ECOLOGY OF BLOOMING JELLYFISH IN THE GULF OF MEXICO

A Dissertation

by

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ABSTRACT

Jellyfish (Scyphozoa, Cnidaria) are important components of marine food webs and form problematic blooms that negatively impact humans, but are understudied in the Gulf of Mexico (GoM). Scyphozoans have a multi-modal lifecycle where the benthic polyp produces seasonal pelagic medusae. We used DNA barcoding and multigene phylogenetic analyses to present evidence of a new *Aurelia* species, *A*urelia sp. 17, in the northern GoM. Using controlled laboratory experiments, we determined the temperature and salinity limits for polyp survival of two GoM species, *A*urelia sp. 17 and *A*urelia sp. 9, and *A*urelia *coerulea* from Japan. *Aurelia* sp. 9 and *A*urelia *coerulea* are tolerant of a broad range of temperatures and salinities but have different tolerance limits. The narrower thermal tolerance range of *A*urelia sp. 17 suggests adaptation to thermally stable marine environments. To address the lack of knowledge on polyp distribution, we constructed habitat suitability maps for *A*urelia sp. 9 and *A*urelia sp. 17. GoM coastal waters are suitable for *A*urelia sp. 9, but not *A*urelia sp. 17, and water temperature, not salinity, limits the distribution of both species. While 94% of GoM artificial reefs and 97% of gas platforms are suitable for *A*urelia sp. 9, only 37% of reefs and 40% of gas platforms have conditions suitable for *A*urelia sp. 17. Summer-high water temperatures restrict *A*urelia sp. 17 polyps to deeper offshore waters. To identify trends, bloom events, seasonal and spatial timing in presence for *Aurelia* spp., *Stomolophus* sp., and *Chrysaora* sp., we analyzed Texas Park and Wildlife Department's trawl survey data from 1982 through 2018 across bay and GoM regions of the Texas coast. Interannual numbers of *Aurelia* spp. vary greatly, with multiple blooms recorded over the survey period in the

GoM. Abundance of *Chrysaora* sp. in bays and the GoM was consistent across years, while *Stomolophus* sp. numbers have remained low since about 2006. *Aurelia* spp. were encountered with similar frequency in bays and the GoM, but CPUE was higher in the GoM. Interannual occurrence of *Chrysaora* sp. was similar among bay and GoM regions. This work advances our understanding of blooming jellyfish in the GoM.

DEDICATION

This dissertation is dedicated to my dad, Dmitry Frolov. Thank you for always supporting me, encouraging me to aim high, and being the first to see the scientist in me.

A huge thank you to my wonderful husband Michael Ruthenbeck, for your love, patience and enthusiastic support of all my endeavors, including this one.

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All other work conducted for the dissertation was completed by the student independently.

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NOMENCLATURE

GoM	Gulf of Mexico
TPWD	Texas Parks and Wildlife Department
ºC	Degrees Celsius
ppt	parts per thousand
GIS	Geographic Information System
COI	cytochrome c oxidase I
ITS1	internal transcribed spacer 1
CLM	chronic lethal thermal method
CTM	critical thermal method
CTMax	critical thermal maximum
CLMax	chronic lethal thermal maximum
CLMin	chronic lethal thermal minimum
CLSMin	chronic lethal salinity method
SIMPER	one-way similarity percentage analysis
PERMANOVA	permutational multivariate analysis of variance

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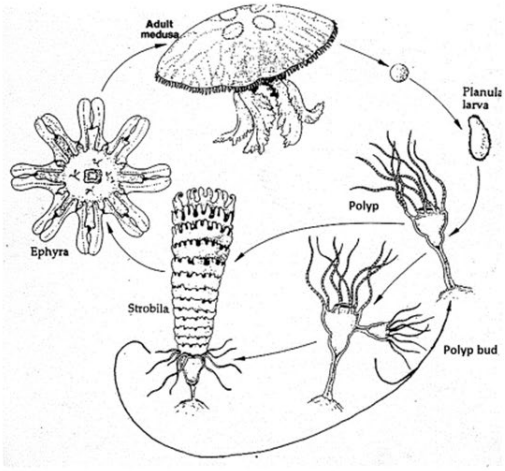
presence in the second area leads presence in the first. No meaningful function (NMF) indicates that correlation did not peak during the ± 100 day window. All values in the table were significant at the 0.05 level. 94

1. INTRODUCTION

1.1 Introduction to Scyphozoan jellyfish

Jellyfish belonging to Class Scyphozoa (Phylum Cnidaria) have a unique life-cycle (Figure 1.1), where an asexual polyp stage precedes and alternates with the pelagic sexually-reproducing medusa stage (Brusca and Brusca 2003). Young medusa, termed "ephyra", are produced by polyps via an asexual transverse fission process, known as "strobilation". The tiny ephyrae mature into the large and conspicuous medusae, which are usually gonochoristic and eventually spawn to produce planulae larvae that settle on the benthos and metamorphose into polyps. Most research of Scyphozoan ecology has focused on the medusa life stage, however studies of polyp ecology may help explain how jellyfish populations persist over years and longer spans of time.



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Large aggregations of medusae, termed “blooms”, are associated with numerous negative socio-economic impacts. Medusae clog fishing nets (Nagata et al. 2009), reduce catch quality (Lynam et al. 2006), obstruct power plant cooling intakes (Azis et al. 2000), and sting beachgoers (Purcell et al. 2007). Blooms can cause problems for aquaculture by fouling net pens or causing fish gill disorders (Purcell et al. 2013). Severe jellyfish blooms can deplete zooplankton stocks and alter ecosystem function and structure at local and regional levels (Huntley and Hobson 1978; Daskalov, Grishin et al. 2007; Møller and Riisgård 2007).

1.2 Role of Scyphozoan jellyfish in marine ecosystems

Jellyfish are ecologically important members of marine food webs and fisheries. As consumers, jellyfish have versatile carnivorous diets, and are able to feed on protists, fish eggs and larvae (Möller 1984; Purcell & Sturdevant 2001; Riascos et al. 2014) with high clearance rates (Olesen 1995; Hansson et al. 2005; Acuña et al. 2011). Long considered to be trophic dead-ends, jellyfish are now recognized as common prey for a variety of marine animals (Hays et al. 2018) and are prey for seabirds (Jarman et al. 2013; McIness et al. 2017), sea turtles (Gonzalez Carman et al. 2014; Smolowitz et al. 2015), invertebrates (Ates 2017) and economically valuable fish species (Cardona et al. 2012).

Presence of medusae in the environment can restructure food-webs by shunting carbon to lower trophic levels. By their “bloom and bust” nature, medusae transfer carbon from surface waters to the seafloor as medusae carcasses fall to and accumulate on the seafloor (Lebrato, Pitt et al. 2012). Medusae also regenerate significant amounts of organic and inorganic nutrients via mucous production, excretion, sloppy feeding and fecal material. The dissolved organic matter produced by these processes has been shown to be preferentially consumed by bacterioplankton,

which use it for respiration instead of production, thereby reducing bacterial growth efficiency (Condon, Steinberg et al. 2011). The excreted inorganic nutrients can increase primary production fueling algal blooms (Pitt, Kingsford et al. 2007; West, Pitt et al. 2009) .

Medusae also serve as floating habitats providing shelter, food, and even transportation to vulnerable fish and invertebrates in pelagic areas where shelter and protection is scarce (Doyle, Hays et al. 2014; Muffett and Miglietta 2021). Many species, including at least nine families of fish and 78 species of crustaceans, seek refuge underneath medusae where they are protected from marine and aerial predators (Castro, Santiago et al. 2002; Muffett and Miglietta 2021). In the same way, medusae may aggregate prey in environments where otherwise small prey items would be widely dispersed (Sato, Kokubun et al. 2015).

1.3 Blooms of Scyphozoan medusae in the Gulf of Mexico

There are at least 20 species of Scyphozoan jellyfish in the GoM, representing three orders and eleven families (Segura-Puertas et al. 2009). Medusae blooms in this region have caused significant economic losses, costing millions of dollars, particularly to the shrimping industry as medusae clogged nets leading to harvest loss (Graham, Martin et al. 2003) . Additional economic detriment was almost certainly caused by the extreme predation of the swarming medusae on zooplankton (Graham, Martin et al. 2003). The predominant bloom-forming jellyfish genera in coastal and shelf ecosystems of the northwestern GoM are *Aurelia*, *Chrysaora*, and *Stomolophus* (Larson 1991). Despite the common occurrence of these species and others along the GoM coast, there have been few studies of jellyfish in this region, with the added complication that some of the findings are confounded by the absence of species-level identification of the animals that were sampled or studied. Morphology of medusae and polyp

stages is highly plastic, so genetic methods are required for reliable identification. For example, Chiaverano, Bayha et al. (2016) found that there are at least two species of *Aurelia* in the GoM with overlapping geographical distributions.

Medusae blooms are highly variable in their timing, location, and number of medusae involved (Purcell 2005; Heim-Ballew and Olsen 2019). This variability is observed within as well as between locations and years. Although there is evidence that jellyfish populations are increasing in some ecosystems (Claudia 2001) and may benefit from certain anthropogenic habitat perturbations (Purcell, Uye et al. 2007), on a global scale, medusae abundance has been shown to oscillate with a periodicity of approximately 20 years (Condon, Lucas et al. 2014). This oscillating nature of blooms suggests a possible intrinsic connection to large scale climate forcing (Condon, Duarte et al. 2013). In the GoM, there appears to be a positive relationship between medusae abundance and the El Nino Southern Oscillation, Atlantic Multi-decadal Oscillation, and Pacific Decadal Oscillation (Robinson and Graham 2013). These climatic forces drive weather patterns, which in turn control local and regional environmental parameters that impact polyp growth rates, strobilation timing, as well as ocean currents and wind patterns which transport medusae.

Three studies in the GoM focused on the effects of various environmental parameters on medusae abundance. In a two-decade-long dataset with spring and summer sampling, Scyphozoan jellyfish in the GoM were found to be most abundant when sea surface temperatures (SST) were higher than average in the winter, cooler than average in the spring and warmer than average in the summer and fall (Robinson and Graham 2013). Aleksa et al. (2018) used a 10 years of survey abundance data paired with oceanographic measurements from the northern GoM, finding salinity, surface currents, temperature, chlorophyll a concentrations and distance

from shore to be the most predictive factors in modeling densities of *Aurelia* sp. and *Chrysaora* sp. in coastal areas. Heil-Ballew and Olsen (2018) analyzed a 30-year dataset of jellyfish abundance in Texas bay systems for influence of temperature, salinity, and dissolved oxygen on presence of medusae. They found *Aurelia* sp. and *Chrysaora* sp. to generally have increased abundance during times of above average temperatures and salinities. Interestingly, their results suggest that *Aurelia* sp. and *Chrysaora* sp. may be capable of tolerating salinities and temperatures above those observed for the region during the 30-year monitoring period. This finding suggests that Scyphozoan jellyfish could benefit from increased average temperatures and increased salinity in coastal margins that are associated with climate change.

The previously described studies all focused on medusae, however different developmental stages of the same species may respond differently to environmental parameters and may have different tolerance limits due to differences in physiological demands of pelagic swimming and sexual reproduction (medusa) versus benthic stationary life and asexual reproduction (polyp). There have not been any studies on wild polyp populations, as polyps have not been found for most species in the GoM. But one study used *Aurelia* polyps sourced from planulae and found that hypoxic conditions promoted planulae settlement as well as polyp survival, and that polyps placed on plates at different depths in the GoM had highest survival in deeper, lower oxygen water (Miller and Graham 2012).

Jellyfish have not been thoroughly studied in many ecosystems, including the Gulf of Mexico. Many questions remain regarding the species present, population locations, trends in abundance, and response to climate change. The objective of this dissertation is to address these unknowns in the Gulf of Mexico. In Chapter 2, we used laboratory experiments to determine the temperature and salinity tolerance limits for three species of *Aurelia*, two of which are from the

GoM, and a third species, *Aurelia coerulea*, that has been translocated to numerous locations around the globe. We utilized molecular barcoding to identify polyps at the species level. Chapter 2 has been published in Frontiers in Marine Science (https://doi.org/10.3389/fmars.2020.00093) and is reprinted here with permission. In Chapter 3, we paired the tolerance limits determined in Chapter 2 with publicly available environmental datasets to predict and map the suitable habitats and distributions for the two GoM *Aurelia* species. In this Chapter we also show one of these two species to be new to science and discuss its phylogenetic relationship to all other *Aurelia* species in a global phylogeny using multiple molecular markers. Chapter 3 has been submitted for publication to Limnology and Oceanography on April 28th, 2021. In Chapter 4, we analyzed a 35-year long medusae abundance dataset to determine trends in medusae abundance, presence, seasonal timing, and temporal differences between bay and GoM regions for three bloom-forming Scyphozoan genera, namely, *Stomolophus*, *Chrysaora*, and *Aurelia*. A manuscript of Chapter 4 is currently in preparation to be submitted for publication.

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2. INSIGHTS ON BLOOM FORMING JELLYFISH (CLASS: SCYPHOZOA) IN THE GULF OF MEXICO: ENVIRONMENTAL TOLERANCE RANGES AND LIMITS SUGGEST DIFFERENCES IN HABITAT PREFERENCE AND RESISTANCE TO CLIMATE CHANGE AMONG CONGENERS[*]

2.1 Introduction

Jellyfish are important components of marine foodwebs. They feed on zooplankton and fish larvae (Möller 1984; Purcell, Purcell et al. 2000; Riascos, Villegas et al. 2014) and are food for a variety of marine animals such as penguins, turtles, and tuna (Hays, Doyle et al. 2018). Large aggregations of jellyfish, also known as "blooms," are associated with numerous negative socio-economic impacts. Jellyfish clog fishing nets (Nagata, Haddad et al. 2009), reduce catch quality (Quiñones, Monroy et al. 2013), obstruct power plant cooling intakes (Abdul Azis, Al-Tisan et al. 2000), and sting beachgoers (De Donno, Idolo et al. 2014). Blooms also cause problems for aquaculture by fouling net pens and jellyfish nematocyst-rich mucus is responsible for fish gill disorders (Purcell, Baxter et al. 2013).

In the Gulf of Mexico (GoM), problematic jellyfish blooms in coastal areas are often caused by medusae of the Class Scyphozoa, Phylum Cnidaria. Scyphozoan jellyfish have a multi-modal lifecycle (Ceh, Gonzalez et al. 2015) where the perennial benthic polyp produces seasonal jellyfish. Young medusae (ephyra) are produced by polyps via an asexual transverse fission process called "strobilation". In the GoM, there are 20 reported species of Scyphozoa,

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representing three orders and eleven families (Segura-Puertas et al. 2009). The predominant bloom-forming jellyfish genera in coastal and shelf ecosystems are *Aurelia*, *Chrysaora*, and *Stomolophus* (Larson 1991), which bloom mostly in the summer months (Graham 2001; Robinson and Graham 2013).

Polyps have a key role in maintaining and expanding Scyphozoan populations (Lucas, Graham et al. 2012). Each polyp releases multiple medusae per strobilation event. Medusae production is controlled by the number of strobilating polyps and the rate and duration of jellyfish release (Lucas, Graham et al. 2012), therefore the size of jellyfish blooms is in part determined by the size of the polyp population. Polyps also reproduce asexually, increasing their benthic population size and thus contributing to the magnitude of jellyfish blooms (Lucas, Graham et al. 2012).

Current knowledge on the location, size, and dynamics of natural polyps in the GoM is lacking. Polyps are tiny, and found in sheltered, poorly visible places, making detection difficult. Polyps are known to inhabit hard substrates including biofouling benthic organisms, floating platforms and manmade structures (Duarte, Pitt et al. 2013). Most of the GoM has a soft sandy or muddy bottom, so settlement surfaces are likely limited. However, despite the conspicuous blooms, polyps of even the most common Scyphozoan species have not been found in the GoM. The inability to locate polyp populations in nature hinders the study of jellyfish population dynamics and blooms. For example, triggers of strobilation and jellyfish production cannot be studied *in situ*, and the geographic origins of jellyfish blooms are unknown. Moreover, without knowledge of the current geographical ranges of polyp populations, it is difficult to predict how jellyfish will respond to climate change.

Scyphozoans' response to climate change is not well understood. Medusae presence and abundance in ecosystems is generally highly variable. The timing, location, and number of individuals observed can vary significantly within and between years and locations (Purcell 2005; Heim-Ballew and Olsen 2019). The variability in the frequency and magnitude of jellyfish blooms is due in part to global multi-decadal climate oscillations (Condon, Lucas et al. 2014). However, evidence from some ecosystems suggests that anthropogenic perturbations to ecosystems may facilitate bloom-formation (Purcell, Uye et al. 2007; Purcell 2011). Jellyfish have been shown to increase in abundance in heavily fished ecosystems (Lynam, Gibbons et al. 2006) in areas with benthic hypoxia (Shoji, Kudoh et al. 2010; Miller and Graham 2012) and in areas experiencing eutrophication (Purcell, Malej et al. 1999; Haraldsson, Tönnesson et al. 2012).

In the GoM, climate change is expected to affect temperatures, precipitation patterns, tropical storm activity, and sea levels (Biasutti, Sobel et al. 2012). Bottom temperature increase may impact benthic polyps, their survival capabilities and their strobilation rates, thus influencing bloom magnitude and frequency. Bottom-water temperatures have increased 2°C over a 30 year period on the northern GoM continental shelf (Turner, Rabalais et al. 2017), which is 1.9 times faster than the local increase in air temperatures during the summer months and 6.4 times faster than the global annual sea temperature increase (Turner, Rabalais et al. 2017). Also, the average temperature of GoM water is projected to increase by 4° C by the end of the century (Muhling, Lee et al. 2011; Biasutti, Sobel et al. 2012). How this increase in temperature will affect Scyphozoan populations and bloom frequency is unclear. It has been shown that temperature affects growth rate, asexual reproduction, and strobilation of polyps of *Aurelia* spp. (Purcell 2007; Willcox, Moltschaniwskyj et al. 2007; Hubot, Lucas et al. 2017).

Thermal tolerance limits constrain the biogeographical range where Scyphozoan species can survive. The ability to tolerate regional or local thermal conditions may also impact the potential for a species to become an exotic invader. The invasive Scyphozoan *Aurelia coerulea*, for example, has so far invaded habitats that possess similar seasonal maxima and minima to its native latitudinal range of 30°N to 45°N (Dawson, Gupta et al. 2005; Scorrano, Aglieri et al. 2017).

Salinity is another important environmental factor that can impact the development and survival of Scyphozoan jellyfish polyps (Rippingale and Kelly 1995; Purcell, White et al. 1999; Pitt and Kingsford 2003; Purcell, Hoover et al. 2009). Jellyfish outbreaks frequently occur in coastal environments that experience variable salinity, such as bays, estuaries and partially enclosed marine waters worldwide (Purcell, White et al. 1999). Furthermore, changes in precipitation are predicted to alter the salinity of coastal areas, including the GoM (Biasutti, Sobel et al. 2012), motivating studies on the salinity preferences and limits of jellyfish species. Only a few natural polyp habitats of *Aurelia* spp. have been studied (Gröndahl 1988; Purcell, Hoover et al. 2009; Malej, Kogovšek et al. 2012; Marques, Cantou et al. 2015; Hočvar, Malej et al. 2018; Marques, Darnaude et al. 2019), thus information on the diversity of salinity tolerances within the genus is limited. Many past studies were also confounded by the presence of multiple cryptic species within the *Aurelia* genus (Dawson and Martin 2001; Scorrano, Aglieri et al. 2017). Field and laboratory studies demonstrate *Aurelia* congeners have differing responses to salinity variation (Spangenberg 1964; Purcell, Hoover et al. 2009; Marques, Darnaude et al. 2019). The size of wild populations of *Aurelia coerulea* polyps appeared to be negatively impacted by high salinities especially in combination with high temperatures (Marques, Darnaude et al. 2019). Low salinity retarded growth of wild *Aurelia labiata* (Purcell, Hoover et

al. 2009), while varying the salinity within the range of local environmental fluctuation was found to have no significant effect on polyp growth in *Aurelia* sp. from Tasmania (Willcox, Moltschaniwskyj et al. 2007).

Temperature and salinity tolerance ranges, limits, and capacity for acclimatization strongly influence the distribution of marine species (Pörtner 2002; Stillman 2003; Somero 2005; Somero 2010), but are unknown for most jellyfish species. Yet, tolerance limits are crucial to understanding present jellyfish polyp distribution in the GoM and how distribution may change in climate change scenarios. In this study, using laboratory experiments, we assessed the temperature and salinity tolerance of the polyps of two species of *Aurelia* collected from the GoM and an invasive *Aurelia* species native to the South and East China Seas (Dawson, Gupta et al. 2005). Namely, we focus on *Aurelia* sp. 9 and a new *Aurelia* species reported for the first time in this paper and found offshore in the GoM, as well as *Aurelia coerulea,* an invasive species native to Japan that has invaded the Pacific coast of the USA and other locations around the world (Dawson, Gupta et al. 2005; Scorrano, Aglieri et al. 2017). Our aims are to 1) determine the range and limits of temperatures that each species can likely tolerate in nature, 2) investigate whether the three species have the same or different upper thermal limits and 3) resolve the salinity tolerance ranges and limits for each species. This study aims to identify the temperature and salinity tolerance limits of three *Aurelia* species, predict their biogeographical distribution in the GoM, and to provide insight into how jellyfish populations may fare as ocean temperatures increase.

2.2 Methods

2.2.1 Organism sources and culture establishment

Five female medusae of *Aurelia* sp. 9 were collected in Galveston Bay in October 2017. Medusae were carrying planulae. Soon after collection of the specimens, planulae were isolated and placed into 700ml containers with filtered sea water of ambient bay salinity. Planulae were transported to the Texas A&M University at Galveston Sea Life Facility, where they were pooled into a single culture and allowed to metamorphose into polyps. Approximately 50 polyps belonging to the species *A. coerulea* were provided by the Moody Gardens Aquarium and used to start cultures. A single live adult female *Aurelia* jellyfish carrying planulae was collected by dip net on July 1, 2017 during a research cruise aboard the R/V Pelican. Collection took place approximately 80 miles south of the coast of Louisiana in the Gulf of Mexico (28° 0' 0"N, -89° 4' 8"W). Instruments onboard the research vessel measured water parameters to be 37 ppt salinity and 28.8°C. Tissue from the medusa was preserved in 100% ethanol. The planulae were collected from the medusa and transported to the Texas A&M University at Galveston where they settled into polyps. Polyps of all three species were maintained at the Sea Life Facility at Texas A&M University at Galveston in aerated aquaria at a salinity of 33-35 ppt, ambient temperature of 15-23°C, minimal lighting and were fed once or twice a week with a combination of freshly hatched *Artemia salina* nauplii and algae-enriched rotifers. Seawater of appropriate salinity was made by adding Instant Ocean aquarium salt to filtered seawater of ambient bay salinity until the target salinity was reached. Water in aquaria was changed once a week. A second partial *Aurelia* medusa was collected on July 3rd, 2017 in a neuston net and preserved in 100% ethanol. Both medusae specimens were used for molecular analyses.

2.2.2 Molecular barcoding for species identification

Total genomic DNA was purified from individual polyps taken from the established polyp cultures of each species and from the tissues of two ethanol-preserved medusae samples collected aboard the R/V Pelican. Mitochondrial cytochrome c oxidase subunit I (COI) and nuclear internal transcribed spacer 1 (ITS1) were used for species-level characterization. COI was amplified using the primers LCOjf (Dawson, Gupta et al. 2005) and HCO2198 (Folmer, Black et al. 1994) using the thermal cycling protocol described by Piraino, Aglieri et al. (2014). ITS1 was amplified using the primers KMBN-8 and KMBN-84 from Chiaverano et al. (2016), using the thermal cycling protocol described by the authors. All polymerase chain reactions (PCR) were performed in a BioRad thermocycler. To check the quality and size of amplicons, PCR products were visualized on a 1.5% agarose gel stained with SYBR Safe. PCR products were purified using ExoSAP-IT™ (Applied Biosystems) or GeneJET Gel Extraction Kit (Thermo Scientific). COI amplicons were bi-directionally sequenced by the Texas A&M University Corpus Christi Genomics Core Lab using the PCR primers. Sequences were viewed and assembled in Geneious 9.1.8. To identify species, each consensus sequence was queried, using the BLASTn search algorithm, against the nucleotide collection (nr/nt) database of the National Center for Biotechnology (NCBI, http://www.ncbi.nlm.nih.gov).

2.2.3 Thermal tolerance ranges and limits

We assessed temperature tolerances using two different approaches, the Chronic Lethal Thermal Method (CLM) and the Critical Thermal Method (CTM). Both methods utilize a dynamic approach to thermal tolerance determination where temperature is gradually changed until a predefined endpoint is reached. However, the methods differ in the rate of temperature change and the endpoint used (Beitinger, Bennett et al. 2000) and therefore evaluate different

aspects of thermal tolerance (Beitinger, Bennett et al. 2000). Maximum and minimum limits in both the CTM and the CLM are determined by calculating the arithmetic mean of the endpoint temperatures among biological replicates (Vinagre et al. 2018).

The CLM utilizes a rate of temperature change that is slow enough to allow organisms to reacclimate at each temperature and uses death as the endpoint (Beitinger, Bennett et al. 2000). Temperature change rates are usually set at 1°C / day or slower (Beitinger, Bennett et al. 2000; Eme and Bennett 2009). By incorporating acclimation, the CLM has the advantage over the CTM of producing a more accurate estimate of the actual thermal limits that species can tolerate in nature (Beitinger, Bennett et al. 2000). We used the CLM approach to estimate the maximum and minimum temperatures that each *Aurelia* species can tolerate in the wild, in the forms of the Chronic Lethal Thermal Maximum (CLMax) and Chronic Lethal Thermal Minimum (CLMin) for each species. In order to acquire a more detailed understanding of how each species responds to temperature change, we monitored polyps for signs of stress at regular intervals during temperature increase and decrease during the CLM trials. We used tentacle morphology and polyp response to tactile stimuli (prodding with a metal probe) to monitor stress, and created a ranking system, that we termed "response score" (Table 2.1). This score is based on observations that under standard culture conditions, polyps respond to external stimuli with immediate muscle contractions and maintain tentacles in an extended position ready to feed. The response score was used to track each species' ability to acclimate to thermal increase or decrease and to track the onset of thermal stress leading to death.

Table 2.1 Response scores with corresponding polyp morphology and degree of tentacle and body response.

Response Score	Polyp morphology characteristics	Tentacle/body response to stimuli
5	Tentacles open as in feeding. Polyp is well-formed.	Immediate retraction, followed by re-elongation
4	Tentacles remain partially retracted. OR stomach is inverted.	Immediate retraction, no re-elongation.
3	Tentacles remain significantly retracted, shrunken or closed. OR significant morphological abnormalities present.	Greater than 1 second delay in retraction after a stimulus is applied. Retraction slow.
2	Mouth may be fixed agape. Tissue recoil is maintained.	Tentacles not responsive to touch.
1	Loss of tissue recoil.	Tentacles not responsive to touch.

The CTM is a common method for defining species' thermal tolerance limits (Bennett, Calosi et al. 2018) that has also been used to evaluate invertebrate response to climate change (Madeira, Narciso et al. 2012; Vinagre, Leal et al. 2016; Vinagre, Mendonça et al. 2018; Vinagre, Dias et al. 2019). CTM is particularly useful for more precisely distinguishing tolerances between species (Beitinger, Bennett et al. 2000) and was used in this study to resolve differences in upper thermal tolerance limits between *Aurelia* congeners. In the CTM, temperature is changed at a constant rate that is set fast enough such that acclimation does not occur until a predefined sublethal critical endpoint is reached (Cuculescu, Hyde et al. 1998; Mora and Ospina 2001; Nguyen, Morley et al. 2011; Salas, Díaz et al. 2014; Kaspari, Clay et al. 2015;

Regil, Mascaro et al. 2015; Vinagre, Leal et al. 2016; Vinagre, Mendonça et al. 2018). The critical point is generally specified as a non-lethal but incapacitating point (Lutterschmidt and Hutchison 1997). CTM rates of temperature change are set fast enough so that acclimation does not occur, but slow enough for temperature to be tracked and should be standardized to allow comparison between species (Eme and Bennett 2009; Bennett, Calosi et al. 2018). We used the commonly chosen rate of temperature change of 1°C/15 min (Bennett, Calosi et al. 2018; Vinagre, Mendonça et al. 2018; Vinagre, Dias et al. 2019).

2.2.4 Salinity tolerance ranges and limits

To estimate the salinities that *Aurelia* polyps can tolerate in the wild, we used a chronic salinity change approach similar in concept to the CLM, which we call the Chronic Lethal Salinity Method (CLSM). We tracked individual polyps over gradual increase or decrease in salinity and monitored their apparent stress level at regular intervals using response scores, until the endpoint. The response score data was used to track each species' ability to acclimate to salinity change and to track the onset of salinity stress leading to death. Death was designated as the endpoint. Slow rates of change in environmental variables allow polyps to physiologically acclimate, such that tolerance limits approximate what species would tolerate in the wild. We selected a rate of salinity change of 1 ppt per day to maximize acclimation time within practical limits for the investigators. Using this approach, salinity limits were calculated by taking the arithmetic mean of the lethal endpoint salinity among biological replicates (CLSMin and CLSMax).

2.2.5 Chronic thermal acclimation range and CLM

For each *Aurelia* species, three non-asexually reproducing polyps were placed and allowed to settle in each well of a 12-well culture plate with 7 ml of seawater at a salinity of 33 ppt. Polyps were selected from random locations within the parent culture in an effort to maximize genetic diversity and prevent selection of polyps belonging to the same clonal line. After three days, polyps were checked for attachment, and one healthy, attached polyp was retained in the dish; all others were removed. Three replicate culture plates were used for each species for a total of 36 biological replicates (polyps) per species. The experimental culture plates were placed in an incubator with the lights off. Temperature was gradually increased starting from 21°C and increasing at 1°C per day. 21°C was the average temperature of the culture conditions in the facility where long-term cultures were kept. This acclimation temperature was selected to minimize baseline physiological stress of the polyps prior to the start of chronic temperature acclimation experiments. One 12-well culture plate populated with 12 polyps/species was used as the control and maintained in an incubator at 21°C with the lights off for the duration of the experiment. Polyps were fed approximately 10 *Artemia salina* nauplii and 15 rotifers per well every third morning (every 2°C increase) for 2-3 hours. Complete water changes were performed after feeding. Water for all cultures was made using natural filtered seawater adjusted to the target salinity of 33 ppt using Instant Ocean sea salt. Water was pre-warmed in the incubators to the target temperatures before each water change. Water parameters were checked with a YSI to maintain accuracy. Temperature inside the incubators was logged using HOBO Onset temperature and light loggers. Polyps were observed under a Leica dissecting microscope every 2°C increment until their response scores reached 3, then they were observed at every 1°C increment. Polyps were scored for signs of stress according to a 5-point scale based on their tentacle morphology and response to stimuli (Table 2.1). A metal probe or

plastic pipette tip was touched to the tentacles and body of each polyp to evaluate response to stimuli (Figure 2.1). The temperature, response score of each polyp, and number of polyps in each well was recorded. Any independent child polyps or free-swimming ephyra were removed. Polyps were considered to have reached the lethal endpoint when they lost tissue integrity at a response score of 1, which was defined as the absence of recoil by the tissue upon prodding the polyp body with a probe. The experiment was then repeated, but with decreasing temperature. Temperature was gradually decreased starting from 21°C at 1°C per day. Polyps were cultured, fed, and monitored in the manner described for increasing temperature.

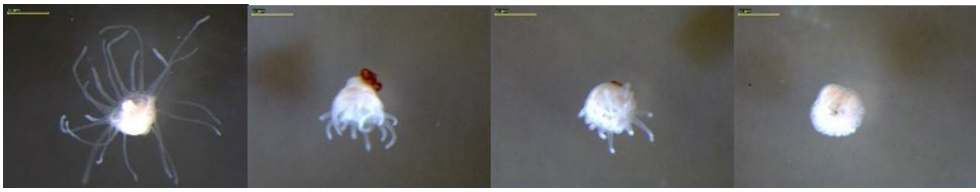

Figure 2.1 Examples of response scores of *Aurelia* sp.1 polyps. Response to stimuli is in decreasing order from left to right 5-1. A response score of 5 indicates optimal polyp response; a response score of 1 indicates compromised tissue integrity (refer to Table 1 for a complete definition of response scores).

2.2.6 Calculating chronic lethal thermal limits (CLMax, CLMin)

The CLMax for each species were determined by averaging the temperatures at which each polyp reached the lethal endpoint using the equation:

$$CLMax_{species}=\Sigma(T_{endpoint})/n$$

Where $T_{end\text{-}point}$ is the temperature at which polyps had a response score of 1 during the CLMax trials, and n is the sample size. The CLMin for each species was calculated using the same equation but using data from the CLMin trials.

2.2.7 Critical thermal maximum (CTMax)

50 to 100 healthy polyps of each *Aurelia* species were transferred from different locations in the master cultures to 700 ml containers with 33 ppt salinity and aeration. Each species was placed in an incubator and kept at 21°C for 2 weeks for acclimation. 21°C was used as the acclimation temperature to approximate the average winter sea temperature along the shelf of the northern GoM (Boyer 2011) where natural polyp populations may be located. Polyps were fed *ad libitum* with newly hatched *Artemia salina* nauplii twice a week for 24 hours. Lights were off in the incubator. Water was changed in the containers on the day following feeding. Polyps were starved for 24 hours before CTMax experiments. Five polyps per well and 10 polyps per species were placed into 24.1 mm diameter propylene wells with 420 µm mesh bottoms (TedPella). Wells were inserted into foam so that they would float and placed into a thermostable water bath with vigorous aeration. Salinity and temperature parameters were maintained the same as during acclimation. Polyps were allowed to settle for 24 hours at 21°C. Temperature was increased at a rate of 1°C/ 15 min. Each polyp's response to stimuli was evaluated at every 1°C by touching the tentacles with a metal probe. If no response was observed, the polyp body was touched with the metal probe. Response to stimuli was observed under a Leica dissecting microscope due to polyps' small size. Water bath temperature was measured using a digital thermometer immediately prior to removing the polyps from the water bath for observation. Individual wells were carefully scooped with the surrounding water from the water bath using a plastic container and placed under the microscope. When no response to the stimuli from either tentacles or polyp body was observed, the polyp was considered to have reached its endpoint and the temperature of the water bath was recorded as the thermal maximum of the polyp. Salinity was maintained at 33 ppt for the duration of the experiment, while dissolved oxygen concentration and pH were both monitored to ensure consistent levels.

2.2.8 Calculating critical thermal maximum (CTMax)

The CTMax for each species was calculated by averaging the temperatures at which polyps lost response to stimuli using the equation:

$$CTMax_{species}=\Sigma(T_{end\text{-}point})/n$$

Where $T_{end\text{-}point}$ is the temperature where polyps lost response to stimuli and n is the sample size. Intraspecific variability of the CTMax was determined by calculating the coefficient of variation given as a percentage for each species, using the equation: (standard deviation/mean)*100.

2.2.9 Chronic salinity acclimation range CLSMin

For each *Aurelia* species, three non-asexually reproducing polyps were placed in each well of a 12-well culture plate with 7 ml of seawater at a salinity of 33 ppt and allowed to attach. After three days, polyps were checked for attachment. One healthy attached polyp was retained in the dish, while the others were removed. Two replicate culture plates were used for each species, with a total of 24 polyps per species. Culture plates were placed in incubators at 19°C, which is the winter average ocean temperature of the northern GoM coast (Boyer 2011). The specific temperature approximates winter thermal conditions of estuaries, bays, and the coastline of the region, where potential habitats for coastal polyps are likely located. Lights were off in the incubators. Salinity was increased by 1 ppt a day, by completing a water change with water of the appropriate salinity. Eight polyps of each species were used for the control and maintained in an incubator at 19°C and salinity of 33 ppt. Water for all cultures was made using natural filtered seawater adjusted to the target salinities using Instant Ocean sea salt. Water was prewarmed in

the incubator to 19°C and water parameters were verified with a YSI prior to water changes. Temperature inside the incubator was logged using HOBO Onset temperature and light loggers. Polyps were fed approximately 10 *Artemia salina* nauplii and 15 rotifers per well once a week for 2-3 hours. Complete water changes were performed after feeding. Polyps were observed for data collection under a Leica dissecting microscope every 2 ppt until their response scores reached 3, then they were observed at every 1 ppt increment. Polyps were scored for visible stress level according to the 5-point response score scale defined above (Table 2.1). A metal probe or plastic pipette tip was used to touch tentacles and body of each polyp to evaluate response to stimuli. The salinity, response score of each polyp, and number of polyps in each well were recorded. Any independent child polyps or free-swimming ephyra were removed. Polyps were considered deceased when they lost tissue integrity, defined as the absence of recoil by the tissue upon prodding the polyp body with a probe, at a response score of 1. The experiment continued until all polyps reached a response score of 1. The experiment was then repeated, but with decreasing salinity. Salinity was gradually decreased by 1 ppt per day starting from 33 ppt. Water of target salinity was made by adjusting natural filtered seawater to target salinities using deionized water. Water quality monitoring, feedings and data collection were performed as described for increasing salinity.

2.2.10 Calculating chronic lethal salinity limits (CLSMin)

The CLSMax could not be determined because polyps' tolerance exceeded the range of the YSI (42ppt). The CLSMin for each species was calculated by averaging the lethal endpoint salinities for each species from the decreasing salinity trial using the equation:

$$CLSMin_{species} = \Sigma(S_{end\text{-}point})/n$$

Where $S_{end\text{-}point}$ is the salinity where polyps lost tissue integrity at a response score of 1, and n is the sample size.

2.3 Results

2.3.1 Identification through molecular barcoding

COI sequences were approximately 650 bp in length, which is a standard length for *Aurelia* COI. ITS1 sequences were around 600 bp. Polyps from coastal medusae collected in Galveston Bay were confirmed to be *Aurelia* sp. 9 according to COI with a percent identity of 99.83% and an E value of 0. Polyps that had been provided by the Moody Gardens Aquarium belonged to *Aurelia coerulea* based on COI with a percent identity 100% of and E value of 0. For the offshore *Aurelia* species, top BLASTn matches for mitochondrial COI were to *Aurelia relicta* (Accession number KX691571), with a percent identity of 91.33% and an E value of 0. The top match for nuclear ITS1 was *Aureli*a sp. Incheon with a percent identity of 84.70% and E value of 1e-158. *Aurelia* sp.5 (*Aurelia relicta*) was also among the top 5 database matches and had a higher percent identity of 88.65% and an E value of 5e-122. Since we are unable to identify the offshore *Aurelia* to any known *Aurelia* species, we refer to this strain as *Aurelia* sp. new. A multigene phylogenetic analysis to clarify the phylogenetic position of *A.* sp. new within the genus is in progress, but outside the scope of this paper.

2.3.2 Chronic thermal acclimation limits (CLMin, CLMax)

Polyps of *Aurelia coerulea* maintained an average response score of 5 from 8°C to 27°C. At temperatures above 27°C, the response scores decreased from 5 to 1 over a span of 3°C. Below 8°C, the response scores of *A. coerulea* polyps decreased to 3 at 5°C. *A. coerulea* polyps did not show visible changes in stress level from 5°C down to 0°C, but response scores fell from

3 to 1 when temperatures decreased from 0°C to -2°C. Loss of response to stimuli occurred at about -1°C (Figure 2.2). For *Aurelia coerulea* polyps, the CLMax was 30.9°C and the CLMin was -2°C; all polyps of this species reached the endpoint at the same temperature during the experiment with decreasing temperature (Table 2.2.2). The thermal range for *A. coerulea* spanned 32.9°C.

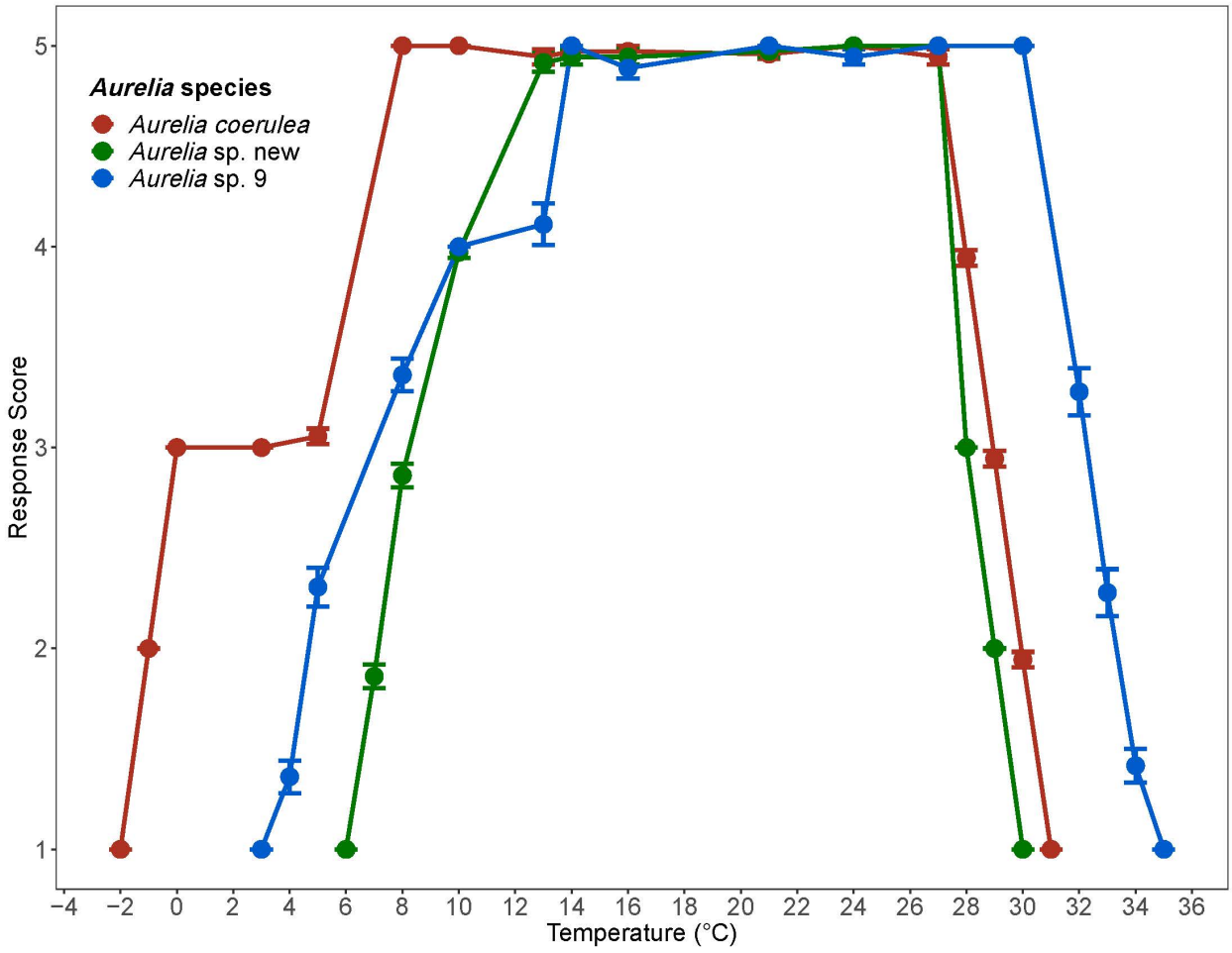

Figure 2.2 Response scores of *Aurelia* species in during 1°C/day temperature change. Response scores 1-5 are described in Table 2.1.1. Temperature is in degrees Celsius (°C). Error bars display standard error. Colors correspond to *Aurelia* species: red is *A. coerulea*, green is *A.* sp. new, and blue is *A.* sp. 9.

Table 2.2 Chronic lethal temperature limits for three *Aurelia* species: Chronic Lethal Minimum (CLMin) and Chronic Lethal Maximum (CLMax) values are in °C. Sample size, standard deviations, and range (CTMax-CTMin) are shown.

Species	CLMin (°C)	n	SD	CLMax (°C)	n	SD	Range (°C)
Aurelia sp. 9	3.1	36	0.5	34.7	36	0.7	31.6
Aurelia sp. new	6	36	0	30	36	0	24
Aurelia coerulea	-2	36	0	30.9	36	0.2	32.9

Polyps of *Aurelia* sp. new maintained an average response score of 5 from 13°C to 27°C. At temperatures above 27°C, the response scores decreased from 5 to 1 over a span of 2°C. Below 13°C, response scores decreased to 4 by 10°C, and steadily to 1 at 6°C. Loss of response to stimuli occurred at about 7°C (Figure 2.2). For *Aurelia* sp. new polyps, the CLMax was 30°C and the CLMin was 6°C; all polyps of this species were observed to reach their endpoints at the same temperatures (Table 2.2). The thermal range for *A.* sp. new spanned 24°C.

Polyps of *Aurelia* sp. 9 maintained an average response score of 5 from 14°C to 30°C. At temperatures above 30°C, polyps' response scores decreased from 5 to 1 over a span of 5°C. Below 14°C, response scores decreased to 4 by 10°C, and to 1 at 3°C. Loss of response to stimuli occurred at about 5°C (Figure 2.2). For *Aurelia* sp. 9 polyps, the CLMax was 34.7°C and the CLMin was 3.1°C (Table 2.2). The thermal range for *A.* sp. 9 spanned 31.6°C.

Polyps in the control groups maintained response scores of 4 or above throughout the experiment. Results for the control polyps can be found in Supplementary Materials (S1).

2.3.3 Critical thermal maximum (CTMax)

Polyps of *Aurelia* sp. 9 had the highest thermal tolerance with a CTMax of 37.2°C. The first polyp lost response to stimuli at 34°C, but most polyps lost their response to stimuli above 37°C with the most tolerant polyps retaining response until 38.4°C. Intraspecific variation for *Aurelia* sp. 9 was 4.0%. *Aurelia* sp. new polyps had a CTMax of 32.1°C with 0% intraspecific variation (all lost response to stimuli at the same time and at the same temperature). *Aurelia coerulea* polyps were the least tolerant to high temperatures with a CTMax of 29.6°C. The first polyps lost response to stimuli at 28.7°C. One polyp tolerated temperatures up to 31.8°C. Intraspecific variability for *Aurelia coerulea* was 4.1%. Results for CTMax experiments are summarized in Table 2.3.

Table 2.3 Critical thermal maximum (CTMax) values for three *Aurelia* species. Sample size, standard deviations, and intraspecific variability are shown. CTMax values are in °C. Intraspecific variability is shown as a percentage.

Species	CTMax (°C)	n	SD	Intraspecific variability (%)
Aurelia sp. 9	37.2	8	1.5	4.1
Aurelia sp. new	32.1	6	0	0
Aurelia coerulea	29.6	6	1.2	4.0

2.3.4 Salinity acclimation range and CLSMin

Aurelia coerulea polyps maintained an average response score of 5 down to about 12 ppt, with response scores falling below 4 at approximately 11 ppt. Polyps lost response to stimuli at about 7 ppt and tissue integrity at 6 ppt. CLSMin for *Aurelia coerulea* was 6.2 ppt. *Aurelia* sp. new maintained response scores of 5, with no signs of visible stress, down to a salinity of 18 ppt, and response scores of 4 or above to 15 ppt. Polyps lost response to stimuli at about 12 ppt and tissue integrity at 10 ppt. The CLSMin for *Aurelia* sp. new was 10 ppt. *Aurelia* sp. 9 polyps maintained response scores of 5 at 10 ppt, and response scores of 4 or above to approximately 7 ppt. Polyps lost response to stimuli at about 4 ppt and lost tissue integrity at 2 ppt. The CLSMin for *Aurelia* sp. 9 was 2.2 ppt. CLSMin values are summarized in Table 2.4. In the chronic salinity acclimation experiment with increasing salinities, polyps of all three species maintained optimal response scores to a salinity of 42 ppt, which was the measurement limit for the YSI salinity meter. The upper acclimation limit for salinity and the CLSMax could not be determined but exceeds ecologically relevant values for the GoM. Polyps in the control groups maintained average response scores of 4 or above for the duration of the experiment. Results for the control polyps can be found in Supplementary Materials (S1).

Table 2.4 Chronic lethal salinity minimum (CLSMin) for three *Aurelia* species. CLSMin values are in are in parts per thousand (ppt). Sample size and standard deviations are shown.

Species	CLSMin (ppt)	n	SD
Aurelia sp. 9	2.2	25	0.6
Aurelia sp. new	10	24	0
Aurelia coerulea	6.2	22	0.9

2.4 Discussion

Environmental changes associated with climate change drive species range shifts. General trends across the globe reveal that species respond to warming ocean temperatures by shifting pole-ward (Thomas 2010). However, in the GoM, the North American continent forms a physical barrier limiting species' northward movement (Stillman 2003; Somero 2010). Due to their complex lifecycle, the success of jellyfish species depends on the ability of the polyp, ephyra, and jellyfish life-stages to tolerate future conditions. Since it is the polyp stage that is responsible for maintaining and expanding jellyfish populations between seasons and years, species' success is influenced by the ability of the polyp to tolerate temperature increases.

In the present study, we barcoded three species of the common bloom-forming *Aurelia* jellyfish, then determined the thermal limits for the benthic stage of each species. To estimate the temperature limits that polyps can tolerate in nature, polyps were exposed to a chronic temperature decrease or increase and tracked until death occurred. To track stress response, polyps were evaluated at each incremental temperature increase and scored for signs of stress using a "response score". Chronic Lethal Thermal Limits approximated from "response score" data were compared to ocean temperature data to infer geographical ranges for polyp populations of species in the GoM. Then, to more closely resolve any differences in upper thermal tolerance limits among the three species, we applied the widely used Critical Thermal Method. Finally, as polyps of two of the three species used in this study have not yet been found in nature, we assessed their salinity tolerance using a gradual decrease or increase in salinity to determine each species' Chronic Lethal Salinity Minimum. The lower salinity tolerance of each species was used to infer possible habitat locations in coastal and offshore areas. Because of the difficulties in

finding polyps in the wild, we acknowledge that we could not control or assess patterns of relatedness within the polyps used in our experiments.

The offshore *Aurelia* species may be a new species as it was not represented among GenBank COI or ITS1 sequences. Additional molecular and morphological analyses are required to confirm the identity of *Aurelia* sp. new as a distinct species. Only two *Aurelia* species have been reported in the GoM: *A.* sp. 9 and *A.* c.f. sp. 2 (Chiaverano, Bayha et al. 2016).

Aurelia coerulea, *Aurelia* sp. new, and *Aurelia* sp. 9 possess distinct thermal tolerance ranges and thermal limits (Table 2.2). More specifically, the thermal ranges of the invasive species *Aurelia coerulea* and *Aurelia* sp. 9 are of similar size, differing only by 1.3°C based on lethal limits. The response scores of both species show a similar trend, as both maintained response scores of 5 over a 21°C span (Figure 2.2). However, *Aurelia coerulea* has a lower thermal tolerance with its experimental thermal tolerance range shifted by about 4°C relative to that of *Aurelia* sp. 9, reflecting a preference for cooler temperature. This is also reflected in the climate of its native geographical origin in the South and East China Seas. The thermal range of *Aurelia* sp. new is approximately 8°C narrower than that of *A. coerulea* and *A.* sp. 9 (Figure 2.2), suggesting that polyps of this species may prefer thermally stable conditions. CLMax values and visible stress as measured by response scores suggest that *A. coerulea* and *A. sp. new* may have similar upper thermal limits. Control polyps that were kept at a constant temperature but otherwise treated in an identical manner, maintained high response scores for the duration of trials, indicating that the observed lethal limits were due to thermal stress.

Unlike *Aurelia* sp. 9 and sp. new, polyps of *Aurelia coerulea* have been found in the wild (Ishii and Katsukoshi 2010; Marques, Darnaude et al. 2019). Interestingly, the experimentally resolved temperature range where polyps of *Aurelia coerulea* maintained

minimal signs of stress (response scores of 4 or above) determined in this study aligns well with published reports of the habitat temperatures for this species derived from field surveys of wild populations. According to our experiments, *Aurelia coerulea* polyps experienced minimal stress from 6.5°C to 27°C, whereas natural polyp habitats in the Thau Lagoon (northwestern Mediterranean) range from 7.6°C to 25.8°C (Marques, Darnaude et al. 2019), 6°C to 30°C for polyps in Lake Verano, Italy (Belmonte, Scirocco et al. 2011), and 9°C to 29°C for *Aurelia coerulea* in Tokyo Bay, Japan (Ishii and Katsukoshi 2010). These are the minimum and maximum recorded water temperatures of wild populations surveyed over the span of approximately one year (Ishii and Katsukoshi 2010; Marques, Darnaude et al. 2019).

The thermal range of *Aurelia coerulea* suggests that it is unlikely to develop a resident population within the coastal GoM. Winter low temperatures along the northwestern and northeastern coasts of the GoM average 13–20°C (averaged from https://www.nodc.noaa.gov/dsdt/cwtg/all_meanT.html), which is well above the lower thermal tolerance limit of -2°C (CLMin) for *Aurelia coerulea.* However, with a CLMax of 30.9°C, this species may be restricted by the summer water temperatures along parts of the northwestern and northeastern GoM coasts, which average 28–31°C (averaged from https://www.nodc.noaa.gov/dsdt/cwtg/all_meanT.html). Summer average temperatures in the coastal GoM are thus likely to be lethal to *Aurelia coerulea* whose response scores indicated damaging levels of thermal stress at temperatures above 27°C.

Aurelia sp. 9, the common bloom-forming *Aurelia* species in the GoM, had a thermal tolerance range of 3.1°C to 34.7°C based on lethal limits and displayed minimal signs of stress between 10°C and 31°C. Monthly annual averages for coastal western and eastern GoM range from 13°C to 31°C suggesting that, from a thermal perspective, the conditions of bays, marinas,

and coastlines are suitable habitats for *Aurelia* sp. 9 polyps. Out of the three species tested, *Aurelia* sp. 9 was tolerant of temperatures at least 3°C higher than the other two species. *Aurelia* sp. new had the narrowest thermal range of 24°C, and displayed signs of stress outside of the range of 10°C to 27°C. The upper lethal thermal limit of *Aurelia* sp. new (CLMax= 30°C) suggests that it may also not be able to tolerate the summer high temperature observed along the northern GoM coast. However, the temperatures in the deeper waters along the continental shelf in the GoM where the medusa of this species was collected, are generally lower than the coastal summer averages, so it is possible that *Aurelia* sp. new is restricted to offshore areas.

We used the Critical Thermal Method to identify CTMax for each species to resolve the relative upper thermal limits of the *Aurelia* congeners (Table 2.3). CTMax values confirm that *Aurelia* sp. 9 is more tolerant of high temperatures than both *Aurelia coerulea* and *Aurelia* sp. new. *A. coerulea* had the lowest CTMax among the congeners, indicating this species to be least tolerant of high temperatures, which is reasonable considering the generally lower temperatures of its native range as compared to the GoM. Fast rates of warming, such as the 1°C/15 min rate used in this study to determine CTMax, may overestimate the actual upper thermal tolerance limits of organisms in nature (Peck, Clark et al. 2009). CTMax values are usually greater than CLMax values, because slower rates of warming in the chronic experiment allow more time at each temperature for lethal physiological effects to accumulate and set in. This relationship was observed for *Aurelia* sp. 9 and *Aurelia* sp. new, where CTMax values were about 2°C greater than CLMax values, but not for *Aurelia coerulea*, where the CTMax was approximately 1°C lower than the CLMax (Figure 2.3). The lower CTMax may indicate that *Aurelia coerulea* polyps are sensitive to rapid temperature changes.

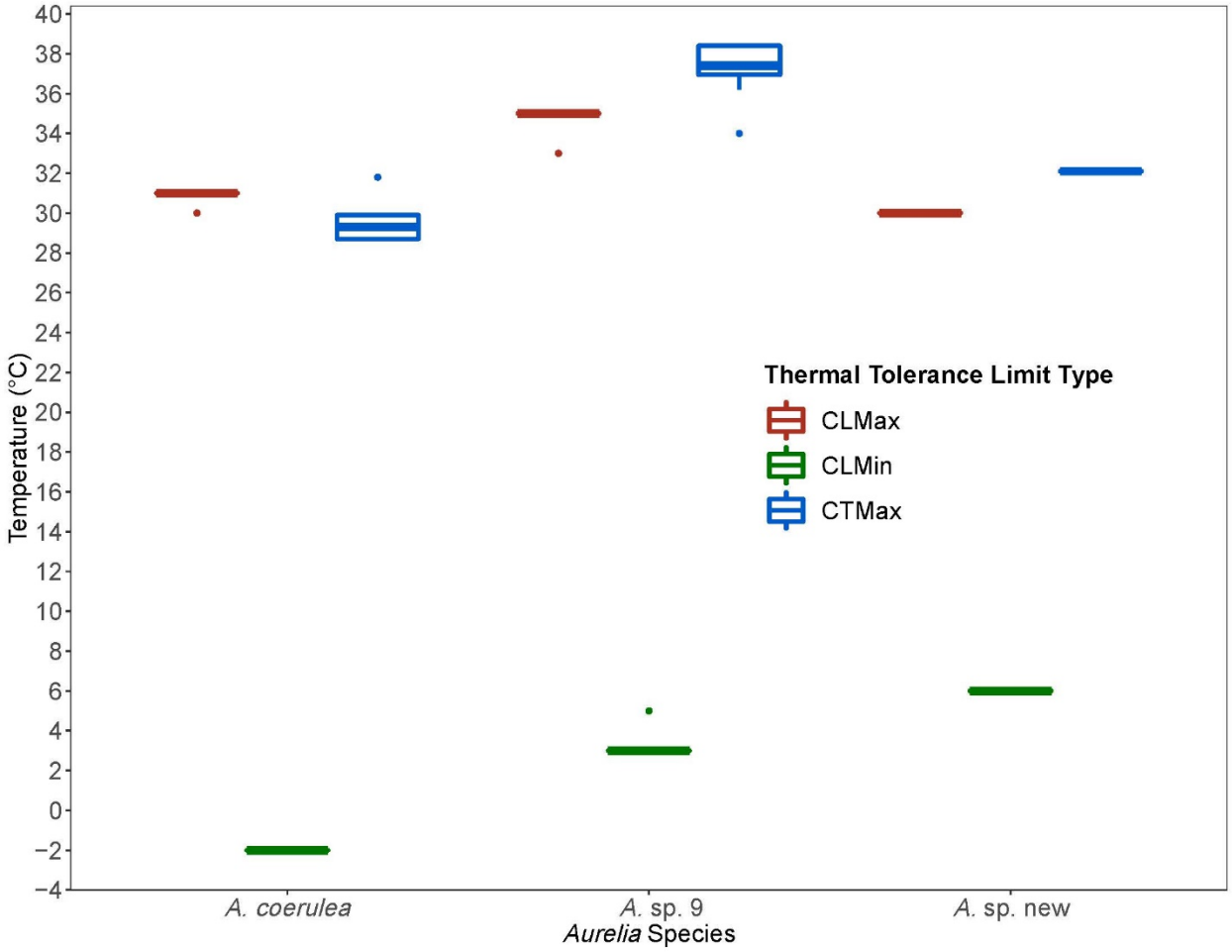

Figure 2.3 Comparison of thermal tolerance values from Chronic Lethal Maximum and Minimum and Critical Thermal Maximum experiments for *A. coerulea, A.*sp. new, and *A.* sp. 9. Thermal limits are in °C. Colors designate thermal limit type: red is Chronic Lethal Maximum (CLMax), green is Chronic Lethal Minimum (CLMin), and blue is Critical Thermal Maximum (CTMax). Boxplots displays the median with the lower and upper hinges corresponding to the 25th and 75th percentiles and whiskers extending to 1.5 times the inter-quartile range. Single points indicate outliers.

Chronic salinity experiments show that polyps of all three species are generally able to withstand a wide range of salinities and are not sensitive to hypersaline conditions (Figure 2.4). Of the three species, *Aurelia* sp. 9, had the lowest CLSMin value of 2.2 ppt. Additionally, polyps of this species had no visible signs of stress during the 1 ppt/ day salinity decrease until salinity dropped below 10 ppt, suggesting that *A.* sp. 9 polyps are tolerant of low salinities and salinity

change. When considered together with the high thermal tolerance of this species, salinity tolerance results suggest that coastal areas of the GoM as well as some bays and estuaries in this region, are suitable habitats for polyps of *A.* sp. 9. *Aurelia coerulea* polyps had a CLSMin of 6.2 ppt with no visible signs of stress until salinity decreased below 14 ppt. Most wild populations of *A. coerulea* have been recorded at salinities above 20 ppt (Belmonte, Scirocco et al. 2011; Marques, Darnaude et al. 2019). *Aurelia* sp. new had a CLSMin of 10 ppt and was therefore the least tolerant of low salinities among the three species. Visible signs of stress were observed for polyps of this species at salinities below 18 ppt, suggesting possible low resilience to salinity change (Table 2.4). Compared to the two coastal species, *Aurelia* sp. new has a considerably more limited ability to withstand both temperature and salinity change, indicating that *A.* sp. new is an offshore species in the GoM.

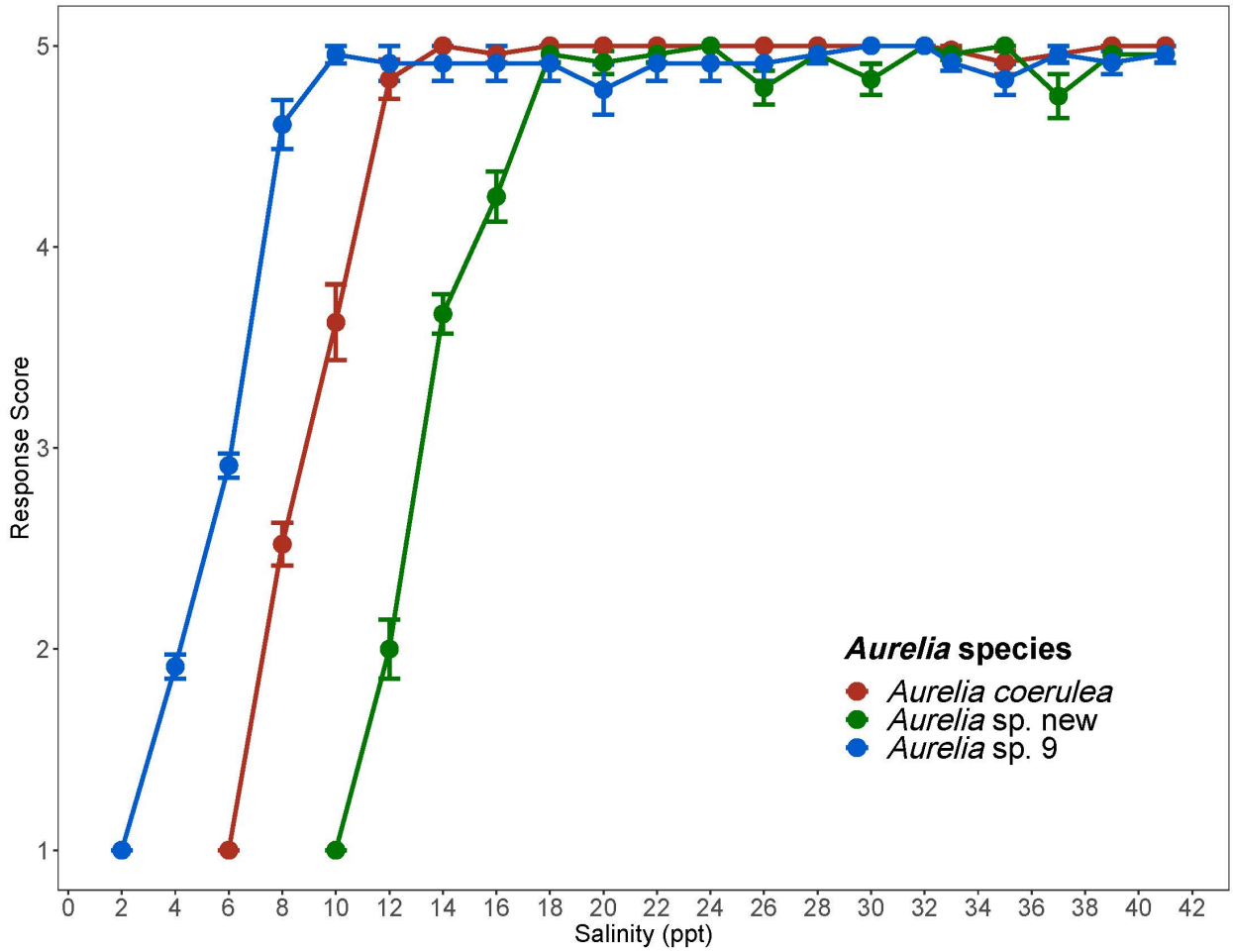

Figure 2.4 Response scores of *Aurelia* species in response to 1 ppt/day salinity change. Response scores 1-5 are described in Table 1. Salinity is in part per thousand (ppt). Error bars display standard error. Colors correspond to *Aurelia* species: red is *A. coerulea*, green is *A.* sp. new, and blue is *A.* sp. 9.

The temperature and salinity tolerance ranges and limits of *Aurelia* congeners can be used to predict species response to future conditions. Ocean surface temperatures are projected to increase by up to 4°C by the year 2100 (Biasutti, Sobel et al. 2012), with benthic habitats becoming even warmer (Turner, Rabalais et al. 2017). A temperature increase of this magnitude may deter *Aurelia coerulea* from invading or becoming established in the coastal GoM, as the upper thermal limit of this species is already at or below current summer average water temperatures. The temperature highs in the South and China Seas (26-29°C) suggest that *Aurelia*

coerulea is currently living fairly close to its thermal limits in its native range, and may be especially at risk in enclosed habitats, which it is known to inhabit. Due to its similar upper thermal limits, *Aurelia* sp. new may also be negatively impacted overall. The Chronic Lethal Thermal upper limit (34.7°C) and Critical Thermal Maximum (37.2°C) of *Aurelia* sp. 9 suggest that it can withstand increasing environmental temperatures. However, an increase of 4°C would bring water temperatures near the upper thermal limit for this species, which would potentially negatively impact *Aurelia* sp. 9 in the warmest extremes of its biogeographical range. Temperature increases are also expected to be greatest in coastal areas (Biasutti, Sobel et al. 2012), indicating that coastal *Aurelia* sp. 9 populations would not benefit and may decline due to habitat temperature increase by the next century.

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3. ECOLOGY OF SCYPHOZOAN JELLYFISH (PHYLUM CNIDARIA) IN THE GULF OF MEXICO: HABITAT SUITABILITY, DISTRIBUTION MODELING, AND A NEW SPECIES

3.1 Background

In the Gulf of Mexico (GoM), jellyfish are common, and blooms occur each year. *Aurelia* is a genus of bloom-forming Scyphozoan jellyfish with worldwide distribution. Like most Scyphozoans, *Aurelia* are meroplanktonic with a multi-modal lifecycle (Ceh, Gonzalez et al. 2015) where a benthic polyp periodically undergoes asexual fission, termed "strobilation", to produce multiple young free-swimming medusae.

Morphology of *Aurelia* medusae can be variable with few reliable characteristics that distinguish species. Still, the application of molecular techniques based on mitochondrial and nuclear markers has uncovered at least 16 phylogenetic species (Dawson, Gupta et al. 2005). Of these, only six species have been formally described: *A. aurita*, *A. limbata, A. labiata*, *A. coerulea*, *A. solida*, and *A. relicta*, and several being identified as *Aurelia* sp. 1 to *Aurelia* sp. 16. Despite being arguably the best studied Scyphozoan genus, new species of *Aurelia* are still being discovered, with the most recent being the identification of two probable new species from the western coast of Mexico and Central America (*A*. sp. 12 and *A*. sp. 14), one species from the Caribbean (*A*. sp. 15), one from the South Western Pacific (*A*. sp. 16) (Gómez Daglio and Dawson 2017), and a potential new species from the GoM, *A*. c.f. sp. 2 (Chiaverano, Bayha et al. 2016). So far, only two *Aurelia* species have been identified in the GoM, namely *Aurelia*

sp.9 and *Aurelia* c.f. sp. 2. These two species have morphologically distinct medusae (differences in the polyps were not examined) as well as distinct evolutionary histories (Chiaverano, Bayha et al. 2016).

Both in the GoM and worldwide, while medusae are frequently observed in the wild, polyps are small, inconspicuous, and rarely found. Polyps are, however, essential for maintaining and expanding the jellyfish population due to their longevity and ability to reproduce asexually (Lucas, Graham et al. 2012). Also, because they produce medusae, polyp locations serve as the geographic origins and sources of jellyfish blooms. Our understanding of bloom formation is thus greatly limited by the lack of information on wild polyp habitats and their geographical distribution. Studies from various ecosystems show that *Aurelia* polyps inhabit natural and artificial hard substrates such as bivalve shells (Miyake, Terazaki et al. 2002), polychaete tubes (Miyake, Terazaki et al. 2002), floating piers (Toyokawa, Aoki et al. 2011), pylons (Marques, Cantou et al. 2015), and metal structures (Di Camillo, Betti et al. 2010). Hard natural substrates are generally rare in the GoM, characterized mainly by a soft, muddy bottom not suitable for polyp survival (Burke 1976). However, the GoM has 6,690 oil rigs and artificial reefs (Schulze, Erdner et al. 2020), which provide abundant potential substrates for benthic organisms, including Scyphozoan polyps, in locations where surfaces are otherwise scarce. Moreover, the presence of man-made structures has been shown to be correlated with an increase in local medusae abundance (Purcell, Uye et al. 2007; Janßen, Augustin et al. 2013; Makabe, Furukawa et al. 2014), and in the magnitude of jellyfish blooms (Duarte, Pitt et al. 2013). In the GoM, coral reefs and artificial reefs and

oil and gas platforms may be suitable habitats for polyps as long as they are located in environmental conditions within the species' tolerance range. Of all environmental parameters, polyp survival has been shown to be affected predominately by water temperature (Lucas, Graham et al. 2012) and depends on a species' thermal tolerance. Salinity tolerance is also likely to be important in coastal and inshore areas where freshwater input lowers environmental salinity levels. *Aurelia* polyps are otherwise tolerant of environmental stressors such as low dissolved oxygen (Ishii, Ohba et al. 2008; Miller and Graham 2012), pollutants (Lucas and Horton 2014), and acidification (Winans and Purcell 2010). For marine ectotherms, thermal tolerance limits correspond to their latitudinal range and can therefore be used to predict species' geographic range boundaries (Sunday, Bates et al. 2012) when the geographical range is unknown, as is the case for *Aurelia* spp. in the GoM.

This study aims to:

1. Present molecular and morphological evidence of a new *Aurelia* species (herein called *Aurelia* sp. 17) from offshore waters of the GoM and infer its evolutionary relationship to other species in the GoM and globally. Polyp and ephyrae morphology was compared to another GoM dwelling *Aurelia* species (namely *Aurelia* sp. 9) and a widely introduced *Aurelia* species (*A. coerulea*).
2. Identify suitable habitats (based on temperature and salinity tolerance) and settlement surfaces (oil and gas rigs, artificial reefs, and coral reefs) for polyps of two bloom-forming Scyphozoan species from the GoM, *Aurelia* sp. 9 and *Aurelia* sp. 17, and determine whether salinity or temperature limit their polyp

distribution in the GoM. To achieve this goal we combine temperature and salinity tolerance ranges for polyps of both species (calculated in Frolova and Miglietta 2020) with World Ocean Atlas long-term mean data for temperature and salinity for the Gulf of Mexico to perform GIS spatial analyses and identify polyp distribution and suitable habitats.

3.2 Methods

3.2.1 Sample collection for *Aurelia* sp. 17

Details on the medusae collection are reported in Chapter 2. We summarize them here and add additional water quality data that are relevant to the geographic analyses carried out in this study. A single live adult female medusa carrying planula was collected by dip net on July 1, 2017, at night approximately 145 km south of the Louisiana coast in the Gulf of Mexico (27.99903, -89.79812) in 37.3 ppt salinity, sea surface temperature of 28.8°C, and dissolved oxygen of 6.44 mg/ml as measured by water quality instruments onboard the research vessel (see also Chapter 2). The bell of the medusa was approximately 7 cm in diameter. A second *Aurelia* specimen was collected in a Neuston Net on July 3rd, 2017 during the same cruise, approximately 230 km off the coast of Louisiana (26.99977, -88.9940) in 37.6 ppt salinity, sea surface temperature of 30.8°C, and dissolved oxygen of 6.47 mg/ml. This medusa specimen was damaged but identifiable by the characteristic horseshoe shaped stomach/gonad complex. The Neuston Net sample contained 3 kg of *Sargassum* seaweed, which was consistently observed in the area during sampling.

Tissue samples from each adult medusa were preserved in 100% ethanol immediately upon collection. No permits were required for collection.

3.2.2 Planulae collection and polyp culture of *Aurelia* sp. 17

Hundreds of planulae were removed from the live adult female medusa and placed in plastic containers with 700 mls of filtered natural sea water. Upon separation from the medusa, planulae were enveloped in a gelatinous material. When viewed under a dissecting scope, planulae appeared sessile and negatively buoyant. Eight hours later, some planulae were observed moving around the bottom and through the water column of the container. Nearly all planulae were free-swimming 12.5 hours after collection.

The swimming planulae were separated from any remaining gelatinous material and moved into plastic containers with fresh filtered natural sea water and settlement surfaces, and transported to the Sea Life Facility at Texas A&M University at Galveston. Over the following 3-5 days, planulae metamorphosed into polyps. Polyps were cultured at a salinity of 33-37 ppt and temperatures ranging 15-23°C. Polyps were fed a combination of freshly hatched artemia and algae-enriched rotifers. Fifty percent of the culture water was changed weekly.

3.2.3 Ephyrae rearing

Polyps strobilated naturally when the temperature was dropped from 21°C to 17°C. Ephyrae were raised at a salinity of 33 ppt and temperature of 17° C. Newly released ephyrae were fed enriched rotifers for the first few days; newly hatched brine

shrimp were then added to the diet for the remainder of the rearing process. One ephyra was raised to a medusa with a final relaxed bell size of 6 cm. The specimen was photographed and small tissue samples were preserved in 100% ethanol for identity confirmation using DNA barcoding. The medusa specimen was then preserved whole in formalin.

3.2.4 Morphological and morphometric analysis

We analyzed and compared the morphology of the polyps of *Aurelia* sp. 17, *Aurelia* sp. 9 the GoM, and *A. coerulea* (obtained from aquaria) (see also Chapter 2 for details on collecting sites for *Aurelia* sp. 9 and *A. coerulea*). The morphometric analyses were done with the intent to compare *A.* sp. 17, with the other GoM dwelling *Aurelia* species and with *A. coerulea*, a successful introduced species found in several locations around the world, but not yet reported in the GoM (Dawson, Gupta et al. 2005). All species were kept at the Sea Life Facility at Texas A&M at Galveston. Prior to morphometric measurements, growing conditions were standardized by culturing polyps of the three species at 21°C for at least 2 months with weekly feedings of *Artemia.* Ten individual non-sexually reproducing, healthy polyps were then selected at random from each culture and photographed for morphological characterization. Additionally, from July 2017 to April 2019, budding was periodically observed for the three species and modes of bud morphogenesis were described. Five six-day old ephyrae of *A.* sp. 17 were photographed for morphometric and morphological characterization.

All photos were taken using a Leica M80 stereo zoom microscope with Leica image software v.3.4.1 for MacOS operating system and morphometric measurements were taken from images using the software ImageJ (Schneider, Rasband et al. 2012). The following standard measurements were taken for polyps and used for statistical analyses: Total Body Length (TBL), the length from tip of hypostome to basal disk; Stalk Length (StL), the length from basal disk to gastric cavity base; Mouth Disk Diameter (MDD), the diameter of the mouth disk; Hypostome Length (HL), the length from the tentacle crown to the tip of the hypostome(Straehler-Pohl, Widmer et al. 2011; Scorrano, Aglieri et al. 2017). The number of tentacles were also recorded. See Supplementary Figure S1 for a diagram of polyp measurements.

Morphometric data for polyps was rescaled to TBL and analyzed with the approach used by Scorrano, Aglieri et al. (2017). Statistical analyses were performed using PRIMER v7. Data were normalized for each variable and a resemblance matrix based on Euclidean distances was calculated between all samples. To determine whether polyps of *Aurelia* sp. 17 are significantly different from *A*. sp. 9 and *A*. *coerulea*, morphometric characters were analyzed together using permutational multivariate analysis of variance (PERMANOVA) with "species" as fixed factor and a 0.05 significance threshold and 9999 permutations. Follow-up pairwise tests were performed to determine which characters were statistically significant in distinguishing *A.* sp. 17 from the other two species.

One-way similarity percentage analysis (SIMPER) was performed to calculate the contribution of each character to the dissimilarity among *A.* sp. 17 polyps and the polyps of *A*. sp. 9 and *A. coerulea*.

3.2.5 DNA extraction, amplification, and sequencing

Total genomic DNA was purified from five live polyps and the two ethanol-preserved medusae of *Aurelia* sp. 17. The nuclear gene 28S rDNA was amplified using the primers Aa_L28S_21 and Aa_H28S_1078 and thermal protocol from Bayha, Dawson et al. (2010). Mitochondrial cytochrome c oxidase I (COI) and the nuclear region, internal transcribed spacer 1 (ITS1) were used for species-level delineation. COI was amplified using the primers LCOjf (Dawson, Gupta et al. 2005) and HCO2198 (Folmer, Black et al. 1994) using the thermal cycling protocol described by Chiaverano, Bayha et al. (2016). ITS1 was amplified using the primers KMBN-8 and KMBN-84 from (Chiaverano, Bayha et al. 2016), using the thermal cycling protocol described by the authors. All polymerase chain reactions (PCR) were performed in a BioRad thermocycler. To check the quality and size of amplicons, PCR products were visualized on a 1.5% agarose gel stained with SYBR Safe. The PCR products were then purified using ExoSAP-IT® (Applied Biosystems) or the GeneJET Gel Extraction Kit (Thermo Scientific). Amplicons were sequenced in both directions using the PCR primers. Sanger Sequencing was performed by the Texas A&M University Corpus Christi Genomics Core Lab or by GeneWiz (https://www.genewiz.com/en). Sequences were viewed and paired reads were assembled and edited in Geneious 9.1.8. To verify

that the correct loci were amplified, each consensus sequence was queried using BLASTn against the nucleotide collection (nr/nt) database of the National Center for Biotechnology (NCBI, http://www.ncbi.nlm.nih.gov).

3.2.6 Molecular analyses

The COI dataset included a total of 76 sequences, four of which belonged to *A.* sp. 17 (including GenBank # MN531714, MN531715, and MN5378 already published in Frolova & Miglietta 2020), 71 belonged to published Genbank sequences of all known *Aurelia* species, and one outgroup sequence belonging to *Phacellophora camtschatica* (family Phacellophoridae, Order Semaestomeae) (see Supplementary Table S1). All COI sequences were translated into amino acid sequences using the invertebrate mitochondrial code using the Translate tool in ExPASy (https://www.expasy.org/) to confirm open reading frames.

The ITS1 dataset includes three sequences from *A.* sp. 17 (MN527964, MN527965, and MN527966, previously published in Frolova & Miglietta 2020) and 20 sequences downloaded from Genbank (Supplementary Table S2). Because ITS1 sequences include flanking 18S and 5.8S fragments and hypervariable regions that do not align well, we narrowed our ITS1 analysis to *Aurelia* species that, based on our COI analyses, were closely related to *A.* sp. 17, namely *A. relicta, A. labiata, A. limbata*, *A. coerulea*, and *A. solida*, *A.* sp. 10, and *A.* sp. 7.

COI and 28S nucleotide sequences were aligned using Clustal Omega using default settings. ITS1 was aligned using MAFFT v.7 with the E-INS-I strategy and the

"leave gappy regions" option selected. Multiple sequence alignments were visually checked and trimmed in Geneious 9.1.8. Phylogenetic relationships for COI, 28S, and ITS1 datasets were generated using both Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. ML trees were constructed in IQ-Tree 2 with branch support calculated based on 1000 bootstrap replicates using the following models: TIM2+F+I+G4 for COI and the HKY+F+G4 for ITS1, as calculated using ModelFinder (Kalyaanamoorthy, Minh et al. 2017). BI trees were generated in Mr. Bayes using the GTR+I+G model for COI and the HKY+I+G model for ITS1, with four runs, 10^5 generations, sampling every 25^{th} generation, with 35% "burnin".

Concatenated analyses (COI+28S) included specimens that had both COI and 28S sequences (Supplementary Table S3) for a total of 55 sequences, including four from *A.* sp. 17. Sequences were concatenated using Geneious 9.1.8. Best-fitting nucleotide substitution models and partitioning scheme were chosen using ModelFinder (Kalyaanamoorthy, Minh et al. 2017) using the Bayesian information criterion (BIC). ML trees were constructed in IQ-Tree 2 (Minh, Schmidt et al. 2020).

All trees were edited and branch support was added in FigTree v.1.4.3 and Adobe Acrobat Pro. Single gene ITS1, and concatenated COI+28S trees were rooted at the midpoint, while COI was rooted using the outgroup *P. camtschatica*. Within and between group mean distances for COI and ITS1 were computed using the Kimura 2 Parameter model of evolution with variance estimated by bootstrapping 1000 replicates in MEGA version X (Kumar, Stecher et al. 2018). Positions containing missing data or gaps were eliminated from the analysis.

3.2.7 Identifying suitable habitats in the Gulf of Mexico

Monthly long-term mean data for temperature and salinity for the GoM region were obtained from NOAA's 2013 World Ocean Atlas (https://www.nodc.noaa.gov/cgi-bin/OC5/woa13/woa13.pl) in July 2020. The datasets consist of monthly means calculated from six decadal averages from 1955 to 2012 using data from the World Ocean Database 2013 (Levitus 2015). Mean fields were provided with 0.25° horizontal resolution (0.25 x 0.25 latitude/longitude grid) for 57 standard levels from surface to 1500 meters depth for all the world's oceans. Temperatures were in the degrees Celsius (°C) and salinity was unitless as measured by the Practical Salinity Scale-1978.

Tolerance ranges for temperature and salinity for polyps of *Aurelia* sp. 17 were previously determined through laboratory experiments in Chapter 2 (Frolova and Miglietta 2020) and are publicly available for download in supplementary materials (https://www.frontiersin.org/articles/10.3389/fmars.2020.00093/full#supplementary-material). In those experiments, polyps were subjected to a continuous temperature or salinity ramp at a rate of 1°/day. Each polyp's response to stimuli was monitored under the microscope at regular intervals to evaluate response to the thermal/salinity stress. The temperature and salinity conditions where polyps maintained optimal response to stimuli with no visible signs of stress were used to define the temperature and salinity parameters of suitable habitats for this species in the Gulf of Mexico. Based on these criteria, the suitable ranges for *Aurelia* sp. 17 and *Aurelia* sp. 9 are defined by temperatures of 13°C to 27°C and 14°C to 30°C and salinities down to 18 ppt and 10 ppt

respectively. Upper salinity limit was not determined as it exceeded instrument sensitivity (42 ppt).

The GoM basin was defined according to the International Hydrographic Organization. A shapefile representing this region was downloaded from marineregions.org. A bathymetry raster for the GoM basin with a resolution of 30 arc seconds was downloaded from https://geo.gcoos.org/data/topography/SRTM30PLUS.html (Becker, Sandwell et al. 2009). A shapefile of coral habitats in the GoM was downloaded from the NOAA Fisheries webpage (https://www.fisheries.noaa.gov/resource/map/coral-essential-fish-habitat-efh-map-gis-data). A shapefile of Artificial Reefs in the USA produced by NOAA was downloaded from https://koordinates.com/layer/20868-us-artificial-reefs/. A shapefile of oil and gas platforms in the Gulf of Mexico was downloaded from the Homeland Infrastructure Foundation-Level Data (HIFLD) webpage (https://hifld-geoplatform.opendata.arcgis.com/datasets/oil-and-natural-gas-platforms). All datasets were analyzed in the WGS 1984 coordinate system.

Habitat suitability was based on the annual minimum and maximum values calculated from climatological monthly means at each depth of the grid in the Gulf of Mexico basin. Gridded point data for environmental temperature and salinity were treated separately. To narrow the dataset to focus only on the GoM region, datapoints of each monthly shapefile that had their centroid in the GoM source layer were selected and exported as new shapefiles. These new files were combined into a single shapefile by merging the gridded point data based on location. Layers representing temperature or

salinity profiles at each depth were created by selecting and saving the data for each depth layer into a separate shapefile. For each standard depth, maximum and minimum temperatures and minimum salinities were calculated at each location in the grid, then interpolated using the inverse distance weighted technique (IDW tool using power of 1, cells size of 0.056232, with the neighborhood defined using a 12 point radius setting) to generate rasters of maximum and minimum temperatures and salinities at the given standard depth for the Gulf of Mexico basin.

These three datasets (maximum temperature, minimum temperature, and minimum salinity) were then analyzed individually to identify suitable areas and associated depths to determine the relationship between each parameter and habitat suitability, as well as together to generate habitat suitability maps and identify suitable settlement surfaces based on all three parameters in aggregate. For the individual parameter analysis, this process is described for maximum temperature but was also repeated for the other two climatological datasets. Each maximum temperature raster was first conditionally evaluated against the bathymetry raster to mask out areas where the standard depth associated with the temperature raster was greater than the local depth of the GoM (necessary because the IDW interpolation includes estimated values for areas immediately adjacent to the footprint of the input points). Suitable areas at each standard depth were then identified by comparing cell values from the masked maximum temperature rasters against the tolerance limits for each species as defined above; suitable areas were assigned a value equal to their associated standard depth.

To generate habitat suitability rasters the minimum salinity, minimum temperature, and maximum temperature rasters were evaluated in a single conditional statement and tagged with the associated standard depth if evaluated to be true (i.e., suitable), and "No Data" if false (i.e., unsuitable). For *Aurelia* sp. 9, grid cells with minimum salinity greater or equal to 10 ppt and minimum temperature greater than or equal to 14°C but less than or equal to 30°C were considered suitable habitats. For *Aurelia* sp. 17 suitable habitats were cells with minimum salinity greater than or equal to 18 ppt, and minimum temperature greater than or equal to 13°C but less than 27°C. For each species, minimum and maximum suitable habitat depth for each grid cell was found by calculating the minimum and maximum standard depth value, respectively, from the stack of co-located suitability raster grid cells. A raster of the height of the suitable habitat water column at each cell was then calculated by taking the difference between the maximum and minimum standard depths for each grid cell. To simultaneously display the minimum suitable depth with the total height of the suitable water column, contours for the minimal habitable depths were generated from the associated rasters for each species.

For each species, potential settlement surfaces consisting of oil and natural gas rigs, artificial reefs, and coral reefs were mapped to the suitable habitat maps. Oil and natural gas platforms with the status "removed" were eliminated from the dataset. The artificial reefs and active oil and natural gas platforms were filtered by location to include only those structures located within the suitable depth layer for each species. To

determine the total area of coral within suitable habitats for each species, the coral layer was clipped to the size of the suitable water column layer and its area was calculated.

3.3 Results

3.3.1 Morphology and asexual budding of *Aurelia* sp. 17

Polyps of *A.* sp. 17 have a total body length (TBL) of 2.33mm (s.d. 0.88) and 31-46 tentacles. Mouth diameter, hypostome length, and stalk length, measured as % of TBL, are 37.23 (s.d. 10.67), 15.24 (s.d. 7.73), 48.11 (s.d. 8.93) respectively (see Table 1). When compared with polyps of *A*. sp. 9 and *A. coerulea*, polyps of *A*. sp. 17 were notably different, being twice as tall, and with significantly more tentacles (31-46 in *A.* sp. 17 versus 16 in both *A*. sp. 9 and *A. coerulea*). Polyp morphometrics also confirmed significant differences between *Aurelia* sp. 17 and both *A.* sp. 9 and *A. coerulea* (Psuedo-F = 6.44, P < .001). TBL distinguished *A.* sp. 17 from both species, while MDD/TBL distinguished *A.* sp. 17 from *A. coerulea* (P < .001) but not from *A.* sp. 9 (P > .1). Neither HL/TBL or StL/TBL were significantly different between *A.* sp. 17 and *A.* sp. 9 or *A. coerulea (*P > .05*)*. SIMPER analysis (Supplementary Table S4) showed that MDD/TBLe was the character contributing the most to the distinction between *Aurelia* sp. 17 and *A. coerulea* (31%), and TBL was the character contributing the most to distinction between *Aurelia* sp. 17 and *Aurelia* sp. 9 (43%). Morphological and morphometric measurements are summarized in Table 3.1.

Table 3.1 Summary of character morphological and morphometric characters for three species of *Aurelia*. Values are averages from 10 polyps of each species with standard deviations shown.

	Species		
Polyp characteristics	*Aurelia* sp. 17	*Aurelia* sp. 9	*Aurelia coerulea*
Total body length (TBL); mm	2.33 ± 0.88	1.20 ± 0.26	0.90 ± 0.25
Mouth diameter (MDD); % of TBL	37.23 ± 10.67	37.15 ± 7.41	67.41 ± 19.35
Hypostome length (HL); % of TBL	15.24 ± 7.73	15.72 ± 6.58	18.74 ± 9.03
Stalk length (StL); % of TBL	48.11 ± 8.93	48.20 ± 6.76	45.25 ± 10.29
Number of tentacles	31-46	16	16
Modes of reproduction	DB	DB	DB, podocyst, FSP

Ephyrae of *A.* sp. 17 had bell diameter (BD) of 3.8 mm (s.d. 0.37). Total marginal lappet length (TMLL), central disc diameter (CDD), lappet stem length (LStL), and rhopaliar lappet length (RLL), measured as % of BD were 27.61 (s.d. 3.42), 46.37 (s.d. 4.72), 15.91 (s.d. 1.18), and 11.78 (s.d.1.88) respectively (measurements summarized in Table 3.2). Although ephyrae measurements of other species were not conducted in this work, they were reported for *A*. *relicta*, *A. coerulea,* and *A. solida* by Scorrano, Aglieri et al. (2017). A comparison shows that *A*urelia sp. 17 body diameter, lappet stem length, and marginal lappet shape are similar (within standard deviation) to the values reported for *Aurelia relicta.* *Aurelia.* sp. 17 central disk diameter and rhopaliar lappet length was proportionally shorter than those of *A. relicta*. *Aurelia* sp. 17 had also similar morphometrics to *A. coerulea*, except for the central disk diameter,

which was proportionally shorter. Ephyrae of *A. solida* were overall smaller in size than *Aurelia* sp. 17, but with proportionally longer lappet stems.

Table 3.2 Average values for ephyrae measurements (see Supplementary Figure S1 for a diagram of which measurements were taken) with standard deviation shown. Values based on five ephyrae.

	Species
Ephyra characteristics	*Aurelia* sp. 17
Bell diameter (BD); mm	3.80 ± 0.37
Total marginal lappet length (TMLL); % of BD	27.61 ± 3.42
Central disk diameter (CDD); % of BD	46.37 ± 4.72
Lappet stem length (LStL); % of BD	15.91 ± 1.18
Rhopalial lappet length (RLL); % of BD	11.78 ± 1.88
Marginal lappet shape	Breadknife
Number of gastric filaments	4

Finally, we observed *Aurelia* sp. 17 direct budding, a mode of asexual reproduction in which child polyps develop from the body of the parent polyp. A single bud develops laterally near the calyx-stalk margin of the parent polyp sharing a gastric cavity (Figures 1a). The calyx and stalk of the secondary polyp develop simultaneously. The early bud typically develops a tendril-like stolon at its base (Figure 1c). The early stolon appears as non-gastric tissue growing at the base of the 17 bud that extends beyond the bud at about the time when tentacles begin to develop in the budding polyp (Figure 1b). The mature stolon appears long and flexible with a thickened tip. As the

column of the secondary polyp develops, the attachment point to the parent polyp slowly shifts from the calyx towards the stalk; as this happens the shared gastric cavity splits into two. Eventually, the child polyp remains bound to the parent only at the base of the stalk (Figure 1d). The child polyp then detaches from its parent. Its stolon, by adhering to the substrate with its tip, seems to facilitate the separation. Once the two polyps are separated, the stolon quickly recedes. Upon detachment, secondary polyps are of similar size to their parents. A new child polyp may begin to develop on the same parent polyp once the child polyp and parent polyp no longer share a gastric tissue or cavity (Figure 1d).

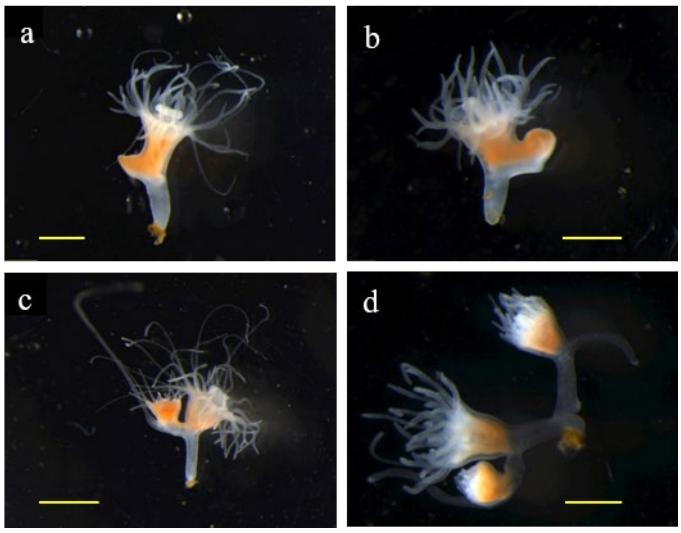

Figure 3.1 New bud formation in *Aurelia* sp. 17 polyps. New bud forms near base of calyx on parent polyp and shares a gastric cavity (a). Stolon forms from non-gastric tissue at base of bud. Stolon begins to extend past budding polyp at about the time when tentacles begin forming (b). When fully formed, stolon is unattached to substrate, flexible, with a thickening at the tip (c). Polyps typically bud one at a time; new buds begin formation only once parent polyp and child polyp no longer share gastric tissue or cavity (1d).

3.3.2 Molecular analyses

The COI dataset consisted of an alignment of 75 sequences of 655 bp with 296 (45.19%) phylogenetically informative sites. The ITS dataset consisted of 20 sequences of 409 bp with 268 (65.53%) informative sites. The COI and 28S dataset consisted of 55 sequences of 1,628 bp with 1206 (74.08%) phylogenetically informative sites. All phylogenetic trees were congruent in showing *Aurelia* sp. 17 as a distinct species, sister to *A. relicta* from the Mediterranean Sea. Because *Aurelia* sp. 1 to 16 have been already identified using a phylogenetic approach, we identify this species as *Aurelia* sp. 17.

In the COI phylogenetic tree (Figure 3.2), the most complete in terms of species (20 *Aurelia* species), shows *A.* sp. 17 and *A. relicta* as sister taxa (with bootstrap values and posterior probability of 99 and 1.0 respectively), and in a major clade with *A. solida* from the Mediterranean Sea, *A.* sp. 10 from Alaska, *A. limbata* from Japan, and *A. coerulea*, the globally introduced species found originally in the western north Pacific and introduced in several locations worldwide (Dawson, Gupta et al. 2005). In this clade there are also several species from Palau (*A.* sp. 3, *A.* sp. 4, *A.* sp. 6), *A.* sp. 7 from Australia, *A.* sp. 14 from Pacific Panama, *A. labiat*a from the Pacific United States, and *A. aurita* from North Europe. The other two species from the GoM, namely *A.* sp. 9 and *A.* cf. 2, are recovered as sister species, and fall in a second clade that also includes *A.* sp. 2 from Brazil, *A*. sp. 15 from the Caribbean Sea, *A.* sp. 16 from Argentina, *A.* sp. 12 and *A.* sp. 13 from the Pacific (Baja California and Central America respectively). Maximum Likelihood and Bayesian reconstructions for ITS1 differed in the placement of *Aurelia solida*, *Aurelia labiata*, and *Aurelia* sp. 7, but supported the

identity of *Aurelia* sp. 17 as a distinct species and sister to *A. relicta* with high node support (Figure 3.4). The concatenated COI+28S species tree (Figure 3.3) also supports the placement of *Aurelia* sp. 17 as sister to *Aurelia relicta* (bootstrap value 97%). K2P mean genetic distances for COI between *A.* sp. 17 and other *Aurelia* species ranged from 11.3%, between *A.* sp. 17 and *A*. *relicta*, to 27.1%, between *A*. sp. 17 and *A*. cf. 2 (mean 22.7%, s.d. 4.1%), while the genetic distances for all *Aurelia* species included in the analysis ranged from 2.1%, between *A*. sp 15 and *A*. sp. 2, to 30.5%, between *A*. cf. 2 and *A. limbata* (mean 22.9%, s.d. 4.1%), as shown in Supplementary Table S6. The mean intraspecies K2P genetic distance was 0.12% for *A*. sp. 17 (mean 0.7%, s.d. 1.4%). For ITS1, between group distances ranged from 8.7%, between *A.* sp. 17 and *A*. *relicta*, to 29.3%, between *A*. sp. 17 and *A*. *solida* (mean 20.5%, s.d. 6.9%), while within group distance was 0 for *A*. sp. 17.

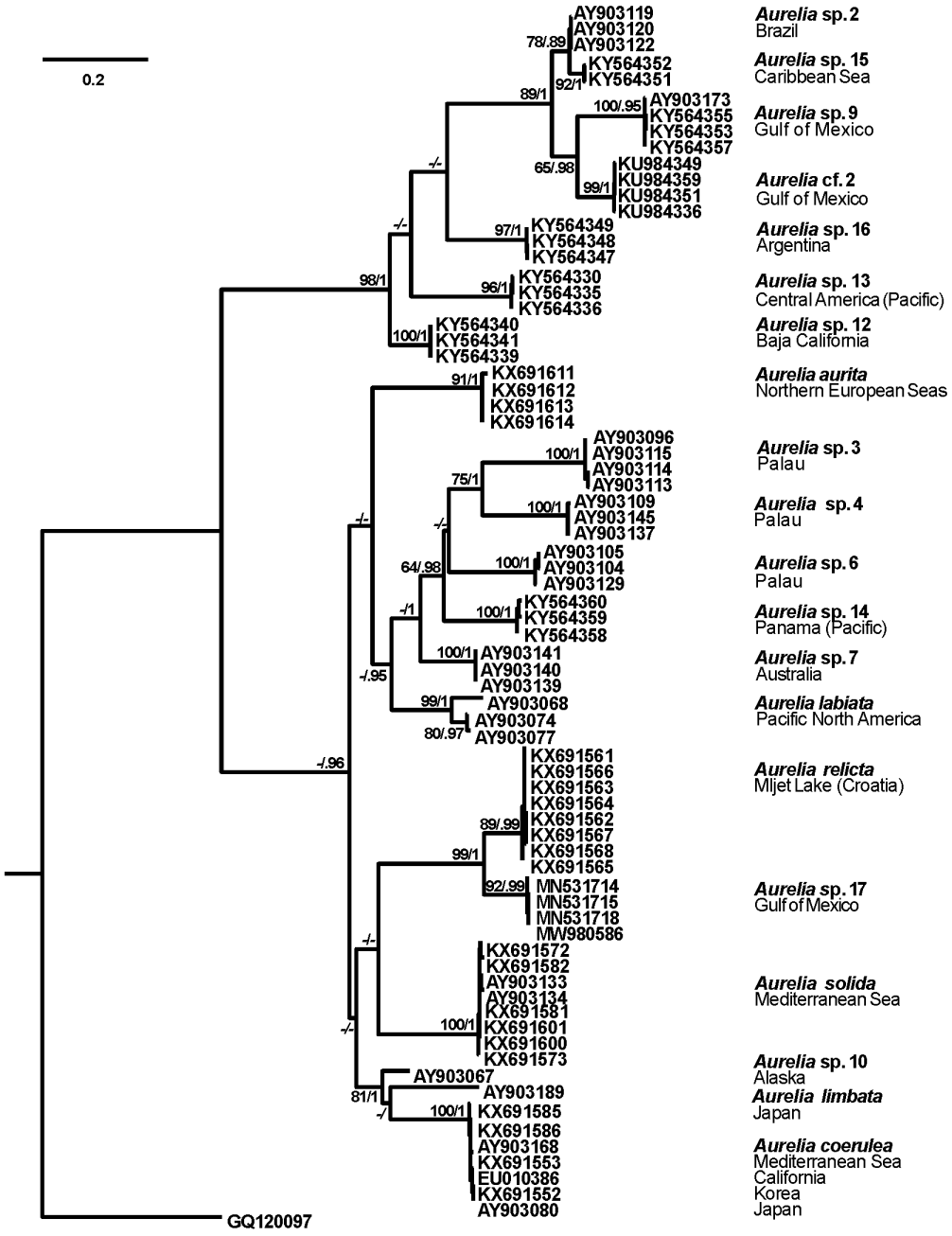

Figure 3.2 COI Maximum Likelihood tree representing the global phylogeny of *Aurelia*. Nodes are labeled with bootstrap support values followed by posterior probability values from BI analysis. Only bootstrap values of 60% or greater and posterior probabilities of 75% or greater are shown. Dashes indicate values below these thresholds, while absence of values indicates node was not recovered in BI tree reconstruction. Branch lengths are measured in the number of substitutions per site.

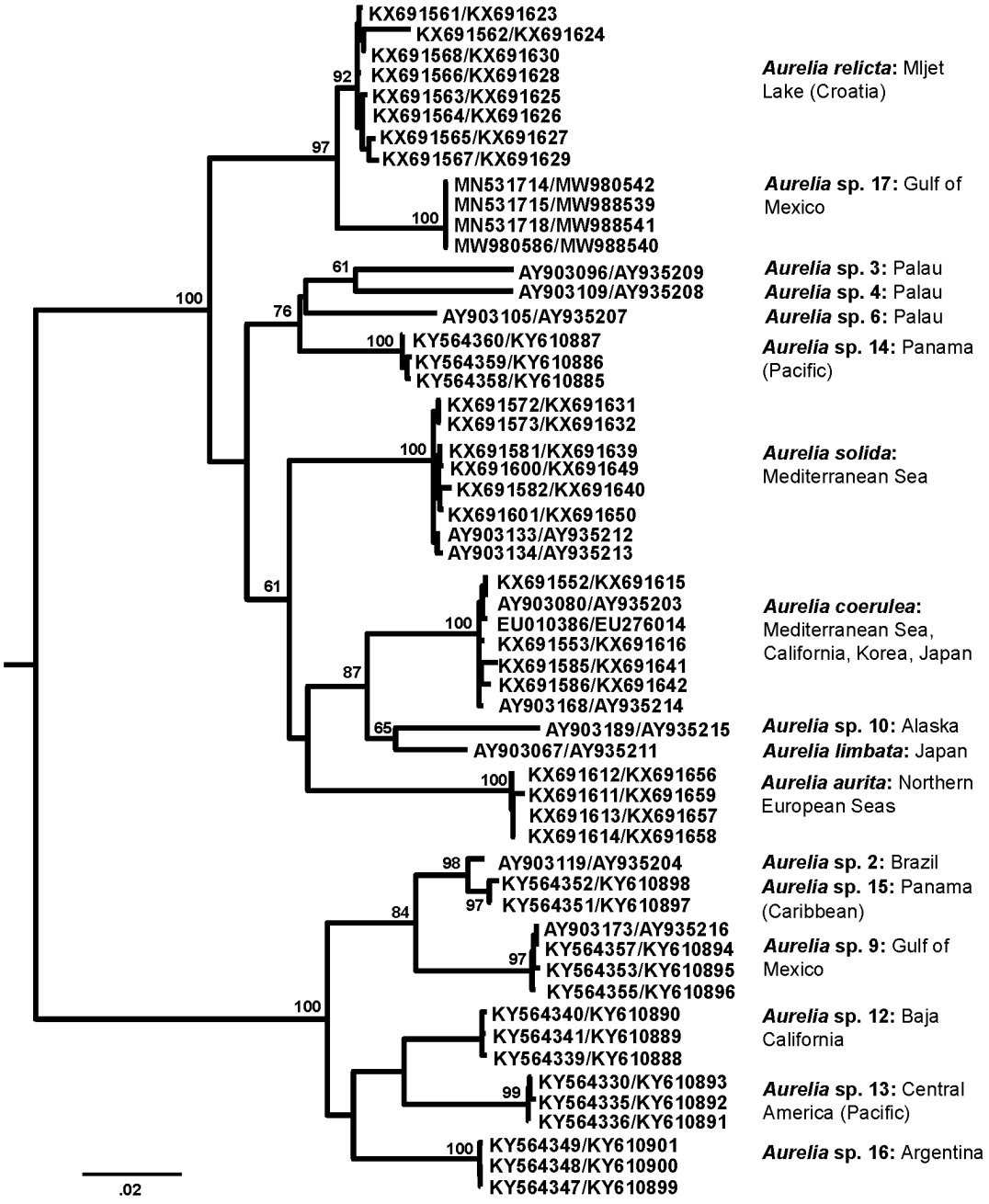

Figure 3.3 Concatenated COI + 28S Maximum Likelihood gene tree of *Aurelia* species. Nodes are labeled with bootstrap support values. Only bootstrap values

60% and greater are shown. Branch lengths are measured in the number of substitutions per site.

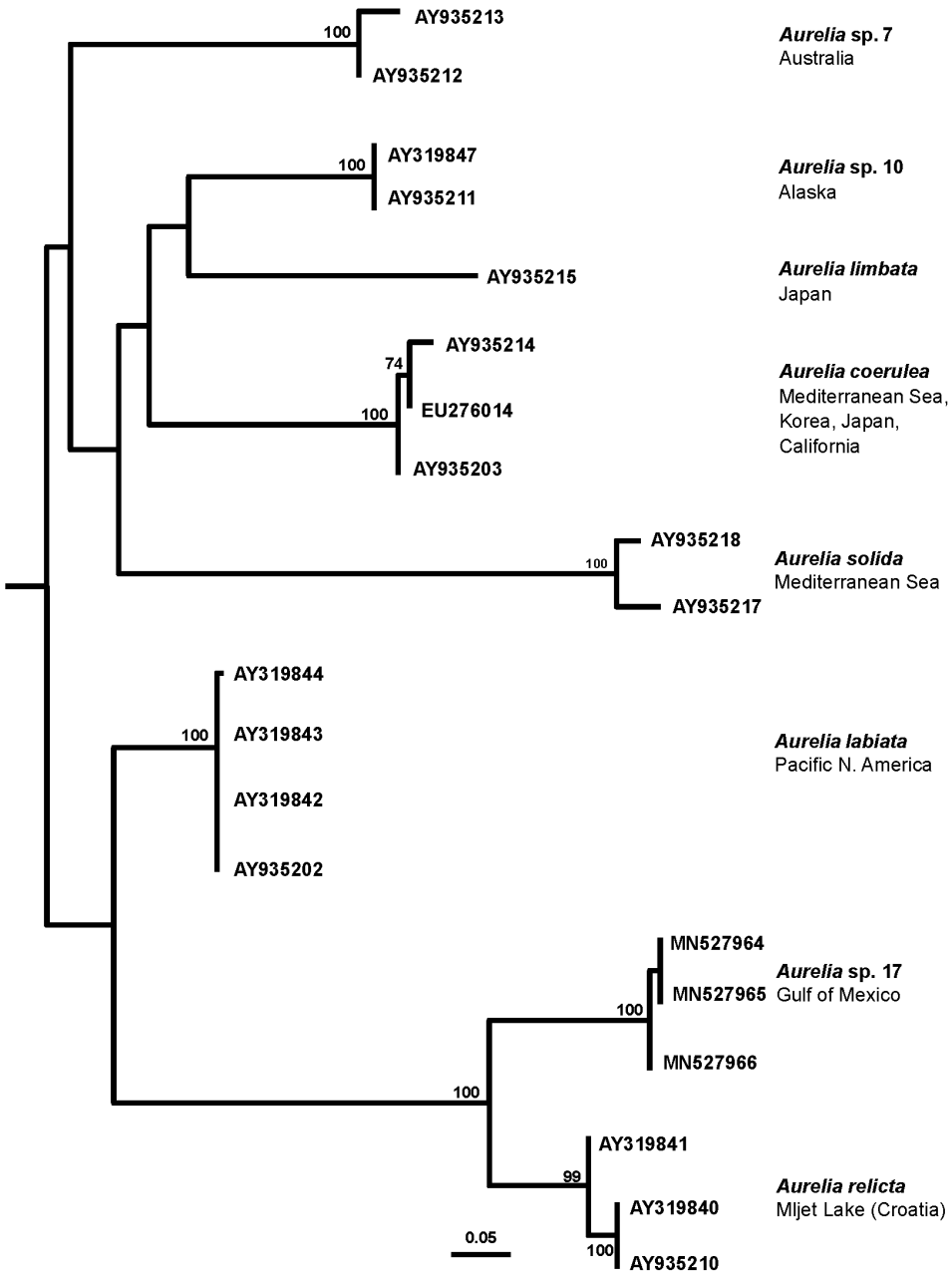

Figure 3.4 ITS1 Maximum Likelihood tree of *Aurelia* species. Only bootstrap values of 60% or greater are shown. Branch lengths are measured in the number of substitutions per site.

3.3.3 Suitable habitats

Using WOA spatial data for temperature and salinity and environmental tolerance data from Frolova and Miglietta (2020), we generated 160 rasters displaying habitat suitability for each species at 40 depths, from 0 to 650 meters, and for temperature (minimum and maximum), salinity (minimum), and all parameters in aggregate. The 40 rasters constructed using the combination of the three parameters, were used to generate a habitat suitability map for each of two species of *Aurelia* in the GoM, *A*. sp. 17 (Figure 3.5) and *A*. sp. 9 (Figure 3.6). The habitat suitability analysis for *A.* sp. 17 indicated that environmental temperature, but not salinity, limits the distribution of its polyps in the GoM. Maximum water temperatures exceed the upper temperature suitability threshold of *Aurelia* sp. 17, restricting the suitable polyp habitats to depths of 20 meters and greater throughout the GoM. At shallower depths, maximum temperatures exceed 27°C with surface waters temperatures over 30°C in some areas, which are lethal to *A*. sp. 17. At 20 meters, only 3,688 km^2 of Campeche Bank off the coast of Cancun is suitable for polyps of *A.* sp. 17. At 25 meters, suitable habitats expand northward, with a stretch of suitable habitats appearing in the northern GoM tracing the Emerald Coast of the western Florida panhandle. At 25 meters, the total GoM area of suitable habitats for the polyps of *A.* sp. 17 is approximately 28,541 km^2. At 30 meters, this area expands to the south and southeast along the Florida coast to an area of 108,084 km^2; at 35 meters, this trend continues along with an expansion of suitable habitats into the northwestern region of the GoM. At 40 meters, suitable habitats extend across the northern Gulf from the southernmost tip of Texas to the tip of Florida and in

the southwestern Gulf from Ciudad Madrid to Ciudad del Carmen, Mexico with a total area of 399,353 km^2 at this depth. As depth increases, suitable habitats expand in all directions to encompass 1,059,674 km^2 at 55 meters, with most of the GoM suitable for *A.* sp. 17 polyps at this depth. The Loop Current brings very warm waters into the GoM so that the general area of this feature is too warm until depths exceed 55 meters. Suitable habitats gradually appear in the region where the Loop Current intrudes into the GoM as depths progress from 60 to 150 meters. Depths within the range of 150 to 175 meters are universally suitable for *A.* sp. 17 polyps across the GoM. As depths increase from 200 meters to 450 meters, minimum water temperatures start to increasingly limit suitable habitats for polyps. The waters of the southwestern GoM are first to become unfavorable due to the cold temperatures (i.e. <13°C, see Frolova and Miglietta (2020)). The maximum habitable depth for *Aurelia* sp. 17 polyps in the GoM is 450 meters. The height of the water column containing suitable habitats range from 0 (meaning that suitable habitats at that location are only found at one depth - on the seafloor) to 355 meters.

The habitat suitability map for *A.* sp. 9 shows that surface GoM waters are generally suitable habitats for its polyps, with the exception of few northern nearshore areas and bays, such as Galveston Bay, Texas, nearshore waters near the Texas- LA border, and the west coast of northern Florida. In these regions maximum water temperatures exceed 30°C and are thus too high for polyps of this species until depths of 10 to 15 meters. Lake Pontchartrain in Louisiana is also unsuitable for polyps due to both maximum and minimum water temperatures. An area from surface to 5 meters off

the west coast of south Florida is also prohibitive to *Aurelia* sp. 9 polyps due to high maximum water temperatures. Depths of 20 meters down through 175 meters have suitable temperature parameters. At depths greater than 175 meters, minimum temperatures become limiting (colder than 14°C), starting with the shallower northwestern portions of the GoM and expanding to the east with depth. The greatest depth where suitable habitats are found is 425 meters, as greater depths in the GoM are uninhabitable for *A.* sp. 9 polyps due to low water temperatures.

Within the GoM, there are a total of 4,124 artificial reefs, 2,566 oil and natural gas platforms, and about 42,716.86 km^2 of coral reefs. Of these, 1,513 (~37%) artificial reefs, 801 (~31%) oil and natural gas platforms, and 17,263.81 km^2 (~40%) coral reefs are located within the suitable habitat range for *Aurelia* sp. 17. For *Aurelia* sp. 9, there are 3,867 (~94%) artificial reefs, 2,477 (~97%) oil and natural gas platforms, and 42,413.44 km2 (~99%) coral reefs located in locations with suitable environmental parameters.

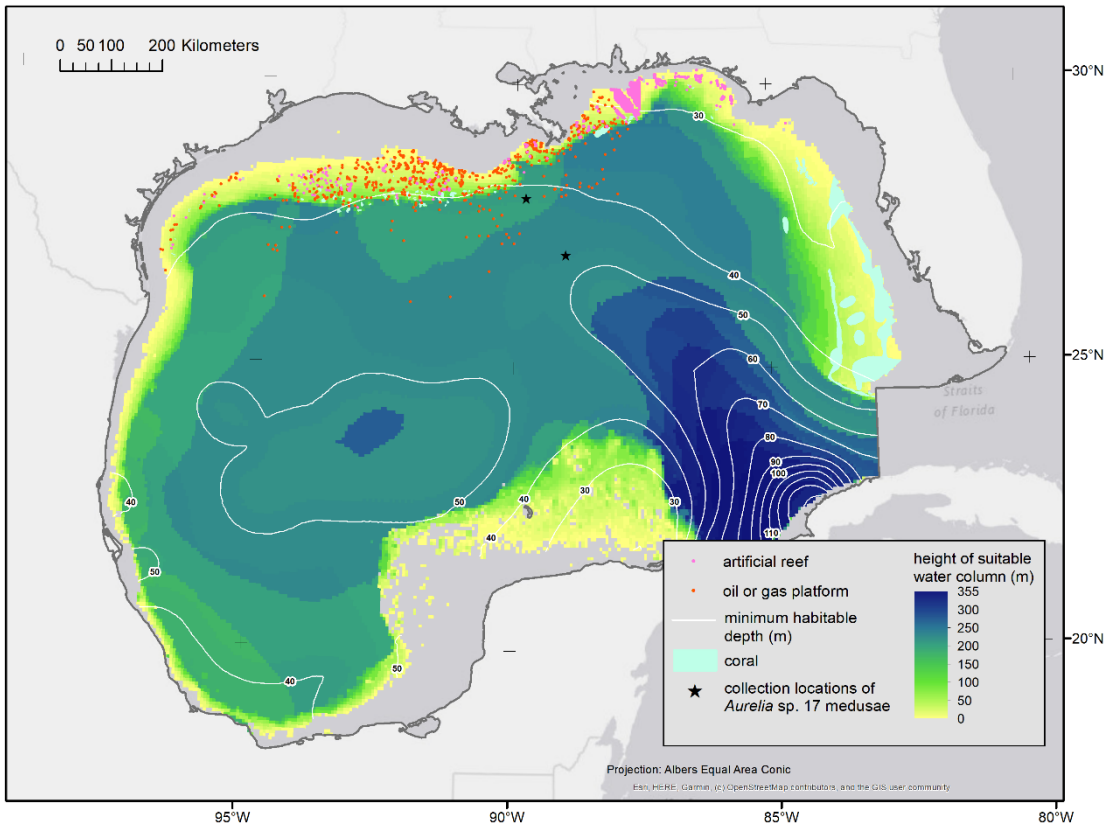

Figure 3.5 Minimum habitable depths (contour lines) in meters and height of water column (color ramp) in meters suitable for *Aurelia* sp. 17 polyps based on temperature tolerance. Hard substrates suitable for polyp settlement: artificial reefs (pink circles), oil and gas platforms (orange circles) and coral (blue patches) are shown. Stars mark the collection locations of the two *Aurelia* sp. 17 medusae.

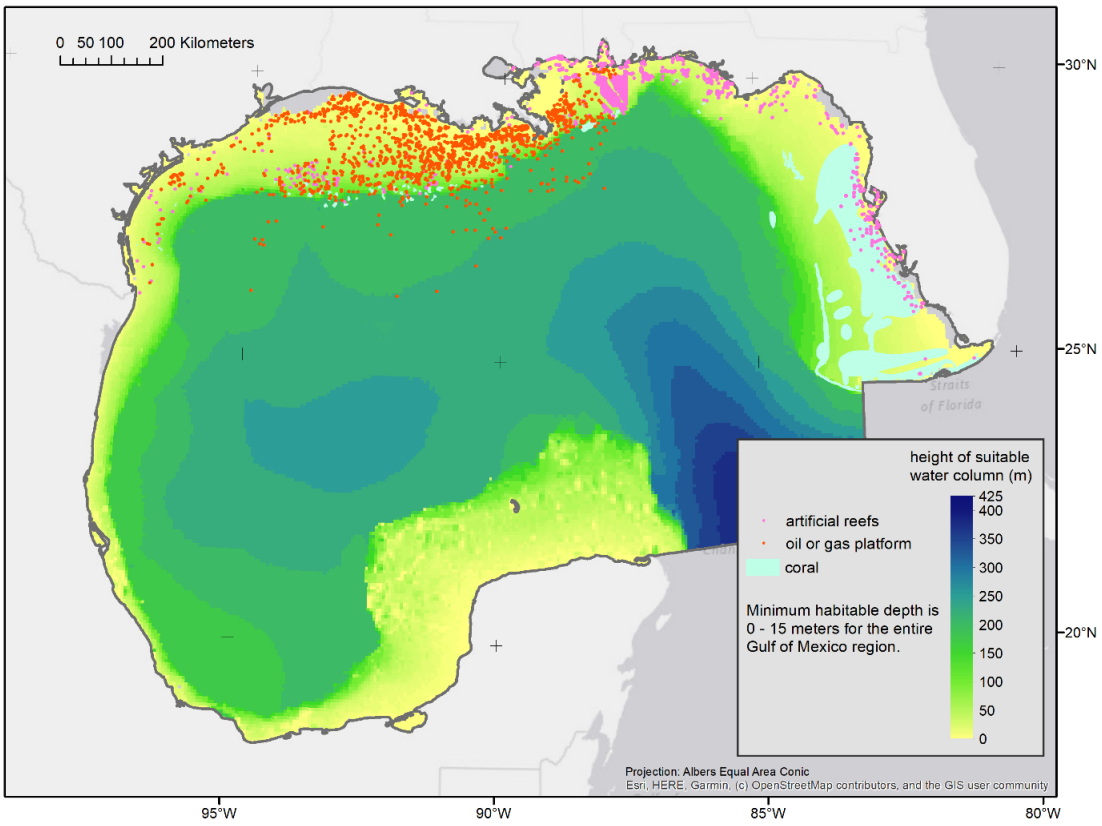

Figure 3.6 Minimum habitable depths (contour lines) in meters and height of water column (color ramp) in meters suitable for *Aurelia* sp. 9 polyps based on temperature and salinity tolerance. Hard substrates suitable for polyp settlement: artificial reefs (pink circles), oil and gas platforms (orange circles) and coral (blue patches), are also shown. Minimum habitable depth is 0 meters for most of the Gulf of Mexico except a few small patches in coastal areas of the northern Gulf and western coast of Florida where minimum habitable depth is at 5-15 meters.

3.4 Discussion

Our phylogenetic analyses based on the mitochondrial and nuclear markers suggest that *A*. sp. 17 is a distinct lineage. All analyses support *A*. *relicta,* a species endemic to the Mljet marine lake in the Adriatic Sea, as a sister-species to *A.* sp. 17. The COI genetic distance between *A*. sp. 17 and *A. relicta* is 11.3%, comparable to the

distance between *A*. *limbata* and *A*. sp. 10 (14.4%) and exceeds the approximate 6% standard "barcoding gap' for distinguishing medusozoan species (Ortman, Bucklin et al. 2010). Morphological differences and budding mode also support *A.* sp. 17 as a distinct species. The COI tree and genetic distances identify 20 distinct *Aurelia* lineages, with 19 phylogenetic species. Only *Aurelia* sp. 2 and *Aurelia* sp. 15 are not resolved as distinct species, with interspecific genetic distances well below the 6% threshold required for species delimitation using COI. Between group genetic distance for ITS1 also supports the identify of *A*. sp. 17 as a distinct species, based on a 5% threshold for species-level separation in *Aurelia* (Dawson and Jacobs 2001).

Our habitat suitability analysis shows that GoM summer high water temperatures often exceed 27°C and are thus prohibitive for *Aurelia* sp. 17 polyp survival in shallow and surface waters. More specifically, we find that coastal environments (between 0 and 15 meters) throughout the GoM, including all bays and estuaries, are too warm during the summer months and therefore not suitable for *A.* sp. 17 polyps. Suitable habitats for this species are restricted to depths of 20-175 meters throughout the GoM (Figure 8). However, at 20 meters, only a small portion of Campeche Bank is suitable, but suitable areas gradually expand with depth in the south GoM, and significant spans of the shelf are suitable by 30 meters. By 40-50 meters, most of the central GoM is suitable for polyps. Maximum water temperatures are the limiting parameter to polyp distribution for *A*. sp. 17 down to 175 meters, then minimum water temperatures become limiting for survival from 200- 450 meters. At depths greater than 450 meters, the GoM becomes too cold for *A.* sp. 17 polyp survival. The waters of the continental shelf are generally not

suitable for this species as well, with the notable exceptions of benthic environments on the western coast of the West Florida Shelf and northern portion of the Campeche Bank; potential habitats begin at the edge of the shelf where cooler conditions are facilitated by the deeper waters of the continental slope (Figure 8). The height of the water column suitable for *A*. sp. 17 polyps range from 0 to 355 meters. 31% of the total number of oil and gas rigs in the GoM are suitable for *A.* sp. 17 polyps. About 22% of the oil and gas rigs that are suitable for *A.* sp. 17 polyps are located within 5 meters or less suitable water column height, meaning that only the bases of these rigs that anchor into the ocean floor have thermal and salinity conditions suitable for polyps.

For *A.* sp. 9, the GoM is suitable from the surface waters to depths of 175 meters, with only a few localized exceptions. This species has a much broader predicted distribution than *Aurelia* sp. 17, which contains most of the available hard substrates, both natural and artificial, available in the GoM (Figure 9). This, along with the recurrent seasonal observations of *A.* sp. 9 medusae and only a single seasonal observation of two *A*. sp. 17 medusae, suggests that *A.* sp. 9 may have more numerous established polyp populations the GoM with an overall greater potential of medusae production. Alternatively, it is possible that blooms of *A*. sp. 17 are restricted to offshore waters due to the unsuitability of coastal thermal conditions, and observers and collection efforts have simply missed medusae of this species. Interestingly, medusae have been found in locations and conditions outside of the suitability zones for their respective species' polyps. The two medusae specimens of *A.* sp. 17 were collected from surface waters, while suitable zones in those areas begin only at 40-50 meters. Water

temperature during collection was 1.8 – 3.8°C above the upper tolerance range determined for *A*. sp. 17 polyps. Similarly, *A.* sp. 9 medusae have been frequently observed along the Texas- Louisiana border, which is unsuitable for polyps (see Figure 9). The differences between calculated suitable geographical distribution of the polyp and actual distribution of the medusae may indicate different environmental tolerances between the benthic and the planktonic stages, and that thermal tolerance of the polyp, not the medusae, limits distribution of this species in the GoM.

Salinity is not limiting to *Aurelia* sp. 9 or *Aurelia* sp. 17 polyp distribution in the GoM. Although salinity in bays and many nearshore areas in the GoM does fall below the minimum salinity tolerance limit of 18 ppt for *A*. sp. 17, these locations are located outside the suitable habitat range based on temperature tolerance.

The actual suitability of bays and shallow areas may be patchier than is suggested by the maps produced from the spatial analysis. The resolution of the World Ocean Atlas dataset does not capture the localized water temperature and salinity variation that are inherently characteristic to the fluctuating hydrological conditions of shallow waters in bays and lagoons. Additional studies would be required to make fine-scale conclusions regarding their suitability. Other factors beyond temperature and salinity profiles and hard substrate presence may also impact habitat suitability and availability. Turbidity of the water column, competition for space or resources with other benthic and biofouling organisms, and food availability may impact habitat suitability. Also, polyp populations must be initially seeded by planulae. Therefore, population locations depend on biotic

and abiotic factors affecting planulae dispersal and settlement, including medusae spawning location, larval supply, current characteristics, and water temperature.

GoM ocean temperature has been increasing over the last decades, with warming expected to continue and impact surface waters at faster rates (Pachauri, Allen et al. 2014). Our results implicate that an increase in temperature at depths of 20 meters and greater, would shrink the thermally suitable areas available for colonization and survival of *A*. sp. 17 polyps. Warming in surface waters would also expand the areas of unsuitable habitat in coastal areas for *Aurelia* sp. 9 and likely push polyps of this species to deeper waters. Our results are aligned with evidence from other studies (Pruski and Miglietta 2019; Lu, Lucas et al. 2020) that suggest that increasing water temperature may negatively impact jellyfish populations.

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4. TRENDS AND CHARACTERISTICS OF BLOOMING JELLYFISH (CLASS SCYPHOZOA, PHYLUM CNIDARIA) IN THE GULF OF MEXICO

4.1 Background

Jellyfish have historically been considered nuisance organisms, but research continues to show that they have numerous important functions in marine ecosystems with roles as predators, prey, in energy and nutrient flow, and as floating refuges for fish and invertebrates. In the GoM, Scyphozoan medusae are common prey items for the endangered Loggerhead Sea Turtle, *Caretta caretta* (Girard, Tucker et al. 2009), provide refuge for larvae of the commercially valuable Blue Crab, *Callinectes sapidus* (Frolova unpublished data), and form blooms consisting of billions of individuals (Robinson and Graham 2013), which consume enormous amounts of zooplankton and contribute to millions in economic loses by clogging shrimp nets (Graham, Martin et al. 2003). There is also an expanding medusae fishery in the Gulf of Mexico with boats operating out of Apalachicola and Port San Joe, Florida (Brotz, Schiariti et al. 2017). The fishery targets *Stomolophus* sp. medusae, which are harvested and processed in the U.S. then exported to Asia where they are a food item. Information on *Stomolophus* sp. is generally lacking for the region. There has only been one long-term study for the entire GoM that included *Stomolophus* sp. (Heim-Ballew and Olsen 2019).

Long-term studies on jellyfish abundance are lacking for most coastal regions including the GoM (Graham 2001; Purcell et al. 2007). Yet, such studies are essential for identifying blooms, predicting future states of jellyfish populations, and informing

management decisions. There is evidence that jellyfish populations may be increasing in some ecosystems (Mills 2001). An analysis of 66 Large Marine Ecosystems, showed 62% to have increasing jellyfish abundance trends over a 50-year time span (Brotz et al. 2012). Jellyfish appear to benefit from anthropogenic alterations to ecosystems, such as the installation of marine structures, eutrophication, and over-fishing. On the other hand, blooms appear to be a natural phenomenon in the jellyfish lifecycle where large scale climatic forces result in periodic pulses of medusae production (Condon, Duarte et al. 2013). Like all animals, jellyfish may also be vulnerable to warming, acidification, and other changes in ocean conditions that are currently taking place on our planet.

There have only been a few long-term abundance studies of medusae in the GoM. These have focused on identifying environmental drivers as well as attempting to tease out long-term trends. Graham (2001) analyzed trawl survey data of *Chrysaora* sp. and *Aurelia* spp. for summer and fall from about 1985 to 1997 for the geographical region bounded by Mobile Bay, Alabama and the northern border of Texas, finding that numbers of both species increased within the study region, however a follow-up study for the same area using a longer dataset, for the years 1987-2007 did not detect any trends in abundance for either genus, but did note that "populations experienced remarkable increases and decreases in size" (Robinson and Graham 2013). Heim-Ballew and Olsen (2019) did not find any significant trends in *Aurelia* spp. or *Chrysaora* sp. abundances and found current year abundance to not be strongly influenced by previous year abundance in Texas bays.

Little to nothing is known about the seasonal patterns and cycles of medusae presence and abundance, long-term patterns, and synchrony in medusae appearance among bays and neighboring GoM regions. Basic information on jellyfish phenology at local and regional scales is still missing for the GoM.

To address these gaps in knowledge for the northwestern GoM, we analyzed a 35-year empirical trawl survey dataset from the Texas Parks and Wildlife Department for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp. medusae abundances for eight bays and five GoM regions in coastal Texas. More specifically, our aims were to 1) identify patterns and blooms in long-term medusae abundance, 2) determine the seasonal timing of medusae occurrence in bays and adjacent GoM regions, and 3) determine whether seasonal medusae appearance is synchronous among bays and GoM regions.

4.2 Methods

4.2.1 Dataset overview

The dataset is a subset of the Gulf and Bay trawl surveys collected by the Texas Parks and Wildlife Department's long-term Marine Resource Monitoring Program. The dataset consists of abundance data for three Scyphozoan genera: *Aurelia* spp., *Chrysaora* sp., and *Stomolophus* sp. from 1983 through 2018. The medusae were not identified to species. However, there are three species of *Aurelia, A.* c.f. sp. 2*, A.* sp. 9*, and A.* sp. 17 (Chiaverano, Bayha et al. 2016; Frolova and Miglietta 2020), one species of *Chrysaora, C. chesapeakei* (Bayha, Collins et al. 2017) and one species of *Stomolophus, S. meleagris* (Gómez Daglio and Dawson 2017) known to inhabit the GoM.

The dataset consists of data for eight bays (Sabine Lake, Galveston Bay, Matagorda Bay, San Antonio Bay, Aransas Bay, Corpus Christi Bay, Upper Laguna Madre, and Lower Laguna Madre) and five adjacent GoM regions (Sabine Lake, Galveston Bay, San Antonio Bay, Corpus Christi Bay, and Lower Laguna Madre) along the Texas coast. The dataset contains 73,936 observational data points with 26,347 (36%) and 47,589 (65%) observations of medusae presence and absence, respectively.

4.2.2 Data collection

Trawls were pulled 16 times per month in the Gulf areas of Sabine Lake, Galveston Bay, Matagorda and San Antonio Bays, Aransas and Corpus Christi Bay, and Lower Laguna Madre (4.1.1). With one exception, regions were centered around major passes, extending 44.4 kilometers on either side and 16.7 kilometers offshore. The sampling area around Brazos Santiago Pass extended 14.8 kilometers south and 40.7 kilometers north of the pass. Sampling followed a stratified cluster design where grid locations were randomly selected for sampling without replacement for each month. The trawl used was a 6.1-meter-wide otter trawl with 38 mm stretched nylon multifilament mesh. Each trawl door was 1.2 meters long and 0.5 meters wide. Trawls were pulled at 3 miles per hour for 10 minutes parallel to fathom curve along the sea bottom in alternating directions until sampling for the particular grid was complete.

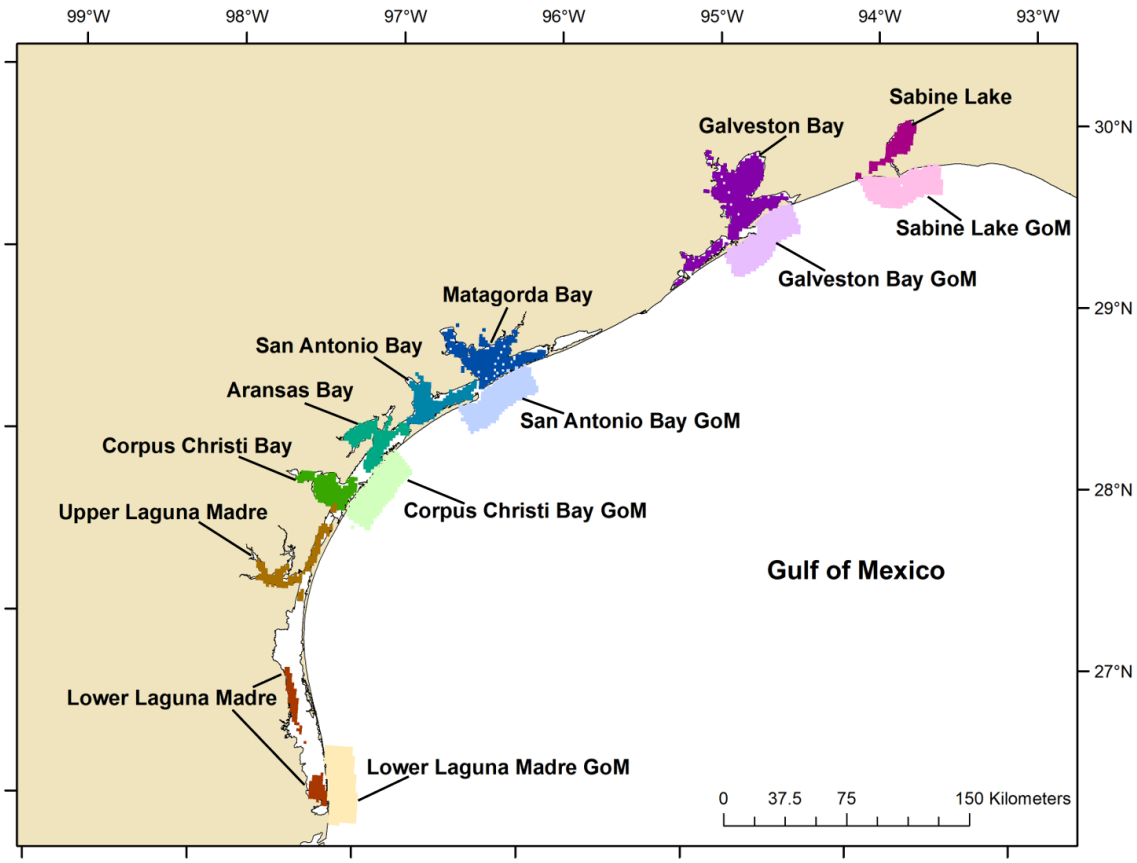

Figure 4.1 Map of Texas coastline with the bay and adjacent GoM region trawl sampling areas labeled. Trawl sampling area grids were centered around 5 major passes, extending 24 kilometers on each side and 16.7 kilometers offshore.

4.2.3 Long-term trends in medusae abundance

Scyphozoans have historically been neglected in long-term sampling studies. Even when reported, individual medusae were rarely counted. In the dataset analyzed here, *Stomolophus* sp. medusae were counted, while *Aurelia* spp. and *Chrysaora* sp. were usually assigned a density code, which introduced significant uncertainty into evaluating abundances. For this reason, count data was used here only to evaluate long-

term patterns where the goal was to identify periods of relative increase or decrease in medusae over time and dates of probable blooms.

Long-term trends across the 35-year dataset in medusae trawl catches were modeled using central moving averages of catch-per-unit-effort (CPUE, catch/trawl hour) data. For each species, data was filtered by area, then ordered by date and aggregated into total daily catch per hour values. Moving averages were calculated using the *rollmean* function in the zoo package in R (version 3.5.1). The width of the rolling window approximated the number of days with observations per year and was determined by dividing the number of rows in the aggregated dataset by the total length of time series dataset in years (34–37 depending on species and geographic region), then rounding to the nearest odd number to ensure equal numbers of days on both sides of the moving average. The resulting moving averages were plotted in R. Moving averages were used because they allow for improved visualization of trends when there is a high degree of fluctuation in the data. 'Blooms' were defined as years when CPUE increased by an order of magnitude or more from that of most years for that area.

4.2.4 Seasonal trends in medusae occurrence

Seasonal trends of medusae presence in each area were modeled using centered 61-day moving averages of presence/absence data, where each proportion is the average of all absences (0s) and presences (1s) for that day, the 30 days before and 30 days after that date. This window length was selected because it resulted in a sufficiently smooth curve. Trends were also described with smooth curves by fitting generalized additive

mixed models (GAMMs) to the presence/absence data using the mgcv (1.8-24) package in R. GAMMs used cyclic cubic regression splines and a binomial distribution. Analyses were performed separately for each species.

To compare timing of medusae presence between bay and neighboring GoM region and check for evidence of directional movement of medusae into bays from the GoM, or from the GoM into the bays, the spacing in time in adjacent bay-GoM systems was approximated using the sample cross correlation function (CCF) with GAMMs to estimate the lag for maximum positive correlation between the time series pairs (ex. Galveston Bay and Galveston GoM region or San Antonio Bay and San Antonio GoM region). For cross-correlation of the driver (ie., Galveston Bay or "x"), and the response variable (ie. adjacent GoM or "y"), then the correlation is computed between x[time + lag] and y[time]. Negative lags indicate events occurring in x before y, while positive lags indicate events occurring in y before x. Medusae "season" for each species was defined as the approximate span of dates from the initial increase in presence after the seasonal minimum to the end of the blooming period when medusae presence leveled off again to its seasonal minimum (Figure S1).

To identify trends in bloom timing across bays or GoM regions, the lag was calculated using CCF as described above, but for pairs of adjacent bays or for adjacent Gulf regions (i.e Galveston Bay and San Antonio Bay or Galveston GoM region and San Antonio GoM region).

4.3 Results

4.3.1 Long-term trends in medusae occurrence

The total number observations of medusae presence for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp. and each region are listed in table 4.2. Based on the total number of observations over the 35-year span, *Stomolophus* sp. were most frequent in the central Texas bays, namely Matagorda Bay, San Antonio Bay, Aransas Bay, and Corpus Christi Bay (1703, 839, 1444, 784 observations in each bay, respectively), with fewer occurrences in Galveston Bay (275 observations), while *Stomolophus* sp. were rarely observed in Sabine Lake and Upper and Lower Laguna Madre (13, 50 and 44 observations of medusae respectively). In the GoM, *Stomolophus* sp. medusae were most frequently observed in the regions adjacent to Sabine Lake, Galveston Bay, San Antonio Bay, Corpus Christi Bay (295, 268, 226, and 304 observations for each GoM region respectively) and only rarely in the region adjacent to Lower Laguna Madre (69 observations).

Based on the total number of observations of medusae presence, *Chrysaora* sp. medusae were commonly found in Galveston Bay, Matagorda Bay, San Antonio Bay, Aransas Bay, and Corpus Christi Bay (2072, 1126, 1365, 1978, 1144 observations in each bay, respectively. They were less frequent in Upper Laguna Madre (614 observations) and rare in trawls in Sabine Lake and Lower Laguna Madre (103 and 62 observations). In the GoM, there were 1173, 1177, 1051, 833, and 411 *Chrysaora* sp. observations for the regions nearby Sabine Lake, Galveston Bay, San Antonio Bay, Corpus Christi Bay, and Lower Laguna Madre, respectively.

Aurelia spp. medusae were observed most frequently in the central Texas bays with 766, 886, 1045, and 838 observations in Matagorda Bay, San Antonio Bay, Aransas Bay, and Corpus Christi Bay respectively. Galveston Bay and Upper Laguna Madre had 237 and 306 total *Aurelia* spp. observations, while *Aurelia* spp. were very rare in Sabine Lake and Lower Laguna Madre with only 19 and 36 total observations of medusae respectively. There were 696, 571, 622, 654, and 248 total *Aurelia* spp. observations for the GoM regions nearby Sabine Lake, Galveston Bay, San Antonio Bay, Corpus Christi Bay, and Lower Laguna Madre, respectively.

Table 4.1 Number of observations of medusae presence for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp. by region. Bay regions are Sabine Lake (SL), Galveston Bay (GB), Matagorda Bay (MB), San Antonio Bay (SAB), Aransas Bay (AB), Corpus Christi Bay (CCB), Upper Laguna Madre (ULM), and Lower Laguna Madre (LLM). Gulf of Mexico regions are that of Sabine Lake (SLG), Galveston Bay (GBG), San Antonio Bay (SABG), Corpus Christi Bay (CCBG) and Lower Laguna Madre (LLMG). Thick black line separates bay and Gulf regions.

	SL	GB	MB	SAB	AB	CCB	ULM	LLM	SLG	GBG	SABG	CCBG	LLMG
Stomolophus	13	275	1703	839	1444	784	50	44	295	268	226	304	69
Chrysaora	103	2072	1126	1365	1978	1144	614	62	1173	1177	1051	833	411
Aurelia	19	237	766	886	1045	838	306	36	696	571	622	654	248

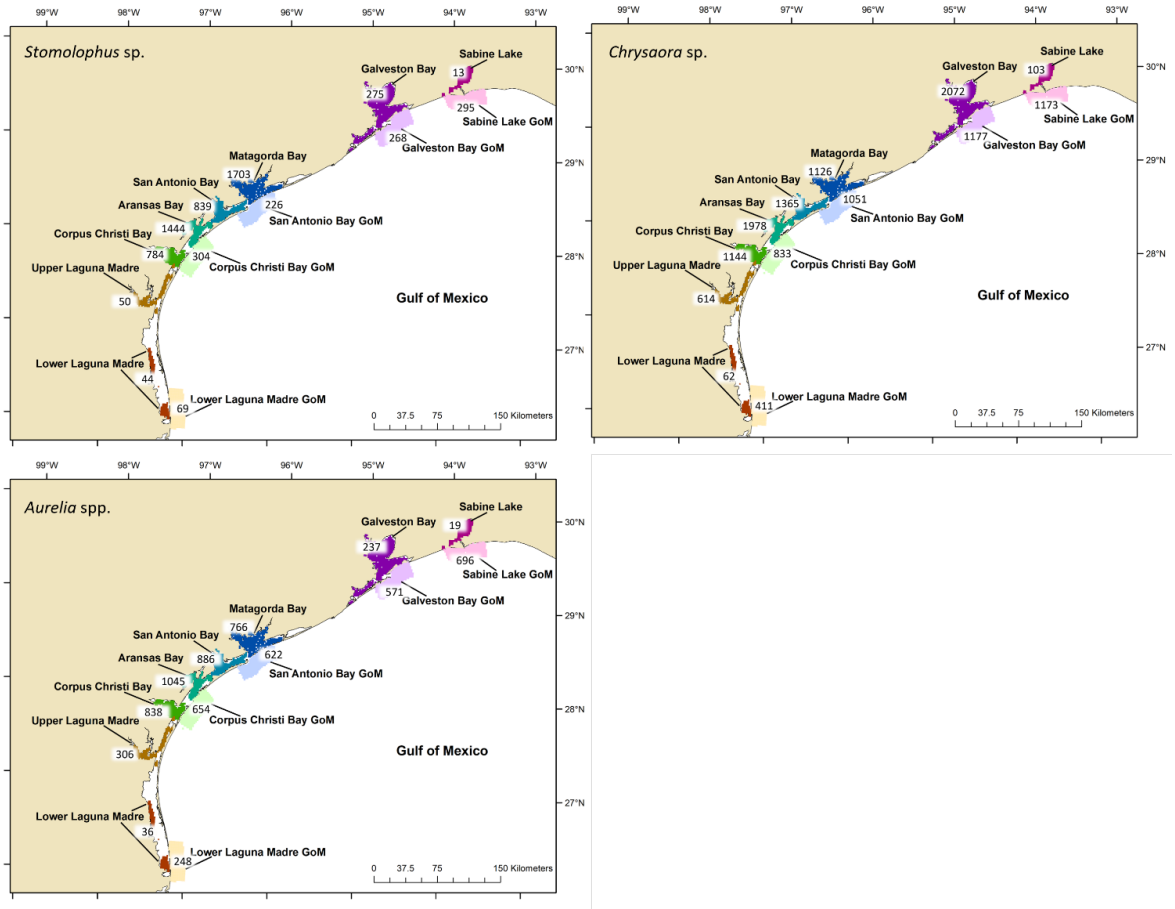

Figure 4.2 Number of observations of medusae presence for each area for *Stomolophus* sp. (upper left), *Chrysaora* sp. (upper right), and *Aurelia* spp. (bottom left).

The three genera had distinct patterns of CPUE (catch/trawl hour) over the 35-year period (Figure 4.3). Medusae CPUE fluctuated between years, with frequent 4 to 7-fold change between consecutive years. Trends were similar among bay and GoM regions for *Chrysaora* sp. and *Aurelia* spp., but differed between bays and the GoM for *Stomolophus* sp., for which numbers were overall higher in bays than in the GoM. For *Stomolophus* sp., there was a prominent spike in CPUE in bays from 1996 to 1997, while in the GoM, there was a series of smaller spikes from 1995 to 2006. *Stomolophus* sp.

CPUE has been consistently low since 2007, especially in the GoM. *Chrysaora* sp. had fairly consistent long-term patterns, at least as compared to the other genera, where CPUE fluctuated from approximately 200 to 700; patterns were highly similar between bay and GoM regions. Long-term trends in *Aurelia* spp. CPUE were highly irregular over the 35 years with multiple CPUE spikes with highly variable magnitude (multiple order of magnitude in consecutive years). Bays had generally lower *Aurelia* spp. CPUE than GoM regions, however there was a large CPUE spike around 2014 in both bays and the GoM (2012–2015 for bays and 2013–2015 for the GoM). Patterns and variation in CPUE in individual bay and GoM regions are shown in Supplementary Figures S1–S3.

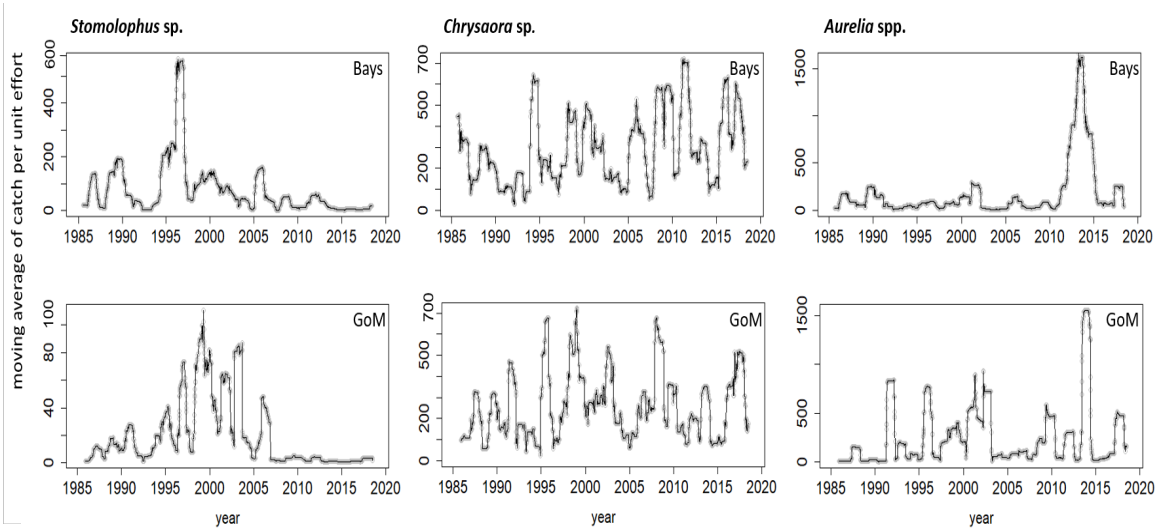

Figure 4.3 Moving annual averages of catch per unit effort (catch/trawl hour) for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp. in bays (top) and Gulf of Mexico regions (bottom) along the Texas coast for the years 1985–2019. Values on y-axis reflect the trends for the particular species and system and are not the same for all graphs.

4.3.2 Seasonal trends in medusae occurrence across bays and GoM regions

Synchrony between bay and GoM regions are depicted in Figures 4.4 and 4.5, 4.6 and 4.7, and 4.8 and 4.9 for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp., respectively. Lag times for positive correlations are listed for each genus and area combinations in Table 4.3. Significant negative correlations were observed for only a few areas and are noted in the text below.

4.3.2.1 *Stomolophus* sp.

Among the bays where medusae were common, seasonal trends were characterized by broad peaks with consistently high medusae occurrence for approximately 150 days of the year. In the bays, presence of *Stomolophus* sp. medusae increased in July and dropped to a seasonal minimum at the end of January. Medusae occurrences plateaued to a seasonal low in bays from February through April. Two-peak patterns, with peaks in August and November, were observed in San Antonio Bay and to a lesser extent in Corpus Christi Bay, with peaks in December and March. In Matagorda Bay, Aransas Bay, and Corpus Christi Bay medusae did not completely disappear between seasons. The number of observations of medusae presence over the 35-year period varied considerably among the bays with most occurrences in Matagorda and Aransas Bays and fewest occurrences in Sabine Lake and Upper and Lower Laguna Madre (Table 4.1).

CCF analyses indicated a north to south sequence in temporal progression of *Stomolophus* sp. occurrence for the northern five bays, Sabine Lake, Galveston Bay, Matagorda Bay, San Antonio Bay, and Aransas Bay, with timing lags of 24, 3, 0, and 6

days between adjacent bays (Table 4.2). This temporal progression between bays was not observed for locations south of Aransas Bay.

The probability of *Stomolophus* sp. presence is low in the GoM, where medusae were generally less common than in the bays. In the GoM, *Stomolophus* sp. medusae were present October through April with peaks in late October (Sabine Lake region), mid-November (Galveston Bay region), end of December (San Antonio Bay and Corpus Christi Bay regions), and at the end of January (Lower Laguna Madre region). Medusae presence in GoM regions nearby Sabine Lake and Galveston Bay peaked twice, once in April and again in November. CCF analyses (Table 4.2) suggest a north to south temporal progression among the GoM regions where *Stomolophus* sp. occurrence in the Sabine Lake region led those in the Galveston Bay region by 13 days, which in turn led the San Antonio and Corpus Christi Bay regions also by 13 days. Trends in San Antonio Bay GoM and Corpus Christi Bay GoM regions occurred simultaneously and led the Lower Laguna Madre GoM region by roughly 17 days.

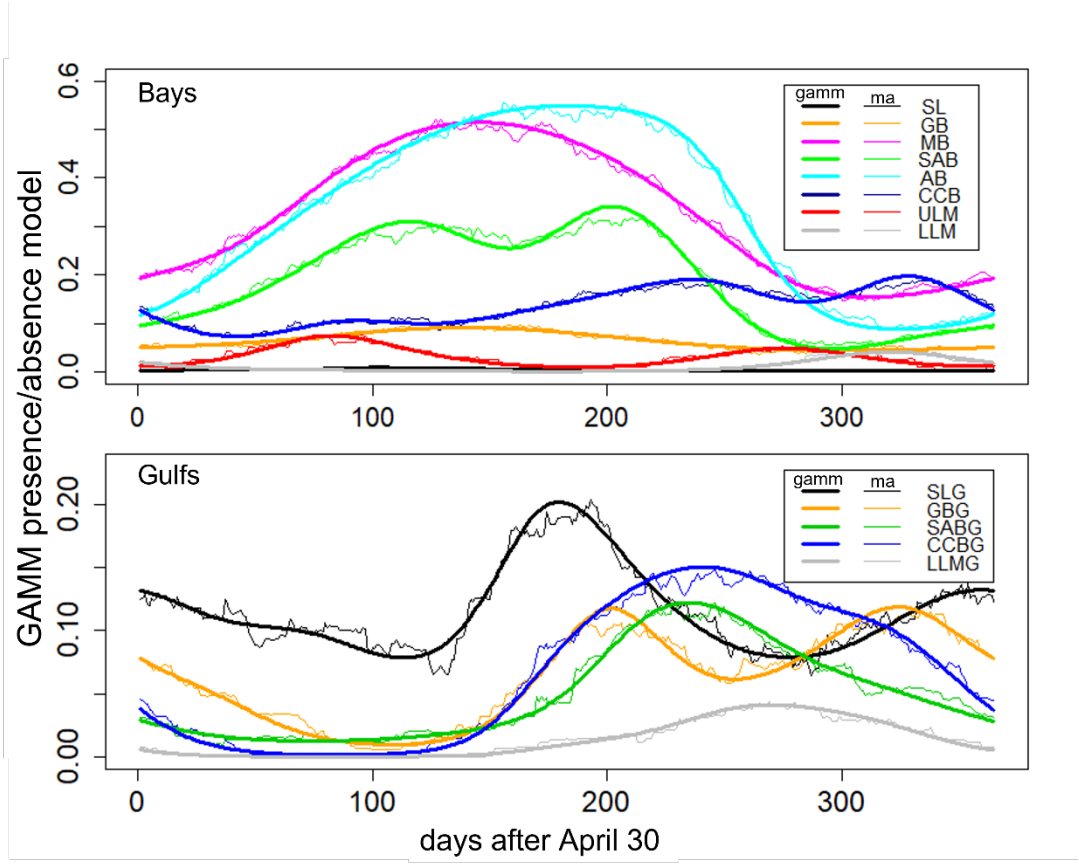

Figure 4.4 Generalized additive mixed models (thick lines) of *Stomolophus* sp. seasonal medusae presence and absence in bays (top) and Gulf of Mexico regions (bottom) along the Texas coast from 1983–2019. 61-day moving averages are included (thin lines) for reference of actual trend. Different colors depict correspond to coastal regions specified by acronyms. Bay regions are Sabine Lake (SL), Galveston Bay (GB), Matagorda Bay (MB), San Antonio Bay (SAB), Aransas Bay (AB), Corpus Christi Bay (CCB), Upper Laguna Madre (ULM), and Lower Laguna Madre (LLM). Gulf of Mexico regions are that of Sabine Lake (SLG), Galveston Bay (GBG), San Antonio Bay (SABG), Corpus Christi Bay (CCBG) and Lower Laguna Madre (LLMG).

Seasonal moving average trends for *Stomolophus* sp. revealed that medusae presence is asynchronous between bays and their adjacent GoM regions (Figure 4.5. See also Table 4.2). For most regions, medusae appeared in bays in the summer, but were not observed in the GoM until fall. CCF analysis showed a significant negative correlation

of -0.8 at a lag of +35 days for Galveston Bay and the adjacent GoM region, meaning that medusae presence in the Bay and GoM is in opposite phases, such that a maximum in presence is observed in Galveston Bay 35 days after a minimum occurred in the GoM.

CCF results confirmed that medusae presence was not tightly linked to presence in the GoM in Matagorda, San Antonio, and Aransas Bays, as lag times were large (lag of -94, 90, and -79 days with correlations of 0.655, 0.537 and 0.648 for Matagorda, San Antonio, Aransas Bays and their adjacent GoM regions, respectively). Medusae presence in Corpus Christi Bay lagged the nearby GoM by 5 days (correlation 0.89); this was the only Bay-GoM system where *Stomolophus* sp. observations were synchronous. Negative correlations were detected for Upper Laguna Madre and the nearby GoM (correlation of -.402 at 13 days, with the GoM leading) and for Lower Laguna Madre and the nearby GoM (correlations -0.947 and 49 days, with the GoM leading).

Table 4.2 CCF results. Magnitude of maximum positive correlation and corresponding lag time in days for each bay and adjacent GoM region for *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* spp. Significant negative correlations were present in only a few cases and are noted in the text. Negative lag times with positive correlation indicate that presence in the first area (as listed in the first column) leads presence in the second area by the number of days indicated by the lag. Positive lag times indicate that presence in the second area leads presence in the first. No meaningful function (NMF) indicates that correlation did not peak during the ± 100 day window. All values in the table were significant at the 0.05 level.

	Stomolophus sp.		*Chrysaora* sp.		*Aurelia* spp.	
Bay and Adjacent GoM region	**Lag**	**Correlation**	**Lag**	**Correlation**	**Lag**	**Correlation**
Sabine Lake & Nearby GoM	-70	0.508	0	0.941	-1	0.954
Galveston Bay & Nearby GoM	NMF	NA	7	0.831	-14	0.93
Matagorda Bay & Nearby GoM	-94	0.655	18	0.684	-52	0.765
San Antonio Bay & Nearby GoM	-90	0.537	-2	0.766	-51	0.685
Aransas Bay & Nearby GoM	-79	0.648	-12	0.731	-42	0.651
Corpus Christi Bay & Nearby GoM	5	0.89	0	0.689	-10	0.488
Upper Laguna Madre & Nearby GoM	NMF	NA	NMF	NA	NMF	NA
Lower Laguna Madre & Nearby GoM	49	0.947	NMF	NA	-30	0.588
Consecutive Bays						
Sabine Lake & Galveston Bay	-24	0.96	-15	0.851	-2	0.962
Galveston Bay & Matagorda Bay	-3	0.976	0	0.978	7	0.935
Matagorda Bay & San Antonio Bay	0	0.948	15	0.94	0	0.966
San Antonio Bay & Aransas Bay	-6	0.944	-8	0.988	-7	0.979
Aransas Bay & Corpus Christi Bay	NMF	NA	4	0.934	0	0.855
Corpus Christi Bay & Upper Laguna Madre	NMF	NA	18	0.879	14	0.753
Upper Laguna Madre & Lower Laguna Madre	NMF	NA	0	0.945	NMF	NA
Consecutive GoM Regions						
Sabine Lake & Galveston Bay	-13	0.453	3	0.871	-11	0.945
Galveston Bay & San Antonio Bay	-13	0.602	7	0.893	-9	0.967
San Antonio Bay & Corpus Christi Bay	0	0.961	-3	0.907	0	0.982
Corpus Christi Bay & Lower Laguna Madre	-17	0.938	-9	0.552	-31	0.605

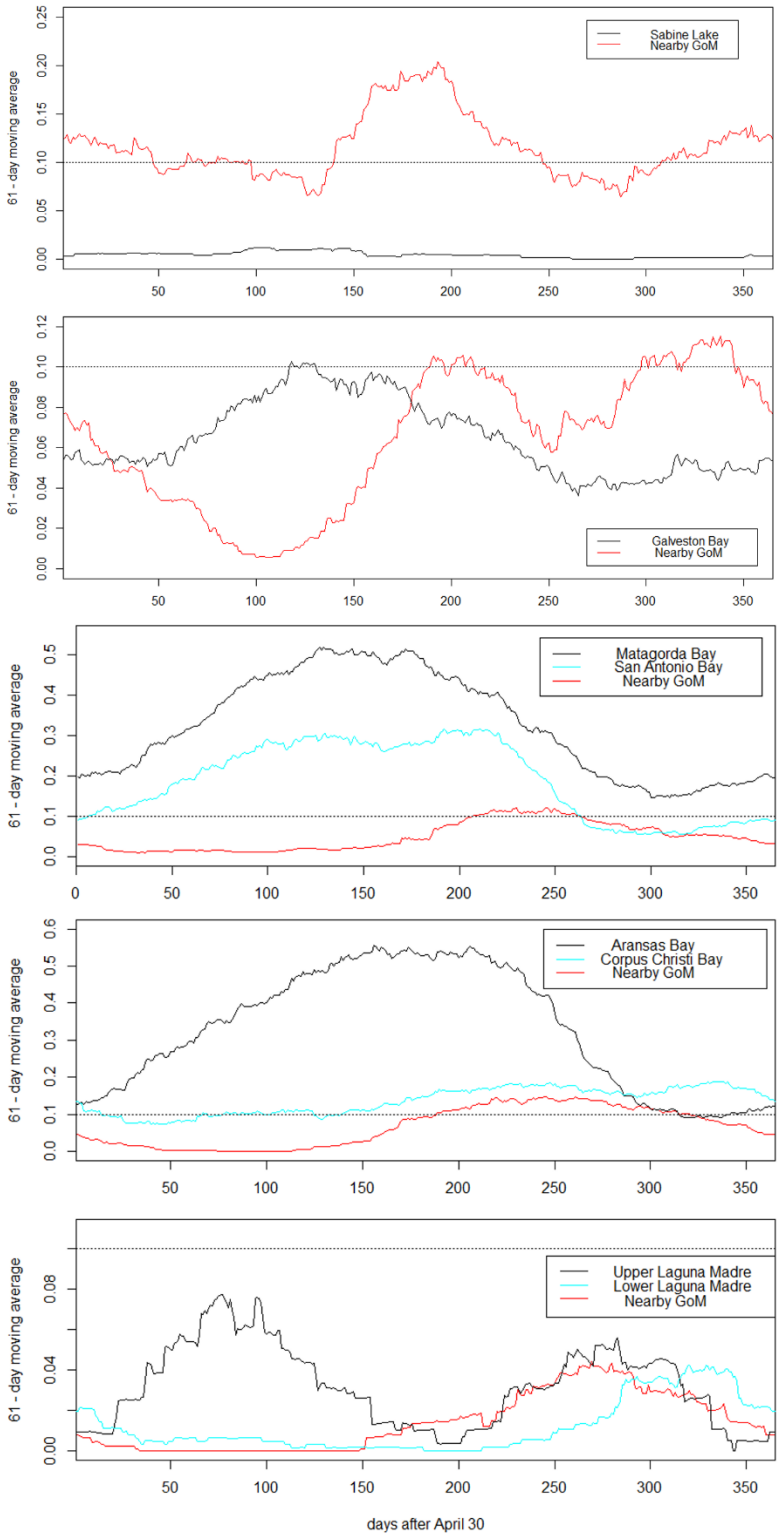
Sabine Lake
Nearby GoM
Galveston Bay
Nearby GoM
Matagorda Bay
San Antonio Bay
Nearby GoM
Aransas Bay
Corpus Christi Bay
Nearby GoM
Upper Laguna Madre
Lower Laguna Madre
Nearby GoM
61 - day moving average
days after April 30

Figure 4.5 61-day moving averages of *Stomolophus* sp. presence and absence for Texas bays and corresponding nearby Gulf of Mexico region. Each plot represents the moving average in the bay and adjacent GoM region organized from north (top) to south (bottom) along the Texas coast. Black and green lines indicate bays, red lines indicate nearby Gulf of Mexico waters. Bays are defined in the legend of each plot.

4.3.2.2 *Chrysaora* sp.

Chrysaora sp. medusae are present in Texas bays and GoM regions from April through December, disappearing completely between seasons in all areas except Upper Laguna Madre as well as the Lower Laguna Madre GoM region. Medusae appear slightly earlier in bays and decrease later than in the GoM. The number of observations of medusae presence over the 35-year period were consistent among the central bays of Texas, but Sabine Lake and Lower Laguna Madre had few occurrences (Table 4.1).

In bays, seasonal trends in medusae occurrence are characterized by broad peaks. *Chrysaora* sp. increase quickly in bay trawls and are found from April through mid-November. Medusae decrease and disappear at about the same time in November in all bays with exception of Aransas Bay, where medusae incidence stays at a maximum for approximately one additional month.

CCF analyses showed consistently strong positive correlation with lag times near zero for pairs of consecutive bays and without directional trends in timing (Table 4.2), indicating synchrony in *Chrysaora* sp. presence.

In the GoM regions, seasonal trends of *Chrysaora* sp. are characterized by the presence of two distinct peaks, the first in early June and a second in mid-October with a

clear decrease in medusae presence during late summer through early fall (Figure 4.6 and 4.7). This trend is observed for all GoM regions except for that of Sabine Lake, where medusae presence was modeled by a single broad peak with a maximum in mid-July. GAMMs show that incidence of *Chrysaora* sp. was highest in the northernmost GoM regions decreasing steadily in each subsequent GoM region down the Texas coast (Figure 4.7). CCF analysis showed strong positive correlation with lag times around zero without directional trends in timing for pairs of consecutive GoM regions, indicating synchrony in *Chrysaora* sp. presence.

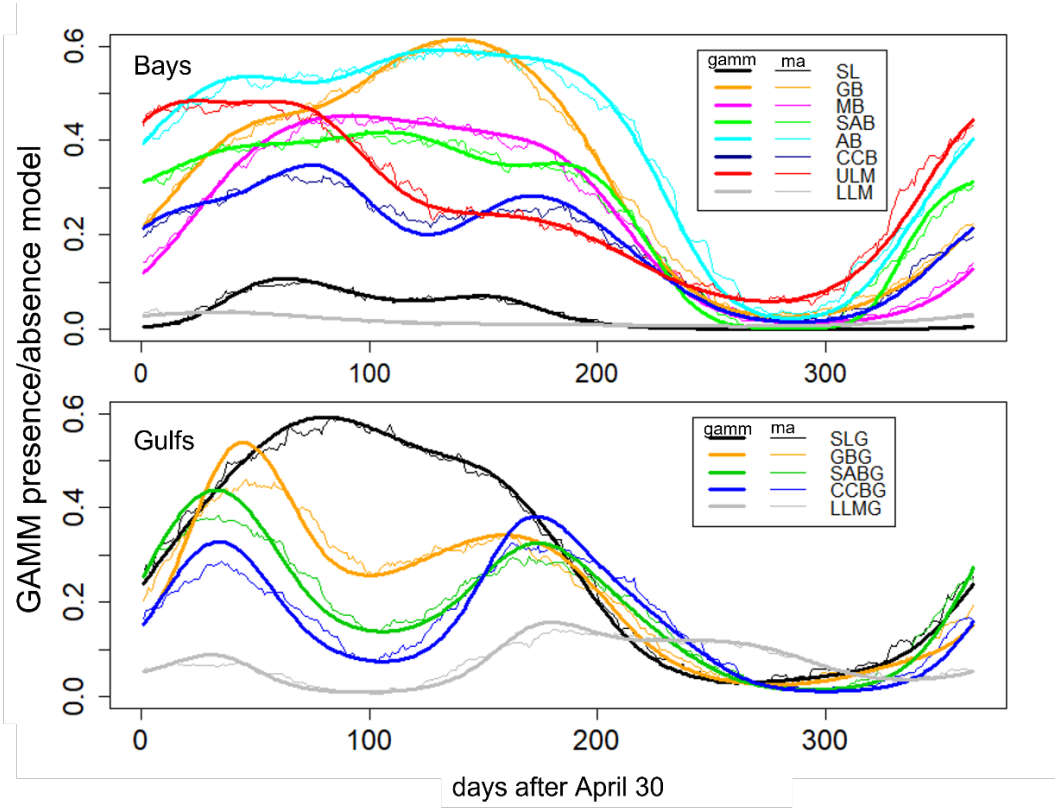

Figure 4.6 Generalized additive mixed models (thick lines) of *Chrysaora* sp. seasonal medusae presence and absence in bays (top) and Gulf of Mexico regions (bottom) along the Texas coast from 1983–2019. 61-day moving averages are

included (thin lines) for reference of actual trend. Different colors depict correspond to coastal regions specified by acronyms. Bay regions are Sabine Lake (SL), Galveston Bay (GB), Matagorda Bay (MB), San Antonio Bay (SAB), Aransas Bay (AB), Corpus Christi Bay (CCB), Upper Laguna Madre (ULM), and Lower Laguna Madre (LLM). Gulf of Mexico regions are that of Sabine Lake (SLG), Galveston Bay (GBG), San Antonio Bay (SABG), Corpus Christi Bay (CCBG) and Lower Laguna Madre (LLMG).

Seasonally *Chrysaora* sp. appeared at about the same time in bays and adjacent GoM regions across the Texas coast (Figure 4.6). Presence in the GoM regions led presence in bays in the Galveston and Matagorda Bay areas, while presence in bays led presence in the nearby GoM region in the Aransas Bay area. Medusae were only rarely observed in Sabine Lake, but were common in the nearby GoM, and presence was simultaneous in the two areas (lag 0, correlations 0.941). In the Galveston Bay and adjacent GoM, medusae were found at about the same time (bay lagged the GoM by 7 days, correlation 0.689) but the GoM had two peaks in medusae presence, while the Bay had one during the same period. A similar trend was observed for Matagorda and San Antonio Bays and their adjacent GoM region, as well as Aransas Bay and its adjacent GoM, where medusae occurred at about the same time (lags of 2 to 18 days), but with two peaks in the GoM whereas presence in the Bays peaked once. *Chrysaora* sp. presence in Corpus Christi Bay was simultaneous with presence in the nearby GoM (lag 0, correlation 0.689) with trends in the two areas having similar magnitude and shape, just as was the case for *Stomolophus* sp. in this area. Medusae were not common in the Laguna Madre area. Positive correlations were not observed for Upper or Lower Laguna Madre and the nearby GoM, however negative correlations were observed for Upper Laguna Madre and the adjacent GoM (lag of 17 and correlation of 0.5 with Laguna

Madre leading) and for Lower Laguna Madre and adjacent GoM (lag of 45 ways with correlation of 0.580 with the GoM leading).

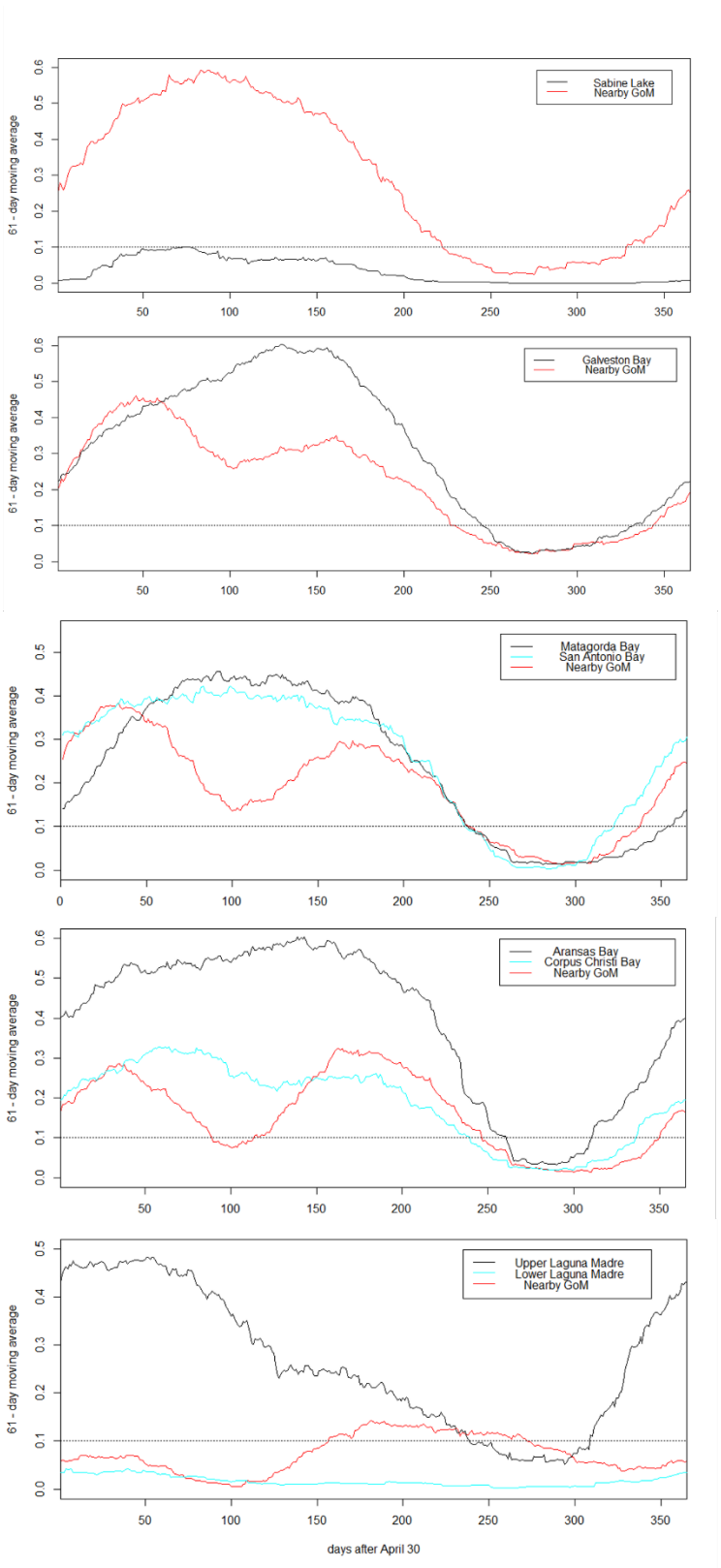
Sabine Lake
Nearby GoM
Galveston Bay
Nearby GoM
Matagorda Bay
San Antonio Bay
Nearby GoM
Aransas Bay
Corpus Christi Bay
Nearby GoM
Upper Laguna Madre
Lower Laguna Madre
Nearby GoM
61 - day moving average
days after April 30

Figure 4.7 61-day moving averages of *Chrysaora* sp. presence and absence for Texas bays and corresponding nearby Gulf of Mexico region. Each plot represents the moving average in the bay and adjacent GoM region organized from north (top) to south (bottom) along the Texas coast. Black and green lines indicate bays, red lines indicate nearby Gulf of Mexico waters. Bays are defined in the legend of each plot.

4.3.2.3 *Aurelia* spp.

Seasonal patterns for *Aurelia* spp. were markedly different between bays and GoM regions (Figure 4.8 and 4.9). Medusae were observed in bays starting earlier in the year than in the GoM. *Aurelia* spp. medusae were found in bays along the Texas coast from April through mid-January. Medusae appeared first in Aransas Bay at the beginning of April, then San Antonio Bay, Corpus Christi Bay, and Upper Laguna Madre in mid-April. In Matagorda Bay and Galveston Bay medusae appeared in late May and mid-August, respectively. CCF analyses showed strong positive correlations with minimal lag times and no directional progression in timing for medusae presence in the first six Texas bays (Sabine Lake, Galveston Bay, Matagorda Bay, San Antonio Bay, Aransas Bay, and Corpus Christi Bay), suggesting near simultaneous appearance of medusae in these bays. Corpus Christi Bay lagged Lower Laguna Madre by approximately 14 days and had a strong positive correlation. Meaningful positive correlations were not observed for Upper Laguna Madre and Lower Laguna Madre within the ± 100 window.

Aurelia spp. medusae were observed in the GoM regions from July through January. Medusae appeared first in the Sabine Lake GoM region in July, then in Galveston Bay, San Antonio Bay, and Corpus Christi Bay GoM regions in September.

Aurelia spp. were rare in the Lower Laguna Madre GoM region and trends in that region were weak. In the Galveston Bay GoM region, *Aurelia* spp. season ends in mid-January and one month later in the other GoM regions. CCF analyses showed strong positive correlations between adjacent GoM regions with a north to south progression in timing, Lag times for adjacent GoM regions were 11, 9, 0, and 31 days for Sabine Lake and Galveston Bay GoM regions, Galveston Bay and San Antonio Bay GoM regions, San Antonio Bay and Corpus Christi Bay GoM regions, and Corpus Christi Bay and Lower Laguna Madre GoM regions, respectively.

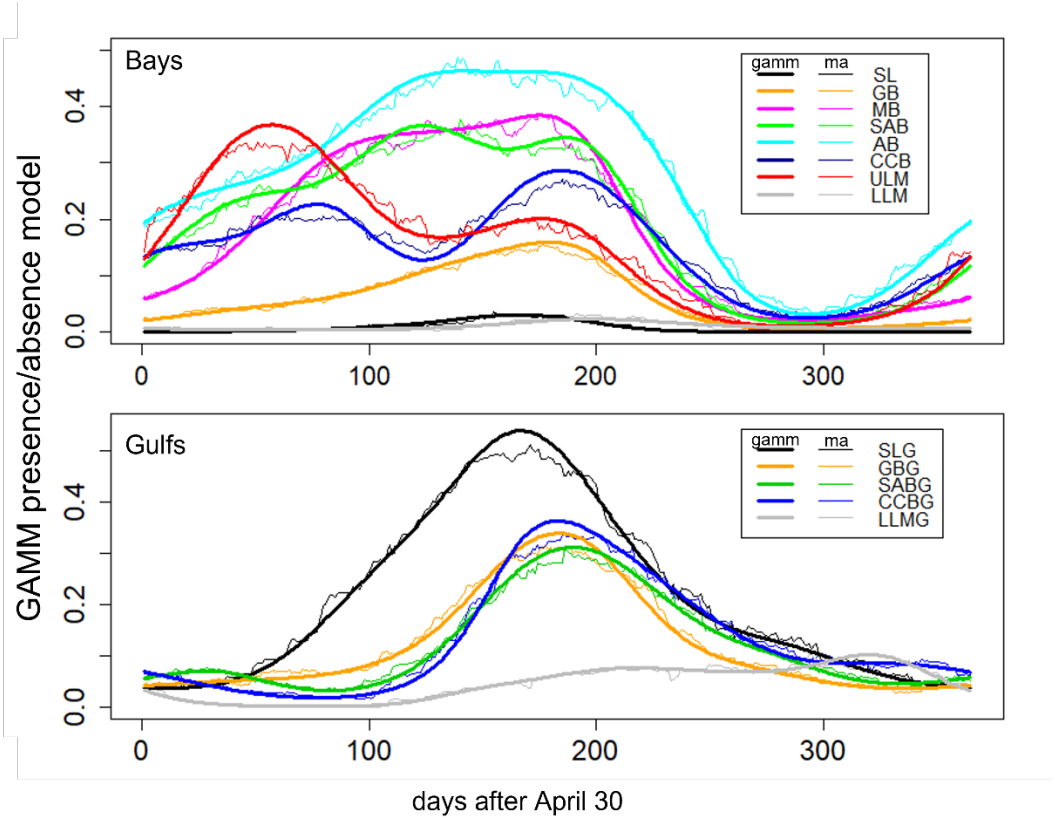

Figure 4.8 Generalized additive mixed models (thick lines) of *Aurelia* spp. seasonal medusae presence and absence in bays (top) and Gulf of Mexico regions (bottom) along the Texas coast from 1983–2019. 61-day moving averages are included (thin lines) for reference of actual trend. Different colors depict correspond to coastal regions specified by acronyms. Bay regions are Sabine Lake (SL), Galveston Bay (GB), Matagorda Bay (MB), San Antonio Bay (SAB), Aransas Bay (AB), Corpus Christi Bay (CCB), Upper Laguna Madre (ULM), and Lower Laguna Madre (LLM). Gulf of Mexico regions are that of Sabine Lake (SLG), Galveston Bay (GBG), San Antonio Bay (SABG), Corpus Christi Bay (CCBG) and Lower Laguna Madre (LLMG).

Occurrence of *Aurelia* spp. were extremely rare in Sabine Lake (Figure 4.8), but medusae appeared at the same time as in the nearby GoM (lag -1, correlation 0.954). During peak season medusae were observed twice as frequently in the GoM region as in Galveston Bay, with presence in the Bay leading presence in the GoM by two weeks.

Matagorda Bay, San Antonio Bay, and Aransas Bay led *Aurelia* spp. presence in the nearby GoM regions by 52, 51, and 45 days, respectively (correlations of 0.765, 0.685, and 0.651 respecitvely). Medusae were observed more frequently in these bays than in the nearby GoM regions. For the Corpus Christi area, the trend was like that observed for *Stomolophus* sp. and *Chrysaora* sp. where the seasonal trend in the Bay closely resembled that in the adjacent GoM; here, presence in Corpus Christi Bay led the GoM region by 10 days.

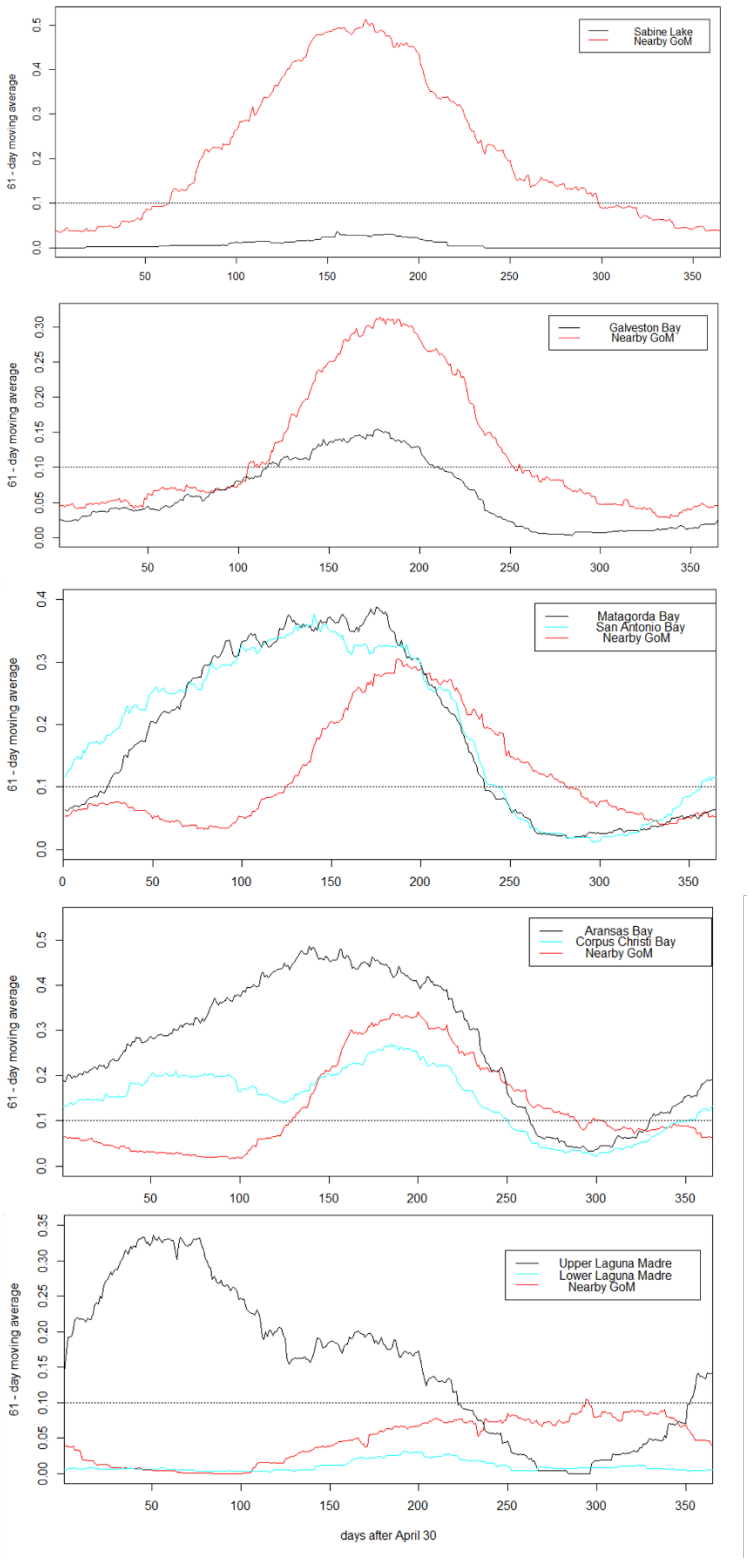
Sabine Lake
Nearby GoM
Galveston Bay
Nearby GoM
Matagorda Bay
San Antonio Bay
Nearby GoM
Aransas Bay
Corpus Christi Bay
Nearby GoM
Upper Laguna Madre
Lower Laguna Madre
Nearby GoM
61 - day moving average
days after April 30

Figure 4.9 61-day moving averages of *Aurelia* spp. presence and absence for Texas bays and corresponding nearby Gulf of Mexico region. Each plot represents the moving average in the bay and adjacent GoM region organized from north (top) to south (bottom) along the Texas coast. Black and green lines indicate bays, red lines indicate nearby Gulf of Mexico waters. Bays are defined in the legend of each plot.

4.4 Discussion

4.4.1 Abundance and blooms

Abundance of medusae belonging to the three genera varied considerably between years. Moving averages of *Stomolophus* sp. CPUE for each area reveal higher numbers of medusae during peak years in bays than in the GoM regions (Supplementary Figures S1–S3). *Stomolophus* sp. presence was consistently "high" during 1995–2006 in all GoM regions, except for Upper Laguna Madre. Similarly, bays experienced increased presence during some or all of the years during this time period. A large spike in *Stomolophus* sp. CPUE occurred in San Antonio Bay and Aransas Bay in 1996–1997 and may have been a true bloom (meaning that an increase in medusae production occurred during this time, as opposed to an apparent bloom where medusae are aggregated by physical oceanographic processes), because CPUE in these bays had otherwise historically been very low. *Stomolophus* sp. medusae CPUE dropped to a minimum and remained at a constant low since 2006 for all areas except Aransas Bay. The cause of this decline in numbers is not known as there is little information about the biology of this species.

The low catch numbers for this species are concerning from a fishery management point of view. The drop in CPUE and absence of fluctuations for over a decade in all GoM regions and most bays raises the question of whether populations of

Stomolophus sp. may have decreased due to over-harvest of the sexually reproducing adults. Primarily considered a nuisance to shrimpers, the *Stomolophus* sp. fishery began in the 1990s in the Florida panhandle in part as a means to remove medusae from the environment (Brotz, Schiariti et al. 2017). Additional information on the biology of this species as well as long-term survey data is greatly needed to clarify whether overharvest is occurring and inform management decisions of the *Stomolophus* sp. industry.

Our analyses show that *Chrysaora* sp. has been seasonally present with consistent CPUE in most of the areas for the 35-years analyzed. Its presence in the Lower Laguna Madre GoM region increased from about 1996 to 2004, with multiple years showing that CPUE increased by a factor of ten or more. There were few *Chrysaora* sp. in San Antonio Bay trawls up until 1995, with significant increase (CPUE between 200 and 600) during most years afterwards. A bloom of *Chrysaora* sp. occurred in Aransas Bay during 1994–1995 (CPUE rose to over 2000). Although *Chrysaora* sp. are generally few in Upper Laguna Madre, blooms occurred in this area during 2011–2012 and 2015–2017 (CPUE of about 1000 and 1500, respectively).

Our analyses reveal strong fluctuations in *Aurelia* spp. CPUE occurring multiple times over the course of the 35-year span, with medusae presence increasing by a factor of ten for about one year then returning to baseline levels each time. A comparison of moving averages between regions also reveals bloom events in most bay and GoM regions in 1991–1992, 2000–2003 and 2013–2014, as well as an additional bloom in the San Antonio and Corpus Christi GoM regions during 1995–1996. The presence of

multiple sympatric species may help explain the great difference in CPUE observed between years only for *Aurelia* spp.

4.4.2 Seasonal patterns between bays and the GoM

Seasonal patterns in bays differ from those in GoM regions for all three genera and especially for *Stomolophus* sp. This suggests that medusae presence in bays is controlled by different factors than in the GoM. There is also evidence that *Stomolophus* sp. medusae move from bays into the GoM. The observed lag times indicate that medusae take multiple months to move into the GoM from the bays.

Stomolophus sp. medusae first appear in the bays in July then in the GoM in October. Unlike *Aurelia* spp. and *Chrysaora* sp., *Stomolophus* sp. are strong swimmers capable of swimming against and across a current, so it is possible that medusae behavior is responsible for the delay in medusae appearance in the GoM. Alternatively, the difference in medusae timing could be explained by the presence of multiple *Stomolophus* species that strobilate at different times and inhabit distinct habitats in bays and GoM. Scyphozoan biodiversity has not been extensively sampled in the northwestern GoM and cryptic species are common in Scyphozoa and in *Stomolophus*. A recent study of species richness in the Tropical Eastern Pacific, Gómez Daglio and Dawson (2017) showed that *Stomolophus meleagris* is a complex of five undescribed species. Finally, it is possible is that medusae observed during the fall in the GoM are transported there by longshore currents from the northeast, possibly from coastal Louisiana. There is an organized westward longshore current and wind stress that begins

in the northwestern GoM in September, which could theoretically facilitate the transport of medusae from coastal habitats from farther up the coastline.

Our results on *Stomolophus* sp. timing in the GoM and its bays are consisten to those described for S. sp. from the east coast of the USA where is has been shown that S. sp. appears first in estuaries in July and then enters the ocean as a mature adult at the end of September (Kraeuter and Setzler 1975).

Our analyses show that across the bays, the presence of all three species are tightly linked across the bays (with a few areas that were exceptions for *Stomolophus* sp. and one for *Aurelia* spp.) as well as among the GoM regions (especially along the north and central Texas coast). We also show a north to south progression in medusae presence among GoM regions and bays for *Stomolophus* sp. and among GoM regions for *Aurelia* spp. *Chrysaora* sp. appear near simultaneously in bays and GoM regions similarly to *Aurelia* spp. in bays. The high degree of temporal correlation observed within each genus in bays suggests that polyp populations are distributed among the bays, and that broad scale forces, as opposed to localized conditions, trigger strobilation of polyps. Furthermore, the high degree of synchrony also means that medusae presence in one bay, can be used to predict medusae presence in other bays, and that presence in north GoM regions can predict presence in GoM locations down the coast to the southwest.

The relationship between presence of medusae in the bays and their adjacent GoM regions is not consistent, and its interpretation is complicated by the long lag times between the appearance of the medusae in the two environments. Relationships also

seem to vary by genera and areas. *Chrysaora* sp. presence appears tightly linked between bays and their adjacent GoM regions, although the correlation strength is weaker than correlation for neighboring bays or for adjacent GoM regions. For *Stomolophus* sp., correlation values suggest a possible link, but the long lag times, which are greater than 70 days for four of the six regions where correlation values were available, and low average presence, especially in the GoM, complicate interpretation. For *Aurelia* spp., the lag magnitudes between bays and adjacent GoM regions vary considerably (-1 to -52 days) depending on the area. These lags are not observed among bays, or GoM regions, which suggests that presence of *Aurelia* spp. medusae may be controlled by different drivers among the different Bay-GoM systems along the Texas coast. It is also possible that the variation is due to the confounding presence of multiple *Aurelia* species in some of the areas. A correlation in medusae presence between Upper and Lower Laguna Madre as well as associated GoM regions could not always be determined, likely due to the small total number of medusa observations in these areas (see Table 4.2).

Despite the existence of an active fishery for *Stomolophus* sp., markedly little information is available on the population size, population dynamics, or phenology of this species in the GoM. The finding of low *Stomolophus* sp. abundances since 2006 along the Texas coast is concerning given the expanding industry and prompts an immediate need for additional abundance and fishery data (eg. catch sizes, dates and duration of season, fleet size) for this species. There is a possibility that the reduced numbers could be linked to the removal of medusae from the environment by the fishery.

Although the current fishery is restricted to the northeastern GoM, and the only other *Stomolophus* sp. fishery in the GoM relocated from the region in 2001 (Brotz, Schiariti et al. 2017), connectivity among jellyfish populations throughout the GoM may be affecting *Stomolophus* sp. catches along the Texas coast. In either case, the low abundances observed in this study may be useful for fishery management if there is interest in expanding or moving the fishery to the northwestern GoM.

In a global change context, patterns of medusae presence in the GoM are likely to be affected by climate shifts. Indeed, changes such as increases in biomass, expansion into new areas, and phenological changes have been reported from other ecosystems (Gibbons and Richardson 2008; Richardson and Gibbons 2008; Van Walraven, Langenberg et al. 2015). Medusae production is tightly linked to water temperature, which has been increasing in the GoM (Turner, Rabalais et al. 2017) with warming attributed mostly to an increase in summer rather than winter temperatures. In the western Wadden Sea, increases in mean summer temperature were linked to extended duration of *Chrysaora hysoscella* occurences (Van Walraven, Langenberg et al. 2015), while increases in winter temperatures were correlated with earlier timing of *Aurelia aurita* appearance. This study provides a synopsis of phenological patterns for three common bloom-forming genera in the GoM, which can be used for developing hypotheses on the effects of climate change on jellyfish phenology.

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5. CONCLUSIONS

5.1 Research summary

Aurelia is one of the best-studied genera of Scyphozoa (Cnidaria). However, its diversity (number of species) is far from settled. In Chapters 2 and 3, we provide molecular evidence, corroborated by anatomical and morphometric features, of a new Scyphozoan species belonging to the well-studied genus *Aurelia* from the northern GoM. This is the third *Aurelia* species identified in the GoM, but its evolutionary history is distinct from *A.* sp. 9 and *A*. sp. c.f. 2. Also in Chapter 2, we determined the temperature and salinity tolerance limits for three *Aurelia* species, two native to the GoM, and *Aurelia coerulea*, a species that has colonized three different continents and is sometimes invasive. This is the first report of temperature and salinity ranges and tolerance limits for *Aurelia* species. Environmental tolerance ranges and limits strongly influence the distribution of marine species and are useful for understanding present polyp distribution and how distribution may change in climate change scenarios. Although polyps of *A*. sp. 9 have never been found, polyps of *A. coerulea* have been located in coastal regions such as bays and marinas. The significantly narrower thermal tolerance range of *A.* sp. 17 as compared to *A*. *coerulea* and *A.* sp. 9, points to preference for a habitat with greater stability in water temperature than the variable conditions characteristic of shallow coastal waters. Taken together with the results of the habitat suitability analysis in Chapter 3, our results strongly indicate *A.* sp. 17 to be an offshore species that prefers deeper waters with little temperature variation. In Chapter 3, we aim

to shed light on the possible locations of polyp populations by using the environmental tolerance results from Chapter 2 to construct habitat suitability maps for *Aurelia* sp. 9 and *Aurelia* sp. 17. We show that suitable habitats for *Aurelia* sp. 17 polyps on the continental shelf generally begin at 30 meters below the surface, while shallower regions are unsuitable. For both *A*. sp. 17 and *A.* sp. 9 polyps, temperature and not salinity, limits the distribution of polyps. Our habitat suitability analyses also show that the vast majority of GoM artificial reefs, coral reefs, and gas platforms are suitable habitats for *Aurelia* sp. 9 polyps. *A.* sp. 17 has, on the contrary, more limited availability, with well under half of the reefs and gas platform offering suitable habitats.

Polyps ultimately produce medusae, which are conspicuous and fortunately are recorded in some marine surveys. Studies of medusae presence and abundance trends in the GoM can shed light on the dynamics of jellyfish populations in the region (eg. whether a population is regularly or rarely producing medusae and blooms). While studies of medusae phenology may indicate where polyp populations are located (eg. bay, GoM, and location along coast). In Chapter 4, we shifted the focus to medusae in the northwestern GoM. We analyzed a 35-year trawl survey dataset, using moving averages and GAMMs and CCFs to model abundance and presence of medusae belonging to *Stomolophus* sp., *Chrysaora* sp., and *Aurelia* sp., across 8 bay and 5 GoM regions of the Texas coast. We show that these three genera have distinct patterns of abundance over the 35-year study span. That *Stomolophus* sp. medusae are observed more frequently and at higher numbers in bays where they first appear in mid July; they then move out into GoM waters in early October, where their occurrence is brief and

numbers are generally low. Abundance trends reveal catch sizes of *Stomolophus* sp. medusae to remain very low since about 2006. *Chrysaora* sp. appear ubiquitously in bays and the GoM April through December in similar numbers from year to year. On the other hand, *Aurelia* spp. experience great interannual variation in numbers of individuals. *Aurelia* spp. appear first in bays in April then in the GoM in July; medusae disappear from both areas in January. Abundance patterns suggest recurring blooms, however interpretation may be confounded by the presence of multiple sympatric species. Medusae presence is linked among the bays and among the GoM regions. We find that in bays, appearance of medusae is generally synchronous, whereas in the GoM, occurrence of *Stomolophus* sp. and *Aurelia* spp. progress from north to south while that of *Chrysaora* sp. is also synchronous.

Our results can be used to form conclusions regarding polyp distributions and jellyfish populations in the northwestern GoM. The initial appearance of *Stomolophus* sp. and *Aurelia* spp. in bays suggests that bays are where polyp populations are located. The near-simultaneous appearance of *Chrysaora* sp. in all areas, suggests that polyp populations of this species are distributed throughout the coastal region, while the consistent CPUE medusae suggests populations of this species have remained stable from 1983-2018 and are likely resistant to local and regional stressors such as rainfall, hurricanes, turbidity, and harmful algal blooms.

5.2 Future work

Given the potential important ecological role of jellyfish in the GoM ecosystem, tracking medusae populations and understanding the long-term regional and local variation in medusae is crucial for successful fisheries and resource management in the GoM. Future research efforts should focus on gathering long-term time-series data of medusae numbers, size, and biomass and should include tissue sampling for identification via DNA barcoding.

Studies of environmental drivers of medusa presence are limited by the fact that they only correlate local environmental parameters from the location where adult medusae were observed, and do not consider the environment of the polyp population that produced ephyrae. Polyps may be located miles away from where adult medusae are observed in a location with significantly different biophysical properties. However, polyp populations have never been found in the GoM and there have not been any empirical studies of medusae movement for the region. Therefore, there is a great need for systematic efforts to locate wild polyp populations in the GoM and beyond. Past attempts to locate wild polyp populations in the northern GoM largely relied on opportunistic sampling of shallow water habitats. The inability to study populations *in situ* is perhaps the main limitation on progress in this field and on our ability to model and predict jellyfish occurrence and blooms. Basic data is missing on population location and size, rates and timing of budding and strobilation, number of ephyrae produced, medusae growth rates, as well as intra- and inter-species variation and differences. For nearly all species, data on rates of asexual reproduction and growth is known only from laboratory studies. Targeted, systematic surveys of coastal and offshore hard substrates

would increase the chance of locating polyps. Utilizing Environmental DNA to screen for Scyphozoan species, especially in benthic samples, would also be worthwhile. This method could be optimized using polyps from lab cultures and could potentially be applied to environmental samples that have already been collected.

APPENDIX A

SUPPLEMENTARY MATERIALS FOR CHAPTER 2

The following supplementary materials are supplied with this document as separate files.

S1: Excel file containing the daily averaged response scores for control and experimental groups for three species of *Aurelia* for the Chronic Lethal Temperature experiments with dates and standard deviations shown.

S2: Excel file containing raw data and final results for Chronic Lethal Temperature Limits, Critical Thermal Maximum, and Chronic Lethal Salinity limits final results for *Aurelia coerulea*, *Aurelia* sp. 9 and *Aurelia* sp. 17.

APPENDIX B

SUPPLEMENTARY MATERIALS FOR CHAPTER 3

The following supplementary materials are supplied with this document as separate files.

S1. Excel file containing GenBank accession numbers and collection locations for *Aurelia* sequences used in global COI analysis.

S2. Excel file containing GenBank accession numbers and collection locations for *Aurelia* sequences used in ITS1 analysis.

S3. Excel file containing COI + 28S Supplementary Table S3: GenBank accession numbers and collection locations for *Aurelia* sequences used in concatenated COI + 28S analysis.

S4. Word document containing the results of SIMPER analysis showing the contribution of each morphometric character to the dissimilarity among *Aurelia* sp. 17 polyps (Group 3) and the polyps of *Aurelia* sp. 9 (Group 9) and *Aurelia coerulea* (Group 1).

S5. Excel file containing the between group K2P genetic distances for COI for all *Aurelia* species.

S6. Excel file containing the within group K2P genetic distances for COI for all *Aurelia* species.

S7. Excel file containing the between group K2P genetic distances for ITS1 for select *Aurelia* species.

S8. Excel file containing the within group K2P genetic distances for ITS1 for select *Aurelia* species.

APPENDIX C

SUPPLEMENTARY MATERIALS FOR CHAPTER 4

The following supplementary materials are supplied with this document as separate files.

S1. Word file containing the moving averages of *Stomolophus* sp. medusae CPUE over 35 years for each bay and GoM region in Texas.

S2. Word file containing moving averages of *Chrysaora* sp. medusae CPUE over 35 years for each bay and GoM region in Texas.

S3. Word file containing moving averages of *Aurelia* spp. medusae CPUE over 35 years for each bay and GoM region in Texas.