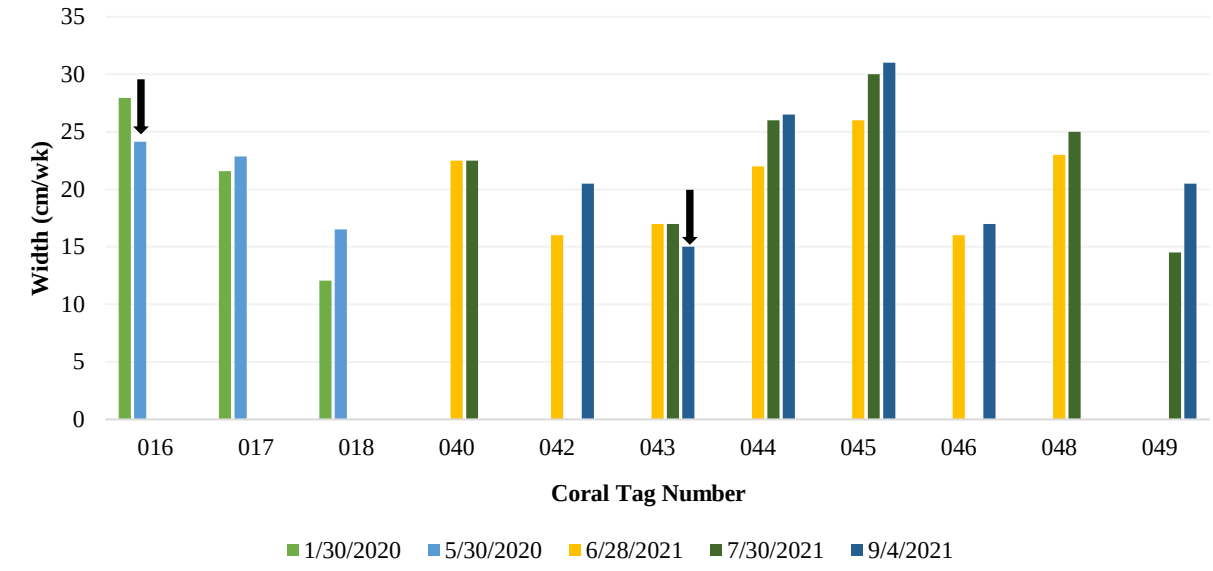


Figure 19. Growth measurements of *Antipathes atlantica* at the Texas Clipper artificial reef. Increases in width measurements occurred in January 2020-May 2020, June 2021-September 2021. Black arrows on the graph indicate a decrease in width.

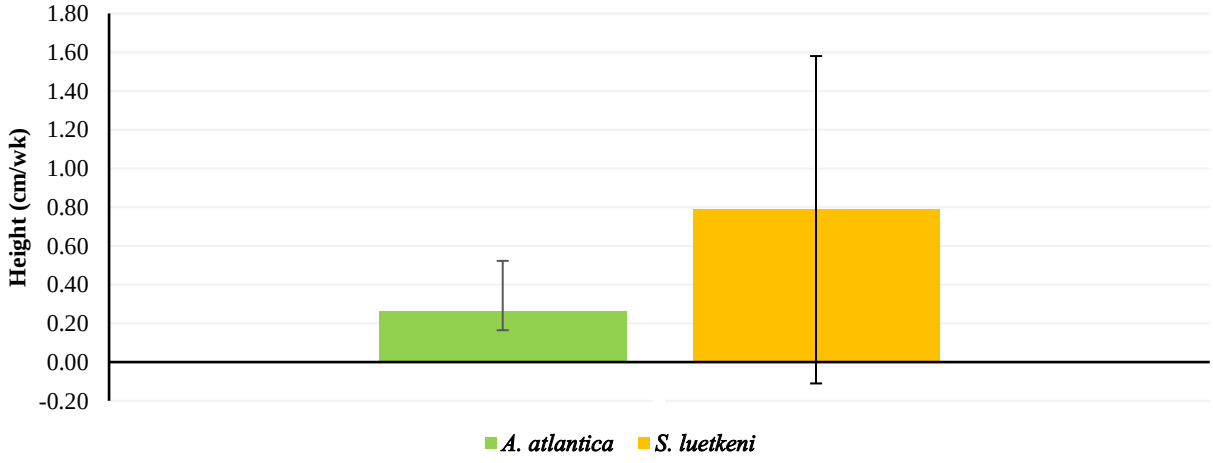


Figure 20. Mean height growth rate of *Stichopathes luetkeni* and *Antipathes atlantica* at the Texas Clipper artificial reef. The difference was not significant (t = 0.58, P = 0.72, n = 25) (bars represent standard error of the mean).

Growth of *Stichopathes luetkeni* in Aquaculture System

Nine *S. luetkeni* fragments averaged 0.09 cm/wk (± 0.04 SE) in aquaculture system. *Stichopathes luetkeni* fragments had a 100% survival rate throughout the 3 mo monitoring project. However, two fragments (6 and 15) experienced breakage inside the aquaculture system and were excluded from statistical results (Figure 21) (Appendix C). Four fragments showed a decrease in height in September 2021 (44%; fragments 6, 8, 14, and 15) (Figure 21) and fragment #14 had a negative overall growth rate (-0.03 cm/wk) (Figure 22). In addition, four other fragments experienced a decrease in height at some point during the monitoring period but had positive overall growth rates (44%; fragments 8, 11, 12, and 14) (Figure 21). One fragment did not experience change in growth due to experiencing breakage (fragment 15). The highest growth rate in the aquaculture system was 0.37 cm/wk for fragment #3.

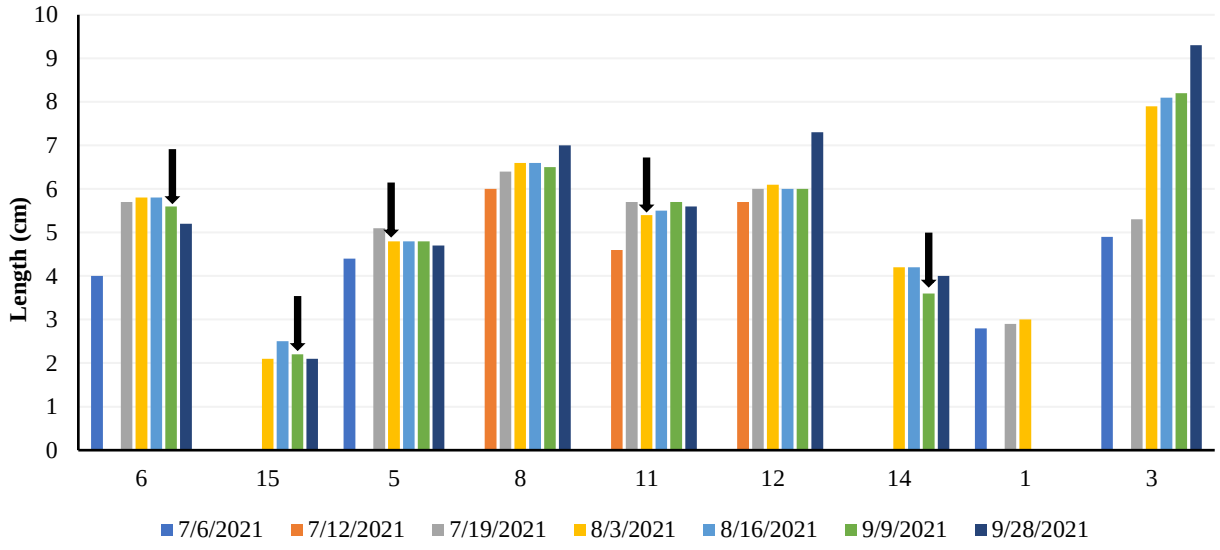


Figure 21. Changes in height in specimens of *Stichopathes luetkeni* transplanted fragments in an aquaculture system. Increases in height were observed July 2021-September 2021. Black arrows indicate a decrease in height.

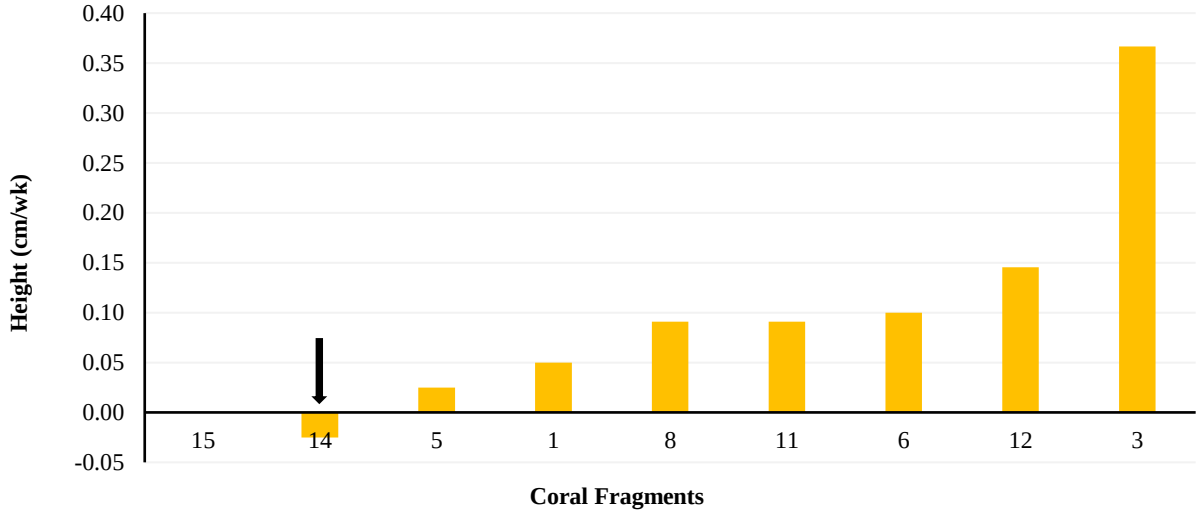


Figure 22. Growth rates of fragmented *Stichopathes luetkeni* in an aquaculture system. Black arrows indicate negative growth rate of fragments.

Growth Comparison Between *in situ* Corals and Aquaculture Corals

The analysis of the recorded data indicates no significant difference (t = 0.77, P = 0.77, n = 23) in *S. luetkeni* growth rates between *in situ* (0.79 ± 0.90 cm/wk) and the aquaculture system (0.90 ± 0.04) (Figure 28).

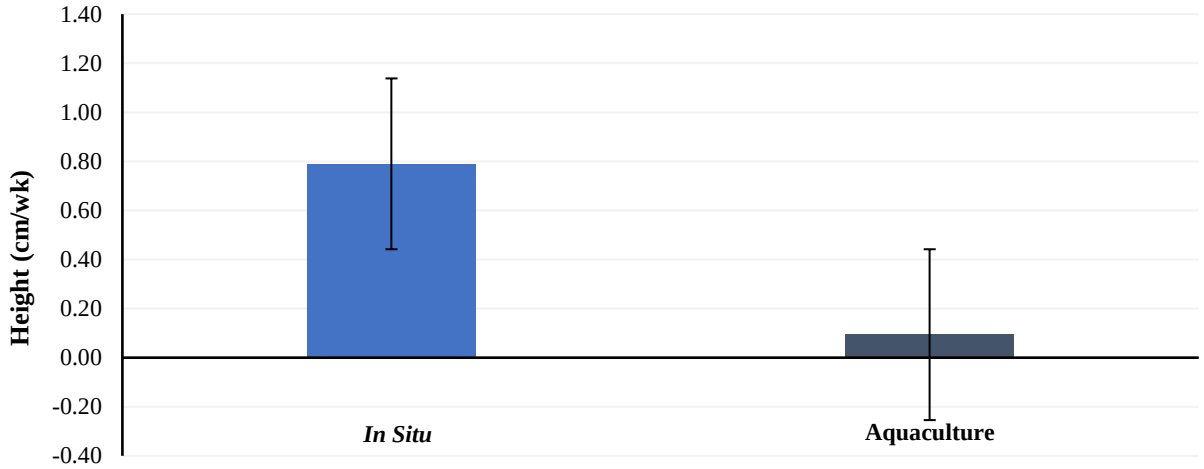


Figure 23. Growth rate comparison of *Stichopathes luetkeni* in an aquaculture system and in situ. Graphic shows the differential growth in an aquaculture (0. 9 cm/week (± 0.04 SE)) and *in situ* (0.79 cm/week (± 0.90 SE)) with no significant difference (t (23) t= 0.77, P= 0.77) (bars represent standard error of the mean).

Growth of *Antipathes atlantica* in Aquaculture System

In the culture conditions used, *A. atlantica* had a slow and consistent decline after collection. Colonies began to lose coenosarc tissue along apical branches at approximately four weeks in aquaculture. As a result, colonies underwent necrosis, polyp bail out, and eventually mortality. Of 20 single-branched *A. atlantica* fragments, 11 fragments survived approximately 80 days and did not experience breakage (Figure 23). Fragments averaged -0.04 cm/wk (± 0.02 SE). Coral fragments #2 and #10 experienced a positive growth rate of 0.03 cm and 0.05 cm over 2-4 weeks (Figure 24). Three fragments number showed no change in growth rate throughout the monitoring project (27%; fragments 7, 8, and 12). Five fragments experienced a negative growth rate from July 2021-August 2021 (45%; fragments 5, 6, 11, 13, 14, and 17) (Appendix D). Five

fragments had a slight increase in height before dying August 20, 2021 (45%; fragments 2, 3, 4, 7, and 9) (Figure 25). Eight fragments decreased in height through the monitoring project (72%; fragments 1, 5, 8, 11, 12, 13, 14, and 15). Only one fragment did not show any change in height from July 2021-August 2021 (frag 10).

In August 2021, the diet was altered by alternating two different feeds and adding five scoops (~1.74 g) of either Reef Cell small microcapsules 15g (New Life Spectrum®, Naturox Series, Reef Cell Coral Food Small 40g, Product code:8126, Item No. FO-NL02602) or Reef Chili (BRS Reef Chili Coral Food, SKU: 205200) to the formula once daily to test whether the additional type of food would improve the condition of *A. atlantica*. Reef Cell contains Zooplankton (80 µm), phytoplankton (2-900 µm), and 50-100 µm artemia nauplii replacement diet, copepods, rotifers, spirulina, and daphnia with 80% of particles being <50 µm. The Reef Chili formula was placed in the refrigerator for 30 minutes before target feeding.

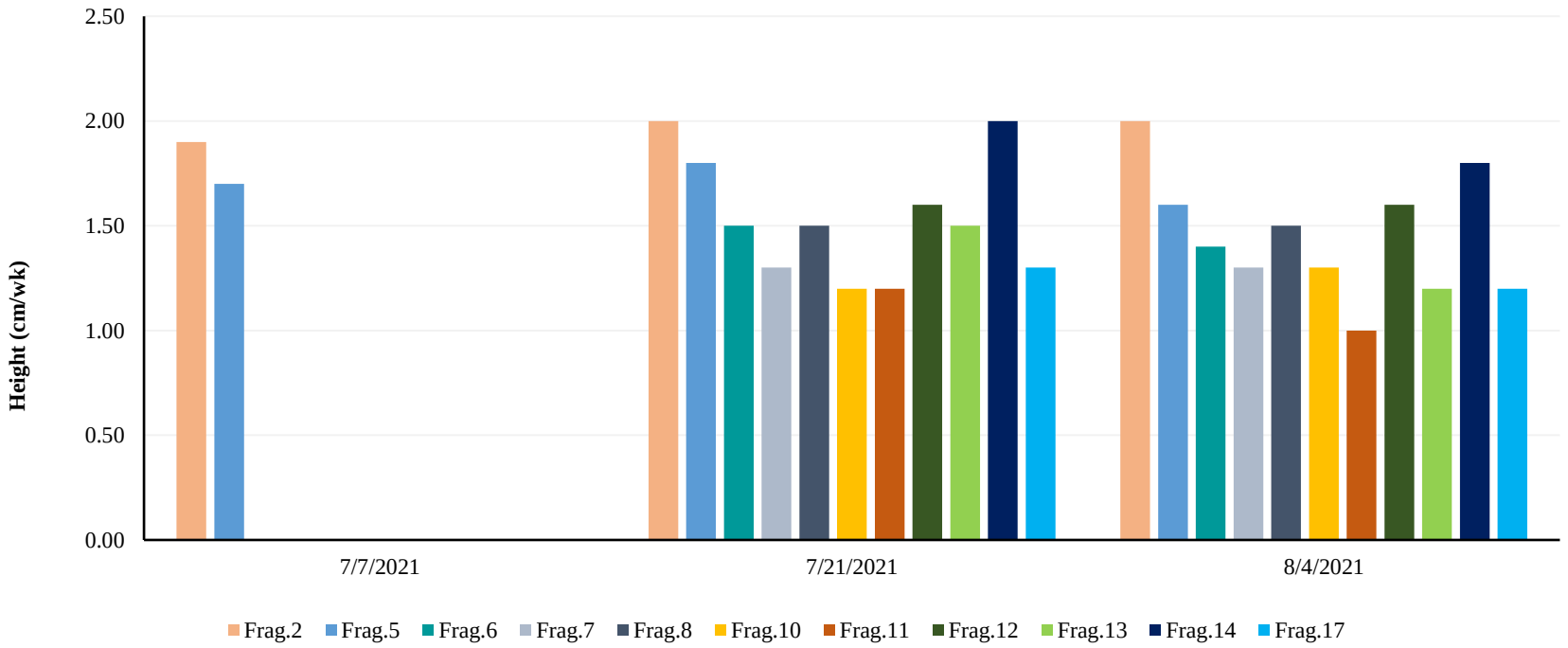


Figure 24. Height of single-branched *Antipathes atlantica* fragments in an aquaculture system July 2021-August 2021.

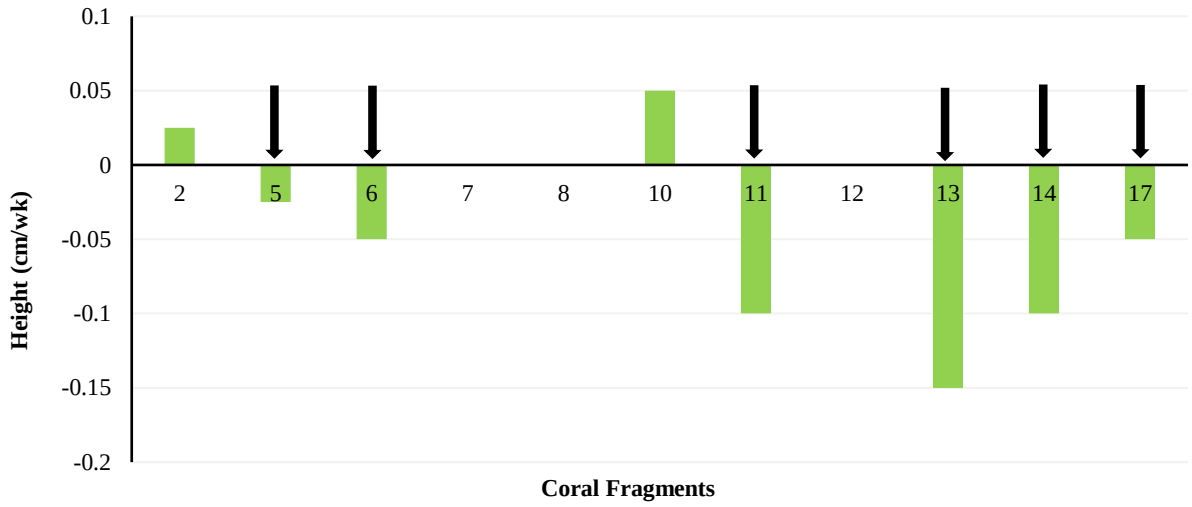


Figure 25. Growth rates of single-branched *Antipathes atlantica* fragments in an aquaculture system. Black arrows indicate negative growth rates of fragments.

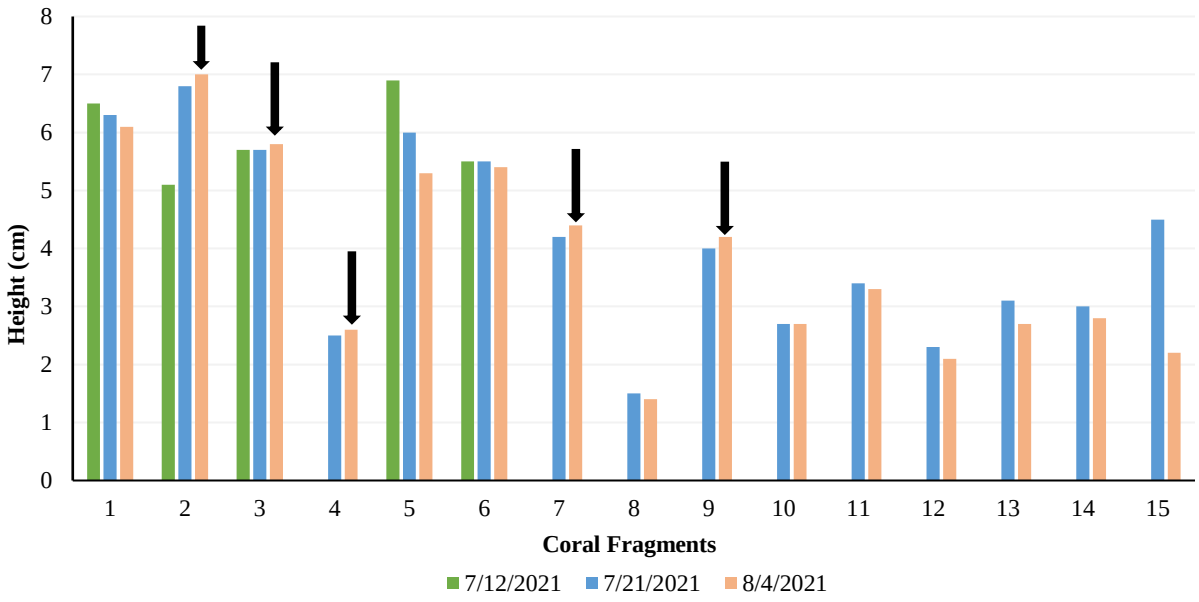


Figure 26. Height of branched *Antipathes atlantica* fragments in an aquaculture system from July 2021-August 2021. Black arrows indicate increases from previous measurements.

In August 2021of fifteen fragments, three showed an increase in width during the final measurement (20%; fragments 1, 3, and 6) (Figure 26). However, the majority showed negative width measurements in August 2021 (67%; fragments 2, 4, 5, 7, 8, 10, 11, 12, 13, and 15). In addition, two fragments did not show any change in width throughout the monitoring project (frag 9 and 14). However, four fragments experienced positive growth rates in height (26%; fragments 2, 3, 4, 7), and three fragments had positive growth rates in width (20%; fragments 1, 3, and 5) (Figure 27).

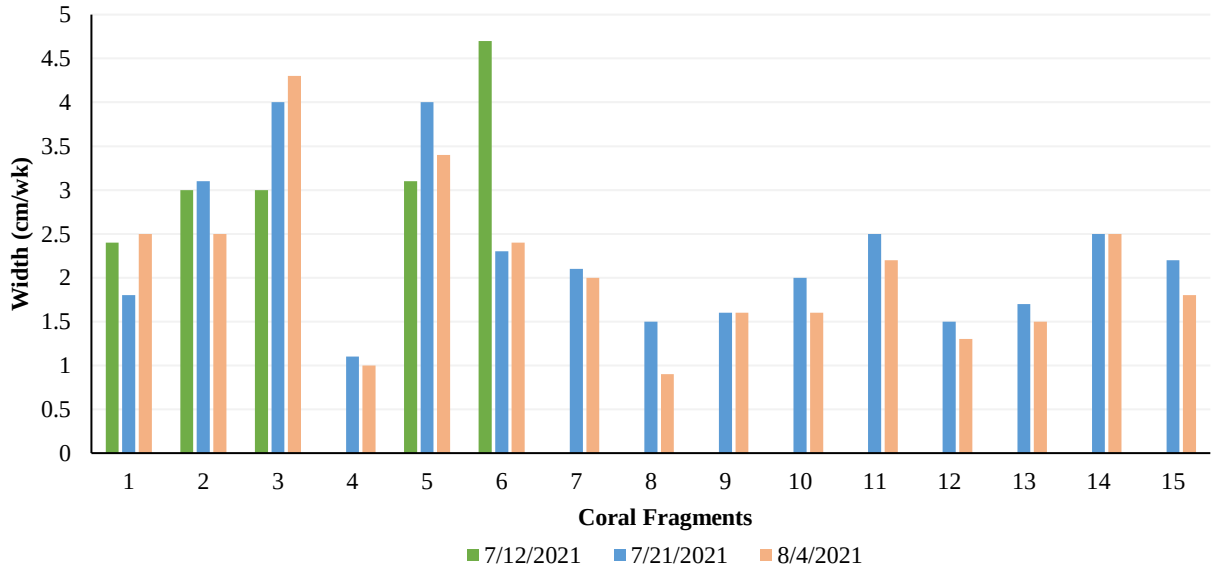


Figure 27. Width of branched *Antipathes atlantica* fragments in an aquaculture system from July 2021-August 2021.

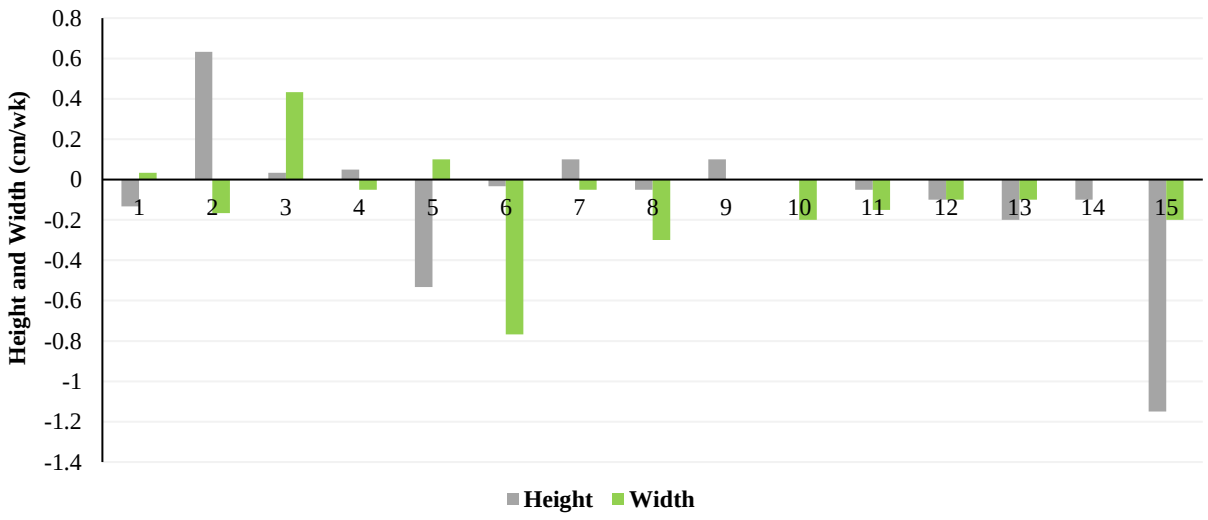


Figure 28. Growth rates of branched *Antipathes atlantica* fragments in an aquaculture system (height and width) over July 2021-August 2021.

Observations of Antipatharians in Aquaculture System

Observations concluded that *S. luetkeni* cannot be within touching distance of *A. atlantica*, they can grow from both ends of the colony, and can produce a thin transparent sheet of skeleton that acts as a basal plate and anchor.

General Reproductive Anatomy

Most *Stichopathes luetkeni* (33 of 35) were found to be gonochoric species, meaning that a colony either produces sperm or eggs. None of the examined polyps contained both oocytes and spermatocytes in an individual polyp; however, two colonies had oocytes and spermatocytes in different polyps within the same colony. Externally, there was no apparent morphological difference between female and male polyps or between mature and immature polyps. The presence of oocytes and spermatocytes after histological analysis was the only method to determine sex. Gametes of both sexes (oocytes and clusters of spermatocytes) were present in the

gastrodermis of the primary transverse mesenteries of the polyp, and spermatocytes extended into the cavity of the lateral tentacles in a few cases.

Sex Ratio and Maturation of *Stichopathes luetkeni in situ*

Of 100 *S. luetkeni* polyps dissected from 35 sampled colonies, 62 polyps (62%) did not contain gametes and were considered *stage 1* and unsexable, 13 polyps (13%) contained oocytes, and 25 polyps (25%) contained spermatocytes.

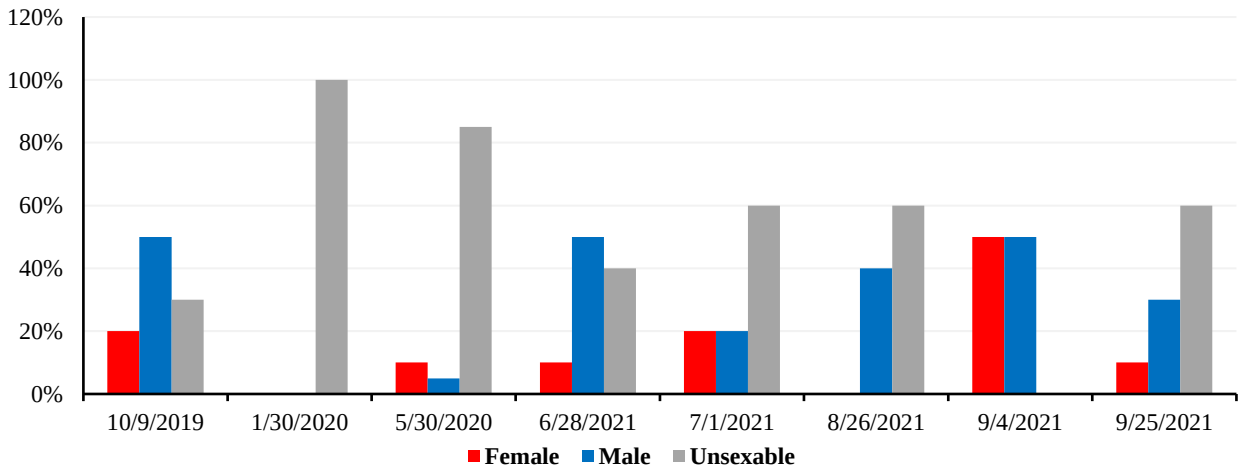


Figure 29. Percentage of polyps containing gametes at the Texas Clipper Artificial Reef, expressed as a proportion of the contribution of female, male, and unsexable colonies to the total amount.

Stage 1 oocytes were first detected in the gastrodermis of the primary mesentery of two samples on May 30, 2020 (Spring) (Figure 30 B). During June, July, and August (Summer 2021) oocytes developed quickly (*Stage 2*: Figure 30 C) and began to present an enclosed membrane around the germinal vesicle (*Stage 3*: Figure 30 D). In September 2021 (Fall) oocytes were shown to be tightly packed together with a uniform ooplasm appearance as they reached maturity (*Stage 4*: Figure 29 E) but not spawned. Female colonies collected on September 25, 2020, showed oocytes had partially spawned in the primary mesenteries (*Stage 5*). Stages of maturity differed slightly for samples within each female or male colony. Stages that differed were most

common within the early stages of gametogenesis maturity, particularly within female colonies, but between two consecutive stages.

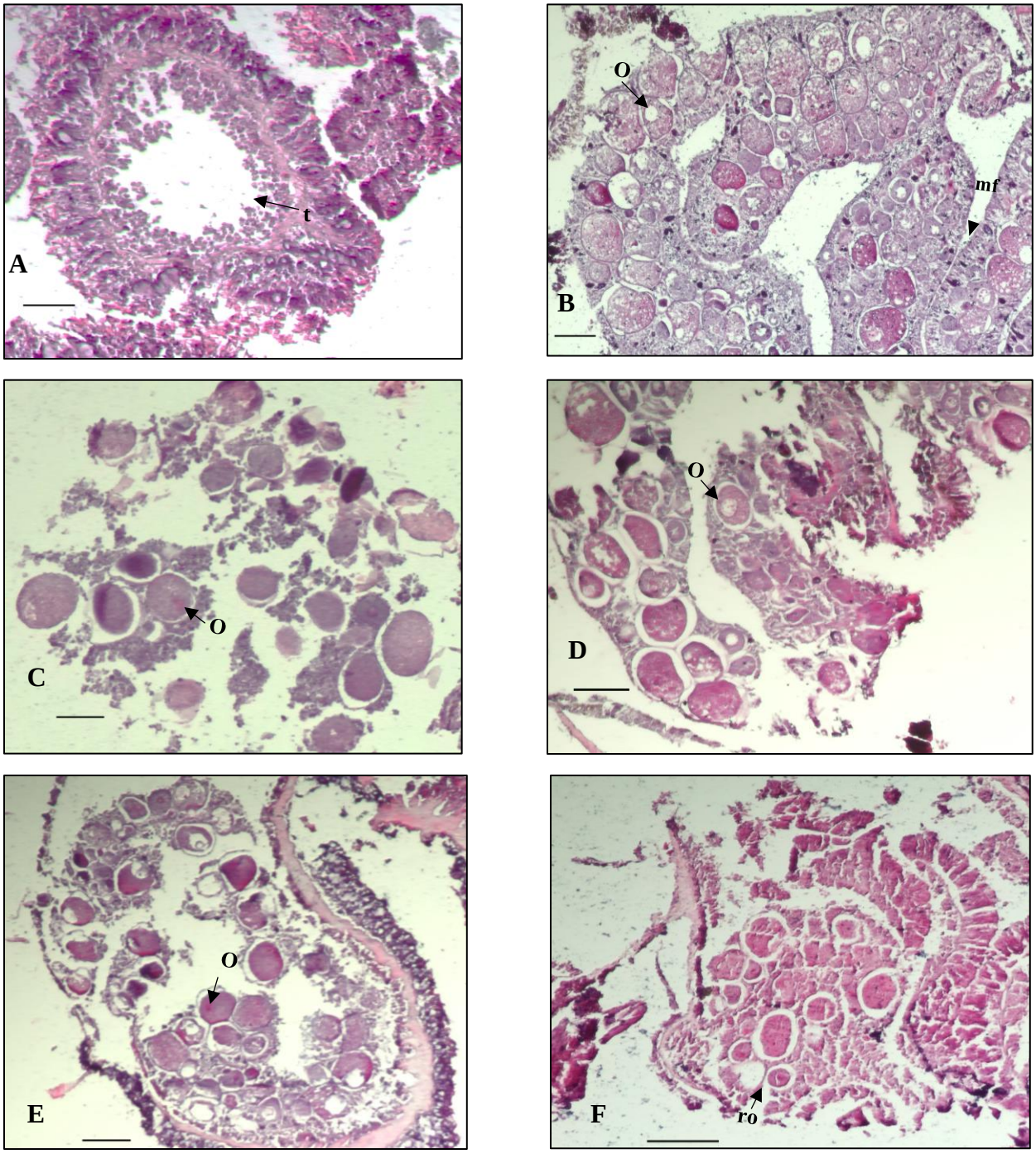


Figure 30. *Stichopathes luetkeni*. Histological observations of stages of oogenesis in black coral. (A**)** Stage 0 (unsexable); (B) Stage 1 (early); (C) Stage 2 (growing); (D) Stage 3 (maturing); (E) Stage 4 (mature); (F) Stage 5 (spent) (*Scale bars* = 500 µm; *mf* mesentery folding; *o* oocyte; *t* tentacle; *ptm* primary mesentery; *ro* relict oocytes.

Stage 1, spermatocytes were first detected in the gastrodermis of the primary mesentery in male samples collected on May 30, 2020 (Spring) (Figure 31 A). During June, July, and August (Summer) spermatocytes appear in spherical clusters, there is a thick layer of stained spermatocytes forming a chamber, and within this chamber darkly stained heads of spermatozoa can be observed (*Stage 2*: Figure 31 B). The tails of the spermatocytes are oriented in similar directions throughout the primary mesenteries. On September 4, 2021, spherical clusters were shown tightly packed with thin layers of spermatocytes and thinning of the lumen (*Stage 3*: Figure C). Male colonies collected on September 25, 2020 (Fall), showed spermatocytes partially spawned in the primary mesenteries with a few relict spermatocytes present (*Stage 4*). Unfortunately, as mentioned previously samples were not collected in October 2020 (Fall) but were collected on October 19, 2019. Results showed that most spermatocytes had experienced a spawning event prior to being collected with a few relict spermatocytes leftover (Figure 31 D).

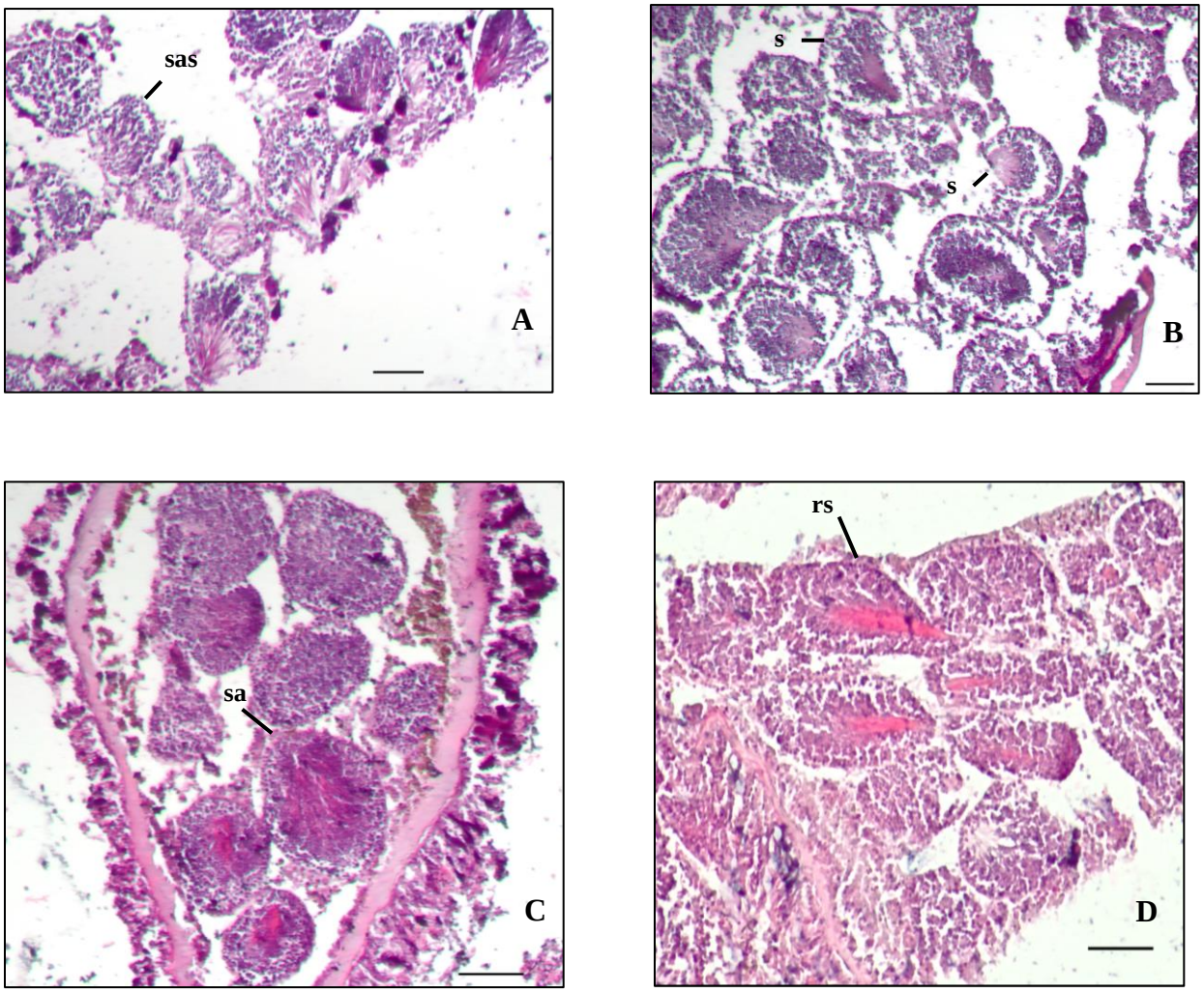


Figure 31. *Stichopathes luetkeni.* Stages of spermatogenesis in black coral. (A) Stage 1 (early); (B) Stage 2 (maturing); (C) Stage 3 (mature); (D) Stage 4 (spent) (*Scale bars* = 500 µm; *rs* relict spermatozoa; *sa* spermatozoa; *sas* spherical aggregations of spermatocytes; *sc* spermatocytes).

Reproductive Cycle

Average oocyte diameter was compared across months and seasons. Average oocyte diameter was 330 µm (± 10 SE) in May 2020 and increased to 482 µm (± 30 SE) in June 2021 before decreasing in July 2021 (393 µm ± 18 SE) through September 2021 (295 µm ± 14 SE) and then increased again in October 2019 (312 µm ± 18 SE) (Figure 32 a). Oocyte diameters were significantly different according to the month sampled (one-way ANOVA, $F = 9.9957$; $df = 4, 517$; $p > 0.0001$). When averaged over seasons, oocytes showed growth in the Spring and reached the largest mean diameter of 402 µm during Summer. Oocyte diameters varied significantly across season (one-way ANOVA, $F = 17.3357$; $df = 2, 519$; $p > 0.0001$) (Figure 32 b).

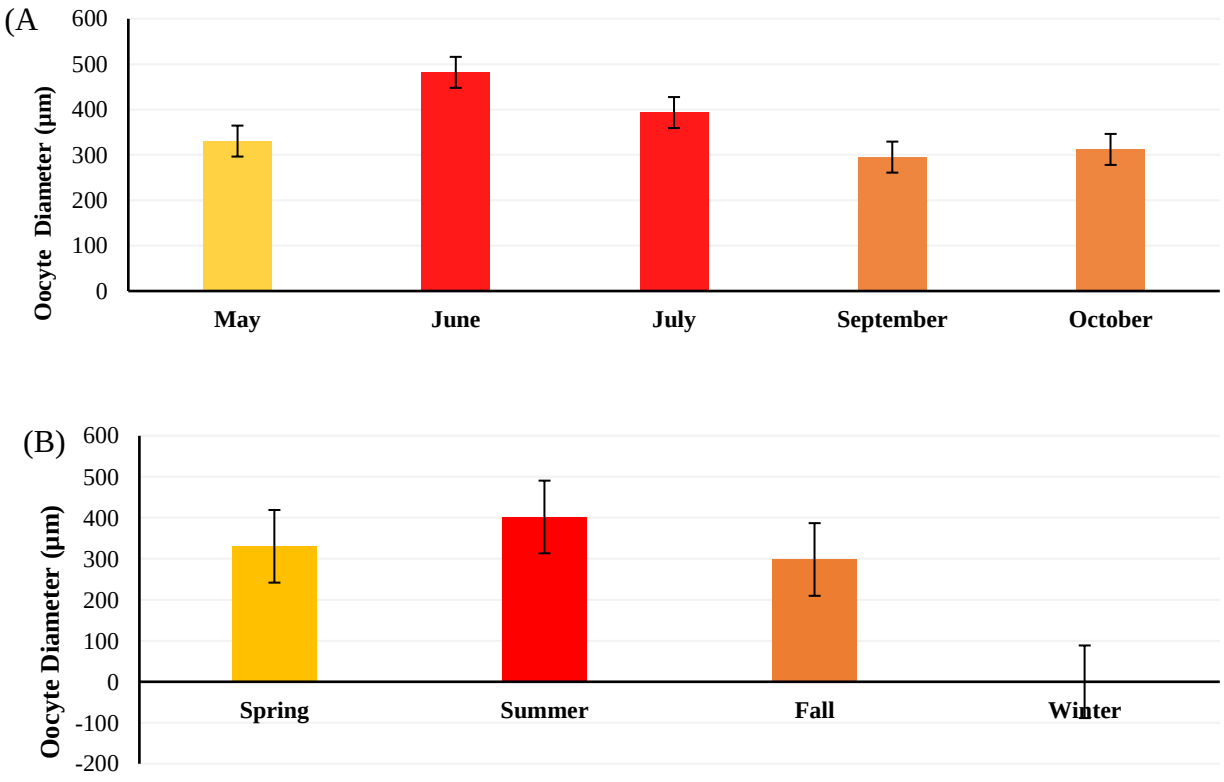


Figure 32. *Stichopathes luetkeni*. (A) Oocyte size-frequency for female polyps from May-October. (B) Oocyte diameters by season: May: Spring (Stage 1: early); June: Summer (Stage 2: growing); July: Summer (Stage 3: maturing); September: Fall (Stage 4: mature); October: Fall (Stage 5: spent) (bars represent standard error of the mean).

Spermatocytes were counted for every male polyp identified from September 2019, May 2021, and June-September 2021. Polyps were observed to have the highest average of 66 spermatocytes per polyp in September 2021 at the beginning of Fall. The lowest average of spermatocytes was in October 2019, producing only 17 spermatocytes per polyp (Figure 33).

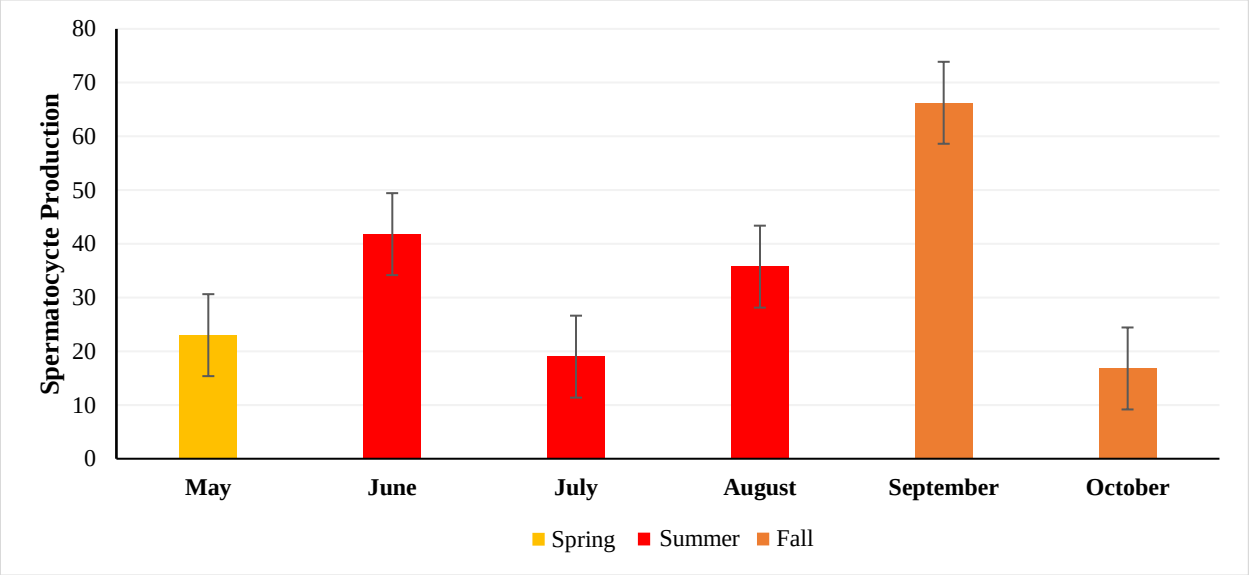


Figure 33. *Stichopathes luetkeni* spermatocyte production/polyp from September 2019, May 2020, and June-September 2021 (bars represent standard error of the mean).

Oocyte production was compared across seasons. Spring oocyte production was highest with an average of 180 (± 127 SE) oocytes per polyp per colony. Fall oocyte production was the lowest with an average of 48 (± 28 SE) per polyp per colony. Spermatocyte production was the highest in Summer with an average of 192 (± 78 SE) and the lowest in Spring 23 (± 23 SE) per polyp per colony. Male colonies averaged 120.7 spermatocytes per polyp and female colonies averaged 197.5 oocytes per polyp per colony, but this difference was not significant ($t = 0.8056$, $df = 5$, $p = 0.457$).

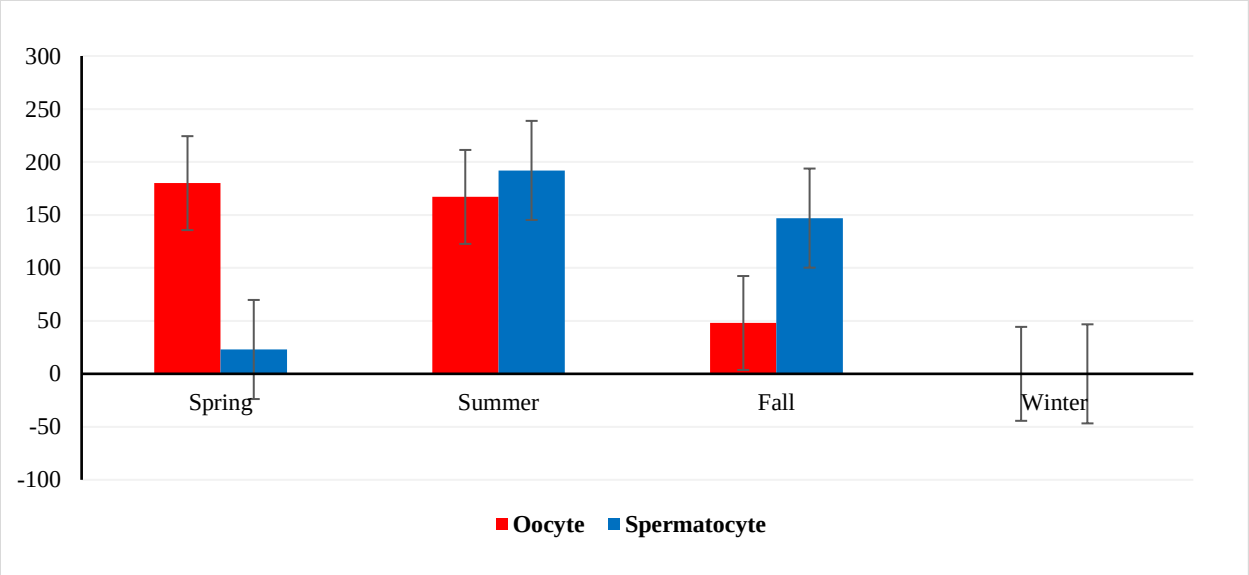


Figure 34. *Stichopathes luetkeni*. Seasonal average oocytes and spermatocytes production from female and male polyps (bars represent standard error of the mean).

Monthly seawater temperatures were documented from September 2015 - July 2016 and July - August 2021. Average water temperatures from 2015-2016 ranged from 13.8-29.5ºC (Figure 35 a-b). Mature gametes were last observed on September 25, 2021, which coincides with timing of the warmest sea temperatures of the year. September 20, 2015, showed the warmest average sea temperatures of the year at ± 29 ºC (Figure 35). It was estimated that a spawning event took place in September (Figure 35).

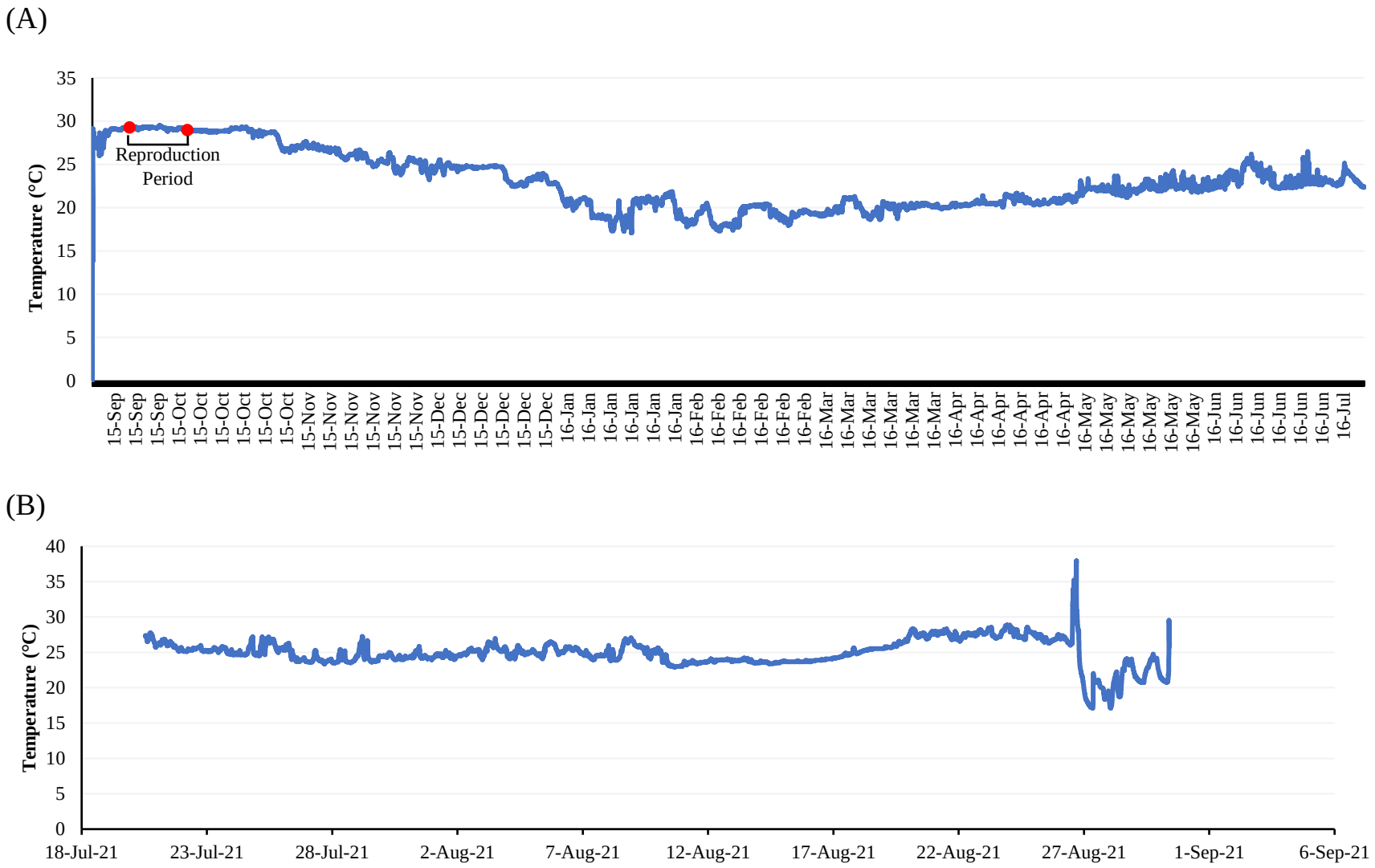


Figure 35. Daily seawater temperature measured at the Texas Clipper Artificial Reef. (A) Seawater temperature taken every 15 minutes from September 11, 2015-July 8, 2016 (bracket shows the potential reproduction period). (B) Seawater temperature taken on 5-minute increments from July 20, 2021-August 30, 2021.

Mode of Reproduction

No developing embryos or larvae were observed during polyp dissection and examination of *S. luetkeni*. In addition, spawning has not been observed *in situ* or in the aquaculture system for either *A. atlantica* or *S. luetkeni*. No evidence was collected to support internal fertilization within the antipatharian colonies. Stage 5 gametes, is indicative that *S. luetkeni* fertilization and larval development is occurring externally in the water column after a mass spawning event and not internally within polyps.

CHAPTER IV

DISCUSSION

Growth of *In Situ* Corals

The growth data taken at the Texas Clipper of tagged colonies revealed no differences among both species. As predicted (unbranched) *S. luetkeni* had a faster growth rate than (branched) *A. atlantica in situ* but the difference was not significant *Stichopathes luetkeni* had an average growth rate that was within the hypothesized range based on previous studies of *Stichopathes cfr maldivensis* in Indonesia and *Stichopathes luetkeni* in Jamaica (Bo *et al.*, 2009). With the exception of a few tagged colonies, most *S. luetkeni* colonies did not show a steady increase or decrease in growth rates. Rather, they showed signs of fracture and evidence of re-growth after, suggesting that breakage resulting from water movement, predation, or recreational scuba diver contact may be a frequent event that whip corals experience at this location. Measurement bias could also be a factor when gathering measurements for *S. luetkeni* causing noise within the statistical analysis. Measurement bias could also be a possibility when there is poor visibility, currents, or the way the diver was extending the colony to its maximum height. However, growth rates can also depend on age, food availability, rearing conditions, and biomass accretion (Coppari *et al.*, 2019). In addition, evidence of tissue damage was seen surrounding the area where the cattle tag and zip ties were placed. In some incidences, it seemed as if that part of the colony had died, so the coral may be susceptible to tissue loss and breakage with contact.

Antipathes atlantica, exceeded the hypothesized growth rates that were based on radial growth rates in *Antipathes griggi* (Hitt *et al*., 2020). Species difference may account for the greater growth rate in *A. atlantica*. The data collected indicated that *A. atlantica* did not experience the magnitude of colony fractures as *S. luetkeni*. Growth rates also showed evidence of *A. atlantica* having a faster growth rate in width than in height. It is a possibility that having a shorter, wider, branch-like morphological structure gives this species of black coral an advantage in fracturing events. Food availability in the GoM may also be greater than in previous study sites provided sufficient nutrients for the colonies to grow faster. It was also observed that *A. atlantica* did not experience the same tissue loss as *S. luetkeni* at the site where the zip tied was placed.

When comparing apical growth rates of both species *in situ*, it was clear that *S. luetkeni* colonies had a greater growth rate although the difference was not significant. This may be possible due to *A. atlantica* having a different morphology and focusing on growing a greater surface area and/or *S. luetkeni* could have experienced increased fracturing and having to regrow, or measurement bias. Unbranched species of black corals can experience evident apical fragmentation due to heavy currents (Bo *et al*., 2009) or as a control mechanism of the colony's growth (Coppari *et al*., 2019).

It is to be noted that light in the development of these two species may play a role in their growth of these two species. Microscopic examinations were not done on the tissues of these corals and no prior evidence has revealed the presence of zooxanthellae. However, when observing the locations of these corals it was noticeable that *S. luetkeni* was mostly if not always located in shaded areas within or underneath the vessel. *Antipathes atlantica* was mostly found at shallower depths and either out in the open or under cover. Observations on *A. grandis* have shown

colonies growing towards light even if located in caves or under overhangs as adults (Grigg, 1965). Further research is needed to determine the presence or lack thereof dinoflagellate symbionts and other factors contributing to dependence or sensitivity to light during various stages of developing black corals to understand their light requirements.

Growth of Corals in Aquaculture System

In this study, I documented, for the first time, fragmentation of *Stichopathes luetkeni* and *Antipathes atlantica* in the aquaculture system.

Although both species of antipatharians live in the same habitat, it was unclear if they could survive in the same aquaculture system. Our data suggest that *S. luetkeni* can easily be transplanted into an aquaculture system with a high survival rate. Transplanted *S. luetkeni* had remarkable success with only one fragment showing a negative growth rate. Many fragments that were not included in the average growth rate were due to fracture or breakage. These fragments were reglued, and, in some cases, these fractures led to new fragments and colonies. *Stichopathes luetkeni* showed great resilience to the change in environment and nutrients available. However, there was no significant difference when comparing *S. luetkeni* growth rates *in* situ to those in aquaculture. Although measurements in aquaculture were shown to be slower than those taken *in situ* there was noise in the data set. Possible measurement bias *in situ,* variation of initial colony sizes *in situ,* and the range of fragment sizes measured in the aquaculture system are a few factors that may have caused noise in the dataset. It should also be noted that "transplant stress may be bigger for juvenile colonies" (Bo et al., 2009) possible due to having thinner tissue and can be easily damaged during transplantation and transportation. Colonies clipped *in* situ were not measured, therefore age was left undetermined. However, observations of fragments in the

aquaculture concluded that the thinner fragments did experience more fracturing events. It was also observed that in some cases new growth was recognizable thinner than the initial fragment (Figure 35 a).

Antipathes atlantica experienced a few setbacks when placed in the aquaculture system. As fragments began to deteriorate, observations showed that the tissue loss was not identical in every colony. Some colonies lost tissue along the tips and others lost tissue starting at the center of the colony. Followed by the loss of tissue, polyps became very defined and started to be expulsed from the colony into free-living propagules. In addition, many polyps continued to stay attached to the mother colony until the final polyp bailout (polyps' fragment) that occurred at approximately two months (Figure 37). However, currently there are three *A. atlantica* fragments in the aquaculture that have survived approximately eight months. The addition of Reef Chili coral food has been the only component changed since the start of the project. Moreover, it has been observed that *in situ A. atlantica* would have organic matter entrapped in their net-like colonies. Reef Chili coral food comes in a powder consistency and can be seen entrapped in branches like observations made *in situ.*

Figure 36. *Antipathes atlantica* in aquaculture experiencing tissue loss and polyp bailout.

Possible stressful conditions known to trigger polyp bail-out in other cnidarians include the decrease in O_2 concentrations, increased temperature, the secondary metabolites produced by macroalgae, changes in salinity and pH as well as low-food availability (Coppari, *et al.*, 2020). However, in this study we can exclude all factors that include water parameters since the water was checked multiple times per week. Therefore, polyp bail-out observed in this study could be related to either lack of nutrients, lack of turbidity, or the potential presence of a competitor (*S. luetkeni*).

Observations that could be crucial to future coral restoration projects was that result of tissue damage to *S. luetkeni* when placed in contact with *A. atlantica* (Figure 35 b). A *S. luetkeni* fragment was also observed expanding from the tip of the colony creating a flat adhesive anchor that attached to the glass (Figure 35 c). Another fragment used multiple anchorages along the colony in a horizontal fashion to adhere to the glass. It can also be noted that even though *S. luetkeni* has polyps arranged on only one side of the colony they will direct their long tentacles upwards to capture food (Figure 35 d). However, when placing fragments in the aquaculture one of the fragments had its polyps facing the glass. It was observed that at some point the polyps had rearranged themselves to the opposite side of the colony to capture food. Essentially, when placing fragments in an aquaculture polyps will rearrange themselves towards in the direction that benefits them when capturing food.

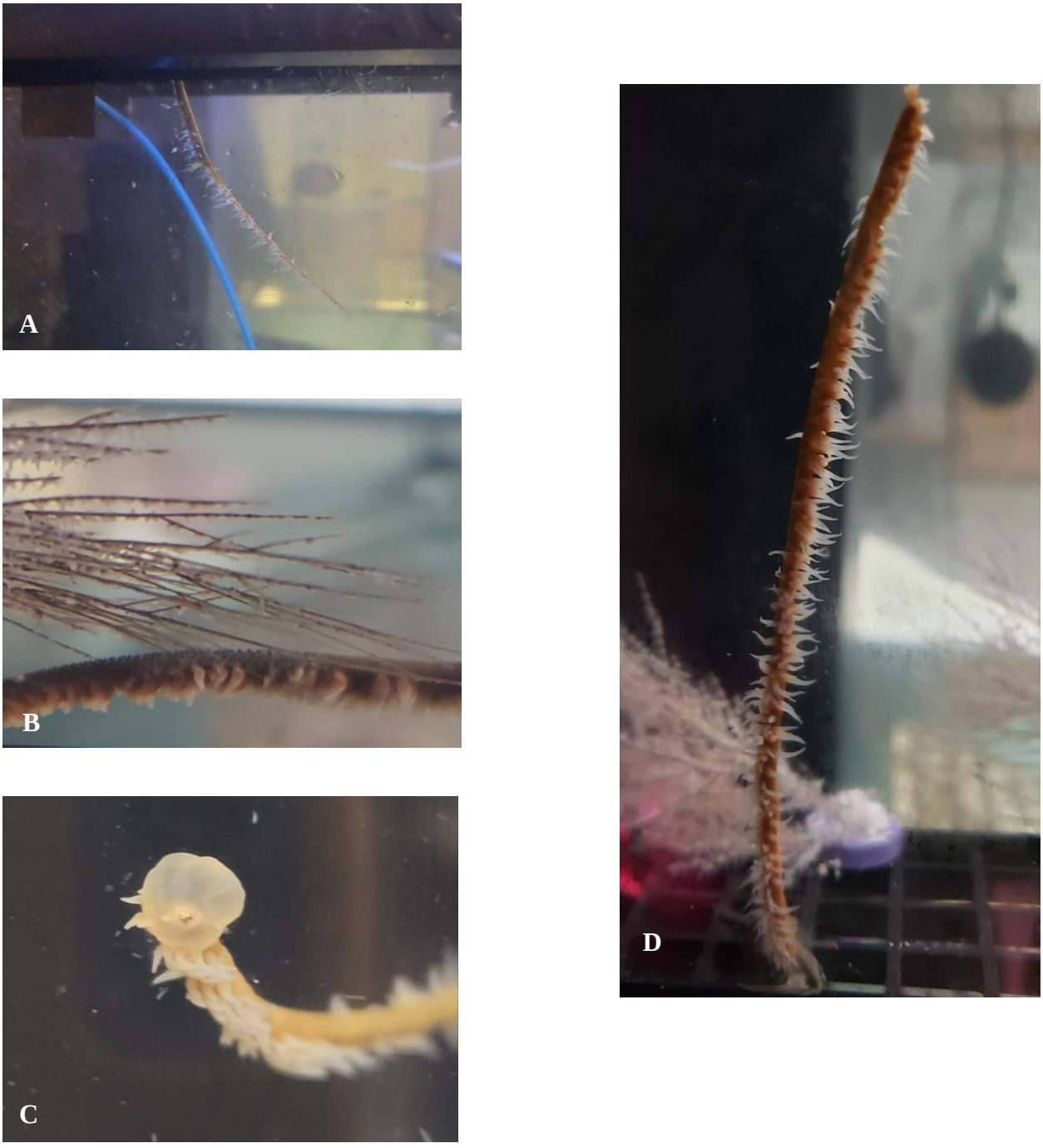


Figure 37. *Stichopathes luetkeni* in aquaculture system. (A) Fragment has shown growth from the bottom end of the fragment in a thinner form (B) Fragment experienced tissue loss from contact with *A. atlantica* (C) Polyp has modified its shape by flattening and creating a basal plate adhering to the glass (D) Fragment rearranged polyps to the opposite of the colony.

Histological Analysis of Gonads

Of 20 colonies sampled, 6 colonies were determined to be female, 12 colonies were determined to be male, and 2 colonies were hermaphrodites. Although, 100 polyps were dissected only 32 polyps were able to be sexed after histological procedures. This low number of examined polyps may have been due to age of the colony, sexually immature, or bad cuts due to histological techniques. Among *S. luetkeni* samples containing gametes, 94% were determined to be gonochoric however it was discovered that in two colonies sampled in October 2019 and June 2021 contained both female and male polyps. It is unclear if *S. luetkeni* is a simultaneously or sequential hermaphrodites (Richmond, 1990). Monitoring was only done over one year, sex changes (sequential hermaphrodites) can occur over longer periods of time (Wagner *et al.*, 2011). It should also be noted that the Texas Clipper (2007) is a relatively new environment for these antipatharian species and may have an influence in sexual reproductive techniques. Further, studies on histological analysis within antipatharians is needed to further clarify sex ratio and reproductive techniques due to inaccuracy in histological methods.

Stichopathes luetkeni gametes were first observed in the gastrodermis of the primary transverse mesentery and during the development of the gametes. However, in one of the histological sectioning it was observed that gametes had also extended into the polyp's tentacle. Gametes were observed and further characterized into maturity stages depending on gamete development (Parker *et al.*, 1997; Wagner *et al*., 2011). Maturity stages were evident during October 2019, May 2020, June-September 2021. During the histological analysis it was found that female and male polyps are relatatively synchronized during maturity stages. It was also appears that reproduction occures during late Summer anually as predicted based on previous spawning at the Texas Flower Garden Banks (Vize, 2006). From a total of 522 oocytes, the

largest average oocyte diameter recorded were during the Summer months (June and July) and the smallest average oocyte diameter was during the Fall months (September and October). Samples collected on October 19, 2019 indicated most oocytes had experienced a spawning event prior to being collected with a few relict oocytes (Figure 30 F). However, oocyte average production with each polyp appeared to be higher during the Spring and lowest during the Fall. Spermatocyte production were determined to highest during the Fall and lowest during the Spring.

Although not observed *in situ* or in aquaculture during this study, broadcast-spawning of gametes is the most likely mode of sexual reproduction in *S. luetkeni*. This reproductive strategy can be concluded from the partially spawned gonads observed on histological sections on September 4, 2021 and the disappearance of mature gametes from all the samples collected on September 25, 2021. Reproduction of *A. atlantica* was not observed *in situ* however a mode of asexual reproduction "polyp bailout" was observed in the aquaculture system. However, it is believed that this bailout was not a result of reproduction cycle cues, but stress induced. This polyp release method can be an effective form of asexual reproduction and dispersal; however, it has not been documented to occur in nature to date. Future sampling during reproduction timing (August-September) typically at night while considering lunar cycles are needed to further analyze mode of reproduction.

It has been widely recognized that environmental changes can induce reproduction. Sea water temperatures, daylength, and lunar cycles are all important cues that are used by corals. There was a correlation between rising sea temperatures and average oocyte diameters in this study. Largest average oocyte diameters were documented in the Summer of 2021 corresponding with the previously recorded sea temperatures at the Texas Clipper. Seawater temperatures

ranged from 13.8-29.5ºC from September 2015-July 2016, highest temperature was recorded September 20, 2015, ± 29 ºC. Although seawater temperatures were not obtained during the entirety of the sampling period it should be noted that the Texas Clipper does not experience drastic temperature differences each year. The results of this study indicate that reproductive output for *S. luetkeni* was correlated with seawater temperature changes.

Coral samples were taken during four seasons and the presence of gonads suggested that the reproductive cycle is annual within *S. luetkeni* colonies, and spawning may occur over several weeks. However, longer coral sampling projects are necessary to properly understand the effect that these abnormal events have on antipatharian corals at the depths they inhabit. In addition, further studies need to be done to understand the correlation between the size of a colony and first reproduction. By estimating the minimum size of the first reproduction, we can estimate the minimum age of a coral.

No developing embryos or larvae were observed within any of the examined polyps, and none showed any sign of fertilization which is consistent with previous histological examination. However, knowing if gametes are being fertilized externally in the water column during a spawning event or fertilized internally and then rapidly spawned is still undetermined. This window is very narrow and requires sampling either before or during a spawning event. Future sampling during August and September nights while considering lunar cycles needs to be done to confirm whether these spawning events at night and if *S. luetkeni* is a spawner or brooding.

It is noteworthy that an extension of the reproduction cycle under unfavorable or abnormal environmental conditions may be considered a response to maximizing reproductive success (Parker *et al.*, 1997). South Texas has experienced freezing temperatures and warmer

spring seasons in the past few years that can alter the reproductive cycle. Ocean warming and climate change will likely affect multiple maturity stages and broadcasting spawning in corals (Schutter *et al*., 2015). Elevated temperature of even a single degree (ºC) above normal range can increase metabolic expenditure of stored energy, increase in premature metamorphosis and reducing recruitment success and mortality (Richmond *et al*., 2018). In addition, to anthropogenic effects on recruitment success, identification of coral source populations and gene flow are crucial to managing mesophotic reefs that have experienced damage (Studivan *et al.*, 2018). Based on the location of the Texas Clipper, antipatharian larvae possibly traveled from Mexico via Mexico current or an inshore eddy current from the Texas Flower Garden Banks southward (Studivan *et al.*, 2018). It could represent an important source of population recruitment and ensuring adequate supply and survival of propagules to eastern reefs in the GoM.

CHAPTER V

CONCLUSION

It was identified that there was no significant difference when comparing *S. luetkeni in situ* to the aquaculture system. No significant difference was found in mean apical growth rates for *S. luetkeni* when compared to *A. atlantica in situ.* This project builds upon literature findings that unbranched corals would have a greater growth rate than branched corals. This study raised many questions on methods used to collect growth rates *in situ* and the need for improvement is evident. Further research needs to be done to correlate growth rates to age, morphology of the colony, and environmental factors.

Our data also concluded that the occurrence and success of fragmentation of antipatharians may play a key role in survivorship and recovery to stressful environmental conditions or negative anthropogenic events. If antipatharians can grow in an aquaculture setting, then it will be possible to transplant them back into mesophotic environments to increase colony number and implement education outreach. Coral restoration can help increase coral abundance and cover, enhance coral sexual reproduction, and study stressors.

Our study identified that the annual reproductive cycle is correlated with sea water temperatures, causing a spawning event in *S. luetkeni* in the Gulf of Mexico. Oocyte average diameters were found to be the largest in size during the Summer months (June & July). However, male polyps were most abundant in spermatocytes during early Fall (September). Both sexes were depleted from the primary gastrodermis in September 2021. Through histological

observations, this study provides evidence that *S. luetkeni* is likely hermaphroditic. However, due to the larger quantity of colonies containing either female or male polyps, sequential hermaphroditism over successive reproductive seasons cannot be ruled out. In addition, no larvae or fertilized oocyte was observed, indicating that *S. luetkeni* is a broadcast spawner. Unfortunately, the reproductive biology of *A. atlantica* was not successful and thus is left undetermined. Further improvements to the histological methodology are needed to successful collect reproductive data from *A. atlantica.*

This is the first study, to the best of our knowledge, on the growth rates of *S. luetkeni* and *A. atlantica* in the GoM and in an aquaculture system. As well as the research is done on the fundamental aspects of the reproductive biology of *S. luetkeni* in the GoM. Therefore, the data that was collected during this study will provide valuable information for future antipatharian growth and reproductive projects. A clear basis has been provided to build an aquaculture system on land and grow coral colonies to further contribute to research and the restoration of these antipatharian corals. It has been highlighted that the need for more effective long-term monitoring is needed to contribute to long-term restoration projects. The effects of ocean warming and environmental stressors on antipatharian corals in the GoM need development to determine their vulnerability.

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APPENDIX A

APPENDIX A

Table 3. *Stichopathies luetkeni* growth statistics at theTexas Clipper artificial reef

Sample Number	1/30/2020	5/30/2020	6/28/2021	7/3/2021	7/20/2021	7/30/2021	8/26/2021	9/25/2021	Growth	Growth Rate	Period Weeks
002	109.22	95.00	-	89.00	93.50	-	-	89.50	-19.72	-0.23	86
003	71.12	93.00	-	-	-	-	-	-	21.88	1.29	17
007	29.21	39.00	-	-	-	-	-	-	9.79	0.58	17
022	-	-	-	27.50	-	-	87.00	-	59.50	7.44	8
025	-	-	-	-	33.00	32.00	-	34.50	1.50	0.17	9
027	-	-	-	-	61.00	58.00	-	-	-3.00	-3.00	1
030	-	-	-	-	26.00	-	71.50	-	45.50	9.10	5
033	-	-	-	-	-	31.50	28.50	-	-3.00	-0.75	4
037	-	-	-	-	-	115.30	116.00	117.50	2.20	0.28	8
038	-	-	-	-	-	125.00	128.00	-	3.00	0.75	4
050	-	-	85.00	-	-	-	-	77.00	-8.00	-0.62	13
051	-	-	-	-	111.50	-	111.50	111.50	0.00	0.00	9
055	-	-	89.00	-	-	92.50	-	-	3.50	0.88	4
057	-	-	58.00	-	-	-	74.50	73.00	15.00	1.15	13
059	-	-	-	-	-	171.70	151.00	-	-20.70	-5.18	4

APPENDIX B

APPENDIX B

Table 4. *Antipathes atlantica* growth statistics at the Texas Clipper artificial reef

Sample Number	1/30/2020		5/30/2020		6/28/2021		7/30/2021		9/4/2021		Growth		Growth Rate		Period Weeks
	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	
016	17.15	27.94	10.16	24.13	-	-	-	-	-	-	-6.99	-3.81	-0.41	-0.22	17
017	21.59	21.59	20.32	22.86	-	-	-	-	-	-	-1.27	1.27	-0.07	0.07	17
018	12.70	12.07	15.24	16.51	-	-	-	-	-	-	2.54	4.45	0.15	0.26	17
040	-	-	-	-	20.20	22.50	21.50	22.50	-	-	1.30	0.00	0.33	0.00	4
042	-	-	-	-	14.40	16.00	-	-	17.00	20.50	2.60	4.50	0.26	0.45	10
043	-	-	-	-	15.30	17.00	18.00	17.00	19.00	15.00	3.70	-2.00	0.37	-0.20	10
044	-	-	-	-	18.00	22.00	21.00	26.00	21.00	26.50	3.00	4.50	0.30	0.45	10
045	-	-	-	-	18.20	26.00	25.00	30.00	21.00	31.00	2.80	5.00	0.28	0.50	10
046	-	-	-	-	17.00	16.00	-	-	20.00	17.00	3.00	1.00	0.30	0.10	10
048	-	-	-	-	17.50	23.00	21.00	25.00	-	-	3.50	2.00	0.88	0.50	4
049	-	-	-	-	-	-	18.50	14.50	21.00	20.50	2.50	6.00	0.50	1.20	5

APPENDIX C

APPENDIX C

Table 5. *Stichopathes luetkeni* fragments growth statistics in anaquaculture system

Frag Number	7/6/2021	7/12/2021	7/19/2021	8/3/2021	8/16/2021	9/9/2021	9/28/2021	Growth	Growth Rate	Period Weeks
1	2.80	-	2.90	3.00	-	-	-	0.20	0.05	4
2	5.50	-	6.00	-	-	-	-	0.50	0.25	2
2f	-	-	-	1.00	0.90	1.00	0.60	-0.40	-0.05	8
2b	-	-	-	5.00	4.90	4.80	5.00	0	0	8
3	4.90	-	5.30	7.90	8.10	8.20	9.30	4.40	0.37	12
4	5.20	-	5.40	-	-	-	-	0.20	0.10	2
4f	-	-	-	1.20	1.40	-	-	0.20	0.10	2
4fr	-	-	-	-	-	0.70	0.90	0.20	0.10	2
4b	-	-	-	4.60	3.90	0.50	0.60	-4.00	-0.50	8
5	4.40	-	5.10	4.80	4.80	4.80	4.70	0.30	0.03	12
6	4.00	-	5.70	5.80	5.80	5.60	5.20	1.20	0.10	12
7	5.50	-	-	-	-	-	-	0	-	-
7b	-	-	4.10	4.20	4.40	4.10	4.20	0.10	0.01	8
8	-	6.00	6.40	6.60	6.60	6.50	7.00	1.00	0.09	11
9	-	5.00	5.20	5.50	5.50	7.00	-	2.00	0.25	8
9f	-	-	-	-	-	-	0.60	-	-	-
10	-	4.50	-	-	-	-	-	-	-	-
10b	-	-	4.40	4.40	4.50	4.40	4.40	0	0	8
11	-	4.60	5.70	5.40	5.50	5.70	5.60	1.00	0.09	11
12	-	5.70	6.00	6.10	6.00	6.00	7.30	1.60	0.15	11
13	-	4.70	0.50			-	-	-4.20	-4.20	1
13r	-	-	-	1.20	0.40	0.40	0.40	-0.80	-0.10	8
14	-	-	-	4.20	4.20	3.60	4.00	-0.20	-0.03	8
15	-	-	-	2.10	2.50	2.20	2.10	0	0	8

APPENDIX D

APPENDIX D

Table 6. *Antipathes atlantica* single-branched fragments growth statistics in an aquaculture system

Frag Number	7/7/2021	7/21/2021	7/23/2021	7/29/2021	8/4/2021	Growth	Growth Rate	Period Weeks
1	1.00	-	-	-	-	-	-	-
2	1.90	2.00	-	-	2.00	0.10	0.03	4
3	1.80	2.10	-	2.00	1.70	-0.10	-0.03	4
4	0.70	-	-	-	-	-	-	-
5	1.70	1.80	-	-	1.60	-0.10	-0.03	4
6	-	1.50	-	-	1.40	-0.10	-0.05	2
7	-	1.30	-	-	1.30	0	0	2
8	-	1.50	-	-	1.50	0	0	2
9	-	1.30	1.20	-	-	-0.10	-	-
9b	-	-	-	-	0.10	-	-	-
10	-	1.20	-	-	1.30	0.10	0.05	2
11	-	1.20	-	-	1.00	-0.20	-0.10	2
12	-	1.60	-	-	1.60	0	0	2
13	-	1.50	-	-	1.20	-0.30	-0.15	2
14	-	2.00	-	-	1.80	-0.20	-0.10	2
15	-	1.00	-	-	-	-	-	-
15b	-	-	0.70	-	0.60	-0.10	-0.07	2
16	-	1.40	-	-	-	-	-	-
16b	-	-	1.10	-	1.10	-	0	2
17	-	1.30	-	-	1.20	-0.10	-0.05	2

APPENDIX E

APPENDIX E

Table 7. *Antipathes atlantica* branched fragments growth statistics in an aquaculture

Frag Number	**7/12/2021**		**7/21/2021**		system **8/4/2021**		**Growth**		**Growth Rate**		**Period Weeks**
	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	
1	6.50	2.40	6.30	1.80	6.10	2.50	-0.40	0.10	-0.13	0.03	3
2	5.10	3.00	6.80	3.10	7.00	2.50	1.90	-0.50	0.63	-0.17	3
3	5.70	3.00	5.70	4.00	5.80	4.30	0.10	1.30	0.03	0.43	3
4	-	-	2.50	1.10	2.60	1.00	0.10	-0.10	0.05	-0.05	2
5	6.90	3.10	6.00	4.00	5.30	3.40	-1.60	0.30	-0.53	0.10	3
6	5.50	4.70	5.50	2.30	5.40	2.40	-0.10	-2.30	-0.03	-0.77	3
7	-	-	4.20	2.10	4.40	2.00	0.20	-0.10	0.1	-0.05	2
8	-	-	1.50	1.50	1.40	0.90	-0.10	-0.60	-0.05	-0.30	2
9	-	-	4.00	1.60	4.20	1.60	0.20	0	0.1	0	2
10	-	-	2.70	2.00	2.70	1.60	0	-0.40	0	-0.20	2
11	-	-	3.40	2.50	3.30	2.20	-0.10	-0.30	-0.05	-0.15	2
12	-	-	2.30	1.50	2.10	1.30	-0.20	-0.20	-0.10	-0.10	2
13	-	-	3.10	1.70	2.70	1.50	-0.40	-0.20	-0.20	-0.10	2
14	-	-	3.00	2.50	2.80	2.50	-0.20	0	-0.10	0	2
15	-	-	4.50	2.20	2.20	1.80	-2.30	-0.40	-1.15	-0.20	2

BIOGRAPHICAL SKETCH

Victoria E. Salinas attended high school at James Pace High School in Brownsville, Texas from 2007 to 2011, where she earned her high school degree. After graduating high school, Victoria attended Texas A&M University in College Station, Texas from 2011 to 2015, where she earned her Bachelor of Agriculture & Life Science (Parks and Conservation) as a student-athlete. Victoria started her work in the marine biological field in 2016 volunteering at Sea Turtle Inc. South Padre Island, Texas and working at the Gladys Porter Zoo in Brownville, Texas, where she worked as aquarist. After working as an aquarist for seven months, Victoria earned her Master of Science in Ocean, Coastal, and Earth Sciences degree at The University of Texas Rio Grande Valley in August 2022. Victoria can be reached by mail at 2974 Old Spanish Trail, Brownsville, TX 78520 or by email at victoriasalinas0@gmail.com