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THE SYMBIOSES OF THE UPSIDE-DOWN JELLYFISH CASSIOPEA XAMACHANA; FROM PROKARYOTE TO EUKARYOTE

A Dissertation in

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by

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ABSTRACT

Symbiosis is the basis of all biological life, from the evolution of eukaryotic cells to animal health driven by gut microbiomes. Holobionts, or the collection of a host and its symbionts, are often difficult to study due to difficulties rearing species of interest in a lab and manipulating the host-symbiont collection. Model organisms on questions of symbiosis can reveal mechanisms applicable to a wide range of related species, and benefit from ease of husbandry in labs. Proper care of these model organisms is critical for ethical considerations and the accurate collection of data.

In this dissertation, I establish husbandry standards for the upside-down jellyfish *Cassiopea xamachana* and carry these standards into an exploration of host-symbiont interactions. My first chapter serves as the first guide to *C. xamachana* husbandry at all points of its life cycle, aiding labs beginning to work on this model and senior labs alike. My second chapter then explores the plasticity of *C. xamachana* with photosymbiotic dinoflagellates in the family Symbiodiniaceae. It both reveals the difference in offspring production when symbiotic with various species, and establishes a list of species and lab-grown strains of Symbiodiniaceae that can form symbiosis with *C. xamachana.* Finally, my third chapter explores the existence of fungal symbionts in adult *C. xamachana*. The work presented here facilitates laboratory research on *C. xamachana* and reveals symbiont diversity in a marine holobiont.

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Chapter 1

Introduction

The universality of symbiosis

The concept of symbiosis has existed since the 1800s (Guerrero et al. 2013). Heinrich Anton De Bary defines symbiosis as when dissimilarly named individuals (i.e. different species) live together (Gilbert and Epel 2015). For centuries, symbiosis was thought to be a rarity, and predominantly found in insects and plants (McFall-Ngai 2024). We now know symbiosis to be the basis of all living organisms, as popularized by the late Lynn Margulis in the 1900s (Margulis 1981). Endosymbiosis, where a symbiont lives within the cells of its host, is accepted as the mechanism behind the emergence of eukaryotic cells as we know them. There is significant evidence that mitochondria and chloroplast originated as bacterial cells that became engulfed and persisted within the predator cell, becoming the first host (Margulis 1981). This revelation brought new attention to biological systems, prompting the examination of the relationship between animal hosts and symbiotic microorganisms. A microbiome is the consortia of microorganisms within and on the surface of a eukaryotic organism and includes not just bacteria, but microbes such as viruses and fungus. Symbiosis became understood as the underlying foundation supporting all life on the plant (McFall-Ngai 2024). This shift in biological doctrine has been accompanied by a rise in microbiome studies on multiple organisms, not just humans or well-established model organisms, analyzing the interactions between a host and its microbial symbionts. The human microbiome, for example, has undergone significant scrutiny as researchers have begun to tease apart the relationship between microscopic symbionts and human health. Gut microbiome studies had revealed that all animals had a bacterial consortium that affected host health, and it was later discovered that plants have equally important microbiomes (McFall-Ngai 2024). With the increased availability of genetic sequencing, particularly metabarcoding and eDNA, it has become apparent that microbial symbionts not only exist in

close association with all biological hosts, but that the diversity of microbial symbionts is greater than previously thought.

Holobionts are the collective association of a eukaryotic host and its population of symbionts (Rosenburg et al. 2007). This term was established to acknowledge a host organism that cannot successfully complete its life cycle without its symbionts and represents the significant place symbiosis has earned in biological studies. In general, a host is considered the larger (often multicellular) organism, while symbionts are smaller and live either on or within the host. Rarely is a symbiotic relationship considered neutral: the majority have one organism benefiting (mutualism) or experiencing detrimental effects from the symbiosis (parasitism) (Gilbert and Epel, 2015). Because symbionts are ubiquitous in living organisms and directly influence the survivorship of hosts, it is critical to study organisms from the holobiont perspective (Gonzalez-Pech et al. 2021). Corals are a classic example of the effects of both mutualism and parasitic relationships. Many coral are in a mutualistic symbiosis with dinoflagellates in the family Symbiodiniaceae, and they cannot complete their life cycle without the photosymbionts. When Symbiodiniaceae leave their host coral, a process known as coral bleaching, the host animal can die of the disruption of symbiosis is prolonged (Gilbert and Epel, 2015). This loss of symbiosis can be triggered by varying environmental parameters (e.g., temperature, light) but also by parasitic relationships, such as bacterial or fungal pathogens. For example, octocorals are susceptible to fungal infections by *Aspergillus sydowii*, which causes tissue loss and mortality. Interestingly, several octocoral individuals have been found with *A. sydowii* living within its tissue without the apparent effects of the disease. These may be cases of a symbiont that becomes parasitic when the correct conditions are met, but it supports the assertion that no symbiotic relationship is truly neutral for one party or the other. There are many such cases of symbioses not well understood. As the catalogue of extant species increases, studying the holobiont of every

organism of interest is an insurmountable task. Problems such as this can be solved by the study of model organisms.

Marine model organisms

Model organisms have been used for decades to explore general principles underlying biological phenomena (Ankeny and Lionelli 2020). The term "model organism" refers to organisms used to study a range of biological functions, but model organisms have several ideal characteristics. Additionally, model organisms need to be easily culturable in a lab environment with short generation times and the ability to produce large populations (Ankeny and Lionelli 2020). For example, *Drosophila* fruit flies have an easily manipulated genome, and have several genes with direct effects on fly phenotype. Studies on their genomes have revealed detailed characteristics on the properties of genomes, chromosomes, and gene expression that can be applied to our understanding of the nature of DNA (Wright and Schaeffer 2022). As our knowledge of global diversity increases, so has the number of model organisms in use by labs.

One ecosystem that can benefit immensely from the use of model organisms as proxies to study life and ecosystems is the ocean. Oceans comprise 70% of Earth's surface area, and they contain incredibly biodiverse ecosystems (Sunagawa et al. 2020). However, many of these species are difficult to study due to decreasing numbers and difficulties rearing in a lab environment. Sea turtles are a perfect example of this issue, as all species are endangered or at risk, and this has led to global regulations limiting interactions with sea turtles. Most of the research done on sea turtles uses capture and release, tagging, or satellite imaging to locate the rare animals (Robinson et al. 2023). This limits the scope of potential research on the animals, as most research is limited to the times when sea turtles are easily found, such as nesting (Robinson

et al. 2023). Another example are reef-building coral, which are in consistent global decline due to the effects of climate change and are difficult to rear in a lab setting (Lehmann 2021). Model organisms can help address these challenges, and new marine species are constantly being suggested as candidates to answer specific questions. One such organism is the upside-down jellyfish *Cassiopea xamachana*.

Cassiopea jellyfish

The family Cassiopeidae has only one genus, *Cassiopea*, which has 12 currently accepted species on the World Register of Marine Species (WoRMS)(Ahyong et al. 2024). *Cassiopea* jellyfish are the only "upside-down" group of jellyfish. The adult medusa rest bell-side down on the seafloor with their oral arms extending upwards from their body. This unique lifestyle had gotten them mistaken for large sea anemones before, but a formal description of the genus was published in 1810 by Péron and Lesueur. In general, *Cassiopea* jellyfish all exhibit an upside-down orientation as adults and are found in warm waters (Figure 1-1). *Cassiopea* medusa have 4 pairs of branching oral arms, named as such because of the multiple oral vesicles lining the arms. Each vesicle has a small opening leading to the branchial canals in the bell of the jellyfish, making a network of small "mouths" across the oral arms. These give *Cassiopea* jellyfish their characteristic "fluffy" oral arms (Mayer 1910; Medina et al. 2021). However, other physical characteristics are variable in *Cassiopea* jellyfish and has made morphological species descriptions difficult. Coloration, size of oral arms, sense organ number, and occurrence of oral appendages all vary within species. Alfred Mayer in 1910 accurately stated that "It is therefore impossible, at present, to classify the forms of *Cassiopea* with any degree of certainty." Fortunately, this issue in species deliniation is slowly getting solved with genetic barcoding (Jarms and Morandini 2019).

The currently accepted species of *Cassiopea* are *andromeda, culionensis, depressa, frondosa, maremetens, mayeri, medusa,* and *mertensi*. Two of these, *C. culionensis* and *C. mayeri* were only officially recognized as species in 2022 with genotyping and through characterization of their stinging cells (cnidae), which proposes an interesting new distinguishing characteristic between *Cassiopea* species (Gamero-Mora et al. 2022). Evidence for the variability and difficulty describing species of *Cassiopea* is noticeable in the publication of the World Atlas of Jellyfish in 2019, which included an additional species (*C. vanderhorsti)* which is not officially accepted due to lack of sampled specimens and similarities in the description to *C. xamachana,* but cannot be fully discounted as a species without further sampling efforts (Jarms and Morandini 2019). The genus as a whole is in need of a more dedicated sampling effort and genetic barcoding to supplement previously made species descriptions.

Historically, several species have been featured more frequently in research studies. The earliest species described was *C. frondosa* in 1774, but *C. andromeda*, described in 1775, went on to be the more highly studied species (Medina et al. 2021). *C. andromeda* is native to the Red Sea and considered alien (e.g. Mediterrean Sea) or invasive (e.g. Hawai'i) in several parts of the globe (Holland et al. 2004). Some have reported it invasive in the Caribbean Sea as well, but this calls to attention the confusing taxonomy of *C. andromeda* and *C. xamachana*. *C. xamachana* is found in the Caribbean ocean and morphologically, has no distinctive characteristics from *C. andromeda* in any life stage. Genetically, *C. xamachana* from the Florida Keys and *C. andromeda* from the Red Sea also group together in a clade. However, they have slight genomic differences and different geographic distributions. Many researchers argue the two species should be grouped together, while others expect the species to differentiate further with the geographical separation (Jarms and Morandini 2019). Morphological variation may also be affected by environmental parameters. I have personally reared several lines of *C. xamachana* collected from the Florida Keys into ephyra and adults, as well as one line of *C. andromeda* originating from the

Red Sea but reared in lab cultures across many years. The *C. andromeda* polyps were consistently larger and underwent asexual budding more often. Also ephyra produced from these polyps had a characteristic blue color surrounding the radial disk immediately upon detachment from the polyp. *C. xamachana* can occasionally have coloration as new ephyra (Muffet et al. 2022), but it is not consistent. However these were personal observations from a limited assortment of the two species, and it is unclear how much of these distinctions would persist or slowly homogenize as the species were kept in the same culture conditions over time. There has also been a debate on which molecular markers are best suited to distinguish between *Cassiopea* species (Muffet and Miglietta 2023), so genetic differences between *C. andromeda* and *C. xamachana* are still being explored. In general, until further evidence is provided either differentiating the species or grouping them into one, *C. andromeda* and *C. xamachana* are considered to be distinct species (Jarms and Morandini 2019).

C. andromeda and *C. xamachana* have been the most utilized of the *Cassiopea* species for experimental research. Both species are relatively easy to find in their geographic regions and often have brightly colored oral appendages, making them easy to spot and identify (Jarms and Morandini 2019). As a contrary example, *C. frondosa*, despite being the first described *Cassiopea* species, has not been frequently studied (Medina et al. 2021). While *C. frondosa* is endemic to the Caribbean, it exists in deeper waters and colder temperatures than *C. xamachana*. Thus while the two species overlap in distribution, it is much easier to find *C. xamachana* medusa (Fitt et al. 2021; personal experience from sampling in the Florida Keys). Additionally, *C. xamachana* is relatively easy to raise in culture conditions compared to *C. frondosa* and other scyphozoan species (Fitt et al. 2021; AZA Aquatic Invertebrate TAG 2021). These characteristics make *C. xamachana* an ideal model organism, and it has gained popularity as a laboratory research animal for many fields of study (Medina et al. 2021).

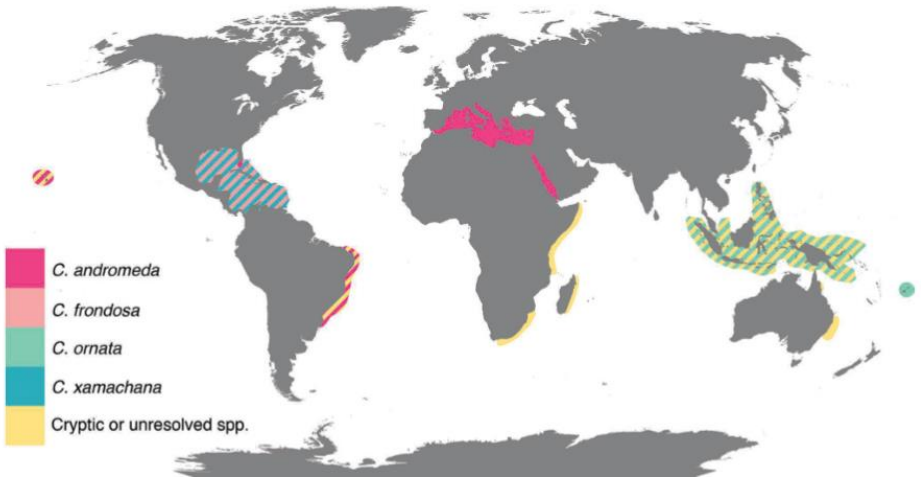

Figure 1-1. Estimated global distribution of major *Cassiopea* species. Published in Medina et al. 2021.

Cassiopea xamachana, life history and use as a model organism

C. xamachana has been utilized to investigate questions as diverse as fluid dynamics and bioaccumulation of toxins, as recently reviewed by Medina, Sharp, et al. 2021. Experimental research on this model began as early as 1908 with studies on the pulsation rates of *C. xamachana* when agitated (Stockard 1908) and the regeneration of medusa when cut in complex ways (Mayer 1910). In 1916, the first study on medusa regeneration was published when it was shown that rhopalia, the sense organs of *C. xamachana*, regenerate when removed (Cary 1916). While other studies on *Cassiopea* species were published during the mid-1900s (Smith 1936; Freudenthal 1959; Gohar and Eisawy 1960; Balderston 1969), the next wave of studies on *C. xamachana* came in the 1970s. There was increased focus on asexual reproduction and polyp development, including assessing the ability of polyps to regenerate (Curtis and Cowden 1971; Curtis and Cowden 1972; Curtis and Cowden 1974). In particular, *C. xamachana* became an organism of interest due to their developmental symbiosis.

Developmental symbiosis refers to when a host relies on the presence of a foreign genome, that of the symbiont, for proper development across the entire life cycle (Trench 1979; Gilbert and Epel 2015). While all marine organisms exist in some form of symbiosis (Gonzalez-Pech et al. 2024), *Cassiopea* jellyfish are unique amongst scyphozoans and marine model organisms as they cannot reach sexual maturity without the presence of a particular symbiont (Medina et al. 2021). All *Cassiopea* species from symbiosis with dinoflagellate microalgae from the Family Symbiodiniaceae (Jarms and Morandini 2019). This family is globally distributed and forms the most common symbioses seen in the oceans. They are the photoendosymbiont found in coral that play a significant role in coral health. Coral bleaching refers to stress-induced loss of a coral's Symbiodiniaceae symbiont, and this process can lead to death of the organism if the symbiosis is not re-established. Symbiodiniaceae contains a diverse assortment of species, but many are mutualistic. In addition to coral, Symbiodiniaceae species are found in symbiosis with sea anemones, tridacnid clams, and or course, jellyfish (LaJeunesse 2020).

For *C. xamachana*, they remain in their juvenile polyp stage until they establish a symbiotic relationship with dinoflagellate endosymbionts in the Family Symbiodiniaceae. Once symbiotic, *C. xamachana* develops into sexually mature medusae (Figure 1-2). The full life cycle of *C. xamachana* can only be completed when symbiotic.

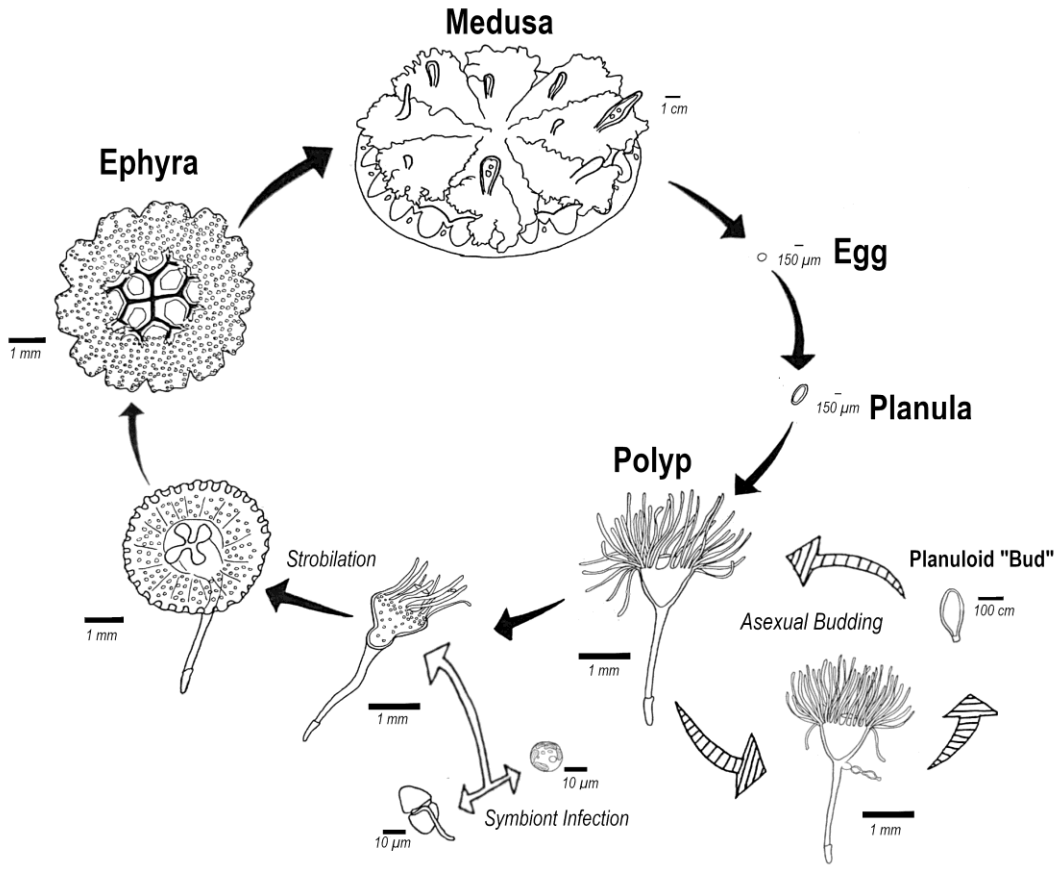

Figure 1-2. Life cycle of *C. xamachana*. Scale bars in black. Striped arrows indicate asexual budding process. White arrow indicates symbiosis establishment. Black arrows indicate life cycle progression. Created by Victoria Sharp for the publication Medina et al. 2021.

C. xamachana life cycle

C. xamachana are the only known genus of scyphozoan that exhibit sexual dimorphism. Female medusa have a concentrated cluster of oral appendages in the center of their oral arms where planula reside (Jarms and Morandini 2018). Zygotes will be housed in a mucus layer secreted by the female planula and when developed, planula will break free from the mucus and swim away from the female. Despite knowing how fertilized embryos develop in female medusa

and knowing what male sperm look like, wild fertilization has never been observed. Additionally, male spawning has never been observed in an aquarium setting (Medina et al. 2021).

Planula are oblong and entirely ciliated, allowing them independent movement. Like all stages in *C. xamachana*, plauna consist of three tissue layers; epidermis, mesoglea, and endodermis. The mesoglea is present in all stages of *C. xamachana*. Planula swim with their aboral end forward and when they are ready to settle, this end forms a pedal disk. The pedal disk securely attaches to a substrate and will not detach unless manually removed (Medina et al. 2021). Once removed, the pedal disk does not regenerate, and the developing polyp will never reattach to a surface (Curtis and Cowden 1972; Mammone and Sharp et al. 2023). Despite this, removal of the pedal disk does not impact polyp development and function. Additionally, planula will not settle and develop into polyps without the presence of a biofilm, often of the genera *Vibrio* or *Pseudoalteromonas* (Ohdera et al. 2023). The hexapeptide Z-Gly-Pro-GlyGly-Pro-Ala was also found to artifically induce settlement after being isolated from biofilms that planula settled on (Hofmann and Brand 1987).

Polyps consist of a long stalk extending from the pedal disk and a calyx, or head. The calyx contains 4 gastric pits and a prominent hypostome, or mouth. There are approximately 32 tentacles extending from the periphery of the calyx that can be retracted or fully extended, often exceeding the length of the polyp. *C. xamachana* does not pass their dinoflagellate symbionts down to offspring and instead polyps acquire symbionts through horizontal transmission, where the symbionts are ingested from the environment. Up until the development of the hypostome, polyps are natually aposymbiotic. Once ingested, Symbiodiniaceae cells avoid digestion and persist within the host cells (Medina et al. 2021). They are uptaken by endodermal cells and engulfed by a lysosome. However, the lysosome does not digest the cell. Instead, the symbiont cells persist and proliferate within the lysosome membrane, which is called a symbiosome at this point (Fitt and Trench 1983). The specific point in the life cycle where microalgae are ingested

allows researchers to manipulate the occurrence and species of symbiosis in *C. xamachana*. Through this, it is possible to better understand how one genome can directly influence gene expression in another species (Trench 1979). Occasionally, endodermal cells with symbiosomes will migrate through the tissue into the mesoglea, where they become motile and move throughout the mesogleal layer. These cells are called amoebocytes and while their presence is known, their function within the animal is currently unknown (Fitt and Trench 1983).

Both aposymbiotic and symbiotic polyps are able to undergo another form of asexual reproduction called budding (Figure 1-2). Budding occurs at the base of the calyx from localized cell proliferation. Buds, also sometimes called planuloids or planuloid buds, can form singlets or budding chains where 2 to 4 buds are attached to each other by ectodermal tubes. Once fully formed, a bud will detach from the polyp through motion with its cilia (Hofmann et al. 1978). Morpholically, planuloids and planula are similar, but planuloids are larger in size than planula (Figure 1-2). Also while planula are aposymbiotic, buds may or may not contain dinoflagellate symbionts. If the buds are formed from polyp cells that contained symbiont cells, these may be passed down to the bud (Hofmann et al. 1978).

Once symbiotic, polyps will undergo strobilation. Strobilation is a form of asexual reproduction in scyphozoan jellyfish where the polyp calyx will morph into a pre-adult stage termed an ephyra. The ephyra develops over the course of a few days and then detaches from the polyp through pulsation inducing lateral fission. The ephyra will then develop into a full medusa (Hofmann et al. 1978). Strobilation in scyphozoans can be mono-disk, where one ephyra is produced at a time, or multi-disk, where stacks of ephyra are produced by a polyp and they detach from the top-down (Marques and Collins 2004). *C. xamachana* is a monodisk strobilator, and the polyp completely regrows its calyx and tentacles in-between ephyra production. One polyp has been reported to produce 3-5 ephyra before the polyp dies (Chapter 2; Sharp et al. 2024), presumably from the expenditure of its energy sources. *Cassiopea* spp. are unique however in that

they only undergo strobilation when symbiotic. If symbionts are kept away from polyps, the polyps will remain in that form indefinitely and never strobilate (Chapter 2; Sharp et al. 2024). As strobilation is required for the production of medusa, *Cassiopea* spp. cannot reach sexual maturity without the presence of a foreign species.

Once ephyra detach from polyps, they are composed of 4 oral arms, their rhopalia, and their bell. Over the course of a few days, the oral arms will bifurcate and gastrovascular canals will fully develop (Medina et al. 2021). Ephyra can form with various coloration and can form their club-like oral appendages as they grow, but oral appendages may not grow on all ephyra. The coloration of oral appendages also varies by individual, and there has not been a correlation currently found between coloration and origin of the ephyra (Muffet et al. 2022). Ephyra are generally considered as medusa when they have developed gonads, which does not happen at a specific time point (Medina et al. 2021). Gonads have been found in individuals as small as 7 cm in diameter (Hofmann and Hadfield 2002), and larger individuals have also been found without fully developed gonads. It is unclear what affects gonad development in medusa and if it is controlled by size, nutrient availability, or other abiotic factors. As medusa, if males and females are kept in the same body of water, fertilization will occur and the life cycle will repeat (Medina et al. 2021).

The symbiosis of the upside-down jellyfish *Cassiopea xamachana*; from prokaryote to eukaryote

This dissertation expands knowledge of the symbioses that can form with *C. xamachana* and establishes laboratory practices to facilitate usage of this developmental symbiosis system. Chapter 1 describes cohesively for the first time sound husbandry practices for *C. xamachana* across all of its life stages in a laboratory setting. Laboratory research organisms have been

utilized for centuries, but regulations on ethical treatment have only increased in recent decades. The Guide for the Care and Use of Laboratory Animals only included aquatic animals in its 8th edition published in 2011, despite aquatic animals like frogs being used in laboratory experiments for decades (National Research Council et al. 2011). Also, definitions of laboratory animals in legislature for animal welfare exclusively refer to vertebrates (National Research Council et al. 2011). Only in 2023 was there any proposed legislature in the United States for regulations on octopi welfare, despite the abundance of evidence that octopi have complex processing skills and experience pain (Reardon 2023). The European Union implemented regulations on laboratory treatment of cephalopods in 2013 (Smith et al. 2013), demonstrating both that laboratory animal welfare regulations are still being established and that there are not consistent standards by country. Other marine invertebrates are not included in United States legislature for the ethical treatment of laboratory animals, and this lack of oversight can lead to inconsistencies in husbandry practices from research labs (Harvey-Clark 2011). Differences in husbandry conditions for an organism in experimental research present both an ethical dilemma and a reproducibility issue. For example, *C. xamachana* polyps are often kept in perpetual darkness when aposymbiotic (i.e. without their Symbiodiniaceae symbiont) to discourage the establishment of symbiosis in case of contamination of the water (Fitt and Trench, 1983; Newkirk et al. 2018; Sharp et al. 2024). However, *Cassiopea* species experience a circadian rhythm (Nath et al. 2017), and there have not been studies on the effect of perpetual darkness in polyps and how this might contribute to animal stress levels and their normal functioning. When I first started working on *C. xamachana*, I had to learn husbandry of the various life stages by a combination of publications and verbal communication with aquarists. Publications on scyphozoan husbandry will include *C. xamachana* medusa (AZA Aquatic Invertebrate TAG 2021; Widmer 2008), but they do not often include planula or polyps. Currently there is no one summary of accepted husbandry parameters for rearing *C. xamachana* across its entire life cycle. Chapter 1 summarizes publications with

methods describing *C. xamachana* care along with experiences from *Cassiopea* researchers and aquarists. Minor experiments run by undergraduate students I mentored in my time undertaking this thesis are also described to support assertions we make on ideal husbandry conditions of the jellies. The chapter follows the life cycle of *C. xamachana* from larvae to adult with proper care conditions for each stage. Additionally, a section is included in the polyp stage to discuss the establishment of symbiosis and how researchers can control the induction of symbiosis in a lab setting for experimental purposes. This chapter encompasses the standards I established for the subsequent chapters of the dissertation. Additionally, it summarizes the consultations I have given multiple labs globally on beginning work on this model organism and the proper care practices needed to produce sound science.

The methods described in Chapter 1 on establishing clonal lines of aposymbiotic (without the dinoflagellate symbiont) polyps were applied to the experiment undertaken for Chapter 2. This chapter describes a mass introduction of varying Symbiodiniaceae species to *C. xamachana* polyps and the phenotypic plasticity of its symbiosis. Phenotypic plasticity, or the change in host phenotype without direct changes to the genome, is an important strategy for survivorship of individuals and species. As a polyp, *C. xamachana* is able to associate with multiple Symbiodiniaceae species from multiple genera (Mellas et al. 2014; Newkirk et al. 2018; Newkirk et al. 2020; LaJeunesse 2020; Mammone and Sharp et al. 2023). Despite this plasticity in symbiont uptake as a polyp, wild adult *C. xamachana* are predominantly found in symbiosis with *Symbiodinium microadriaticum* (Thornhill et al. 2006; Ohdera et al. 2018). There has never been an investigation as to how this symbiont winnowing occurs. Symbiont winnowing is the process where the dominant symbiont of a host shifts over its ontogeny, and can be induced by the host or the symbiont. A well-known marine system that undergoes symbiont winnowing is *Euprymа scolopes*, the bobtail squid. Juvenile squid will have multiple species of bacteria enter their crypt organ but over time, the bacteria are expelled and the crypt is colonized exclusively by *Vibrio*

fischeri (Nyholm and McFall-Ngai 2004). While the mechanisms behind this winnowing are still being explored, there is evidence in other organisms like coral that winnowing may be influenced by apoptosis from the host (Dunn and Weis 2009). In *C. xamachana*, symbiont community composition has not been studied in the same individual from polyp to medusa and thus, there is no knowledge on what may drive this specificity with *S. microadriaticum*. Additionally, while studies introducing symbionts to polyps have looked at strobilation as an indication of the successful establishment of symbiosis, no *C. xamachana* studies have observed the impact of symbiont species on ephyra development (Newkirk et al. 2020). It is possible ephyra associating with different symbionts may not persist to medusa or experience other morphological differences that affect their survival. Also, phenotypic differences between *C. xamachana* polyps associating with different Symbiodiniaceae species have yet to be observed. Chapter 2 looked at the difference in time to produce an ephyra through strobilation and the subsequent mortality of ephyra when symbiotic with various symbionts. We found that while ephyra mortality was not significant, the time to produce an ephyra was. There was a significant difference between symbiont species and the number of days for a polyp to complete strobilation. As rapid production of offspring benefits organisms by increasing the likelihood of survival of a genotype, this plastic response to symbiont species reveals *C. xamachana* may be able to associate with various Symbiodiniaceae at a juvenile to ensure rapid production of ephyra. Additionally, the study utilized several lab-grown strains of the same species, and there were significant differences between strains of the same species and time to strobilation. This knowledge informs future lab experiments on *C. xamachana*, as it shows the strain used has an impact on symbiosis progression.

While Symbiodiniaceae is the dominant symbiont of *C. xamachana's* developmental symbiosis, the jelly has a rich microbiome community. The bacteria of *C. xamachana* has been studied (Muffet et al. 2024, preprint), but other microbial associates have yet to be explored. The

majority of historical studies on marine symbioses have looked at hosts and dinoflagellates but, like all living organisms, marine organisms rely on a diverse array of other microbial symbionts for successful development and completion of the life cycle. For example, many marine larvae rely on the presence of a bacterial biofilm to settle and develop (Gilbert and Epel 2015), and these biofilms are often species-specific (McFall-Ngai et al. 2013). In *C. xamachana*, planula have a higher success rate in development when exposed to bacteria in the genera *Pseudoalteromonas* and *Vibrio* (Ohdera et al. 2023). In coral, not only are bacteria crucial for larval settlement, they have also been found aiding holobiont health and disease resistance (Voolstra et al. 2021). Coral holobionts are known to host a diverse range of Symbiodiniaceae, Archaea, endolithic algae, fungi, bacteria, and a variety of other protists and viruses (Voolstra et al. 2021). Likewise, studies on marine sponge microbiomes have identified a wide diversity of protists, with fungi and bacteria playing an important role in the sponge life cycle (Hentschel et al. 2012). Marine hosts invariably rely on symbionts for development, but the diversity of symbionts, the specificity of hosts and symbionts, and the roles symbionts play within hosts are all questions still being explored in various systems. With the increased awareness of the complexity of marine holobionts and the prevalence of developmental symbiosis in all living organisms, any study on a marine system needs to be aware of the entire holobiont. For a marine model organism like *C. xamachana*, extensive knowledge on the entirety of its holobiont is critical to accurately apply findings on the system to other marine organisms.

Marine fungi are known to play a significant role in the life cycles of coral and sponges (Yarden 2014), but they have not been well-characterized in scyphozoan jellies. There have been relatively few studies on the internal fungal community of scyphozoans (Wright et al. 2003; Yue et al. 2015), and none on a benthic jelly like *C. xamachana*. Fungi needs nutrient-rich environments and a substrate to persist in any environment and are found in high abundance in the sediment and on/inside of marine organisms (Richards et al. 2012). The proximity of *C.*

xamachana to fungus-rich sediment, plant life in the form of mangrove habitats, and human activity due to its near-shore existence, makes it extremely likely to host a rich assemblage of internal fungus. My third chapter explores for the first time the endomycotal community of *C. xamachana* through a culturing approach. Male and female medusa from three wild sampling locations and one lab-reared population were collected. A variety of tissue was collected from each individual for plating; bell, oral arms, rhopalia, and gonads. Additionally, environmental samples were collected through seawater, another invertebrate found in the same location, and *C. xamachana* mucus. *C. xamachana* produces a thick mucus full of stinging cells that spreads out up to several feet away from a single individual. This mucus is hydrophobic (Ames et al. 2020) and was thought likely to collect spores present in the seawater and surrounding sediment. The fungal isolates were sequenced down to genus level, and the diversity of isolates was higher than other scyphozoan jellies that have been sampled (Wright et al. 2003; Yue et al. 2015). There were several isolates that were found in only jelly tissue or only environmental samples, suggesting a mechanism of selectivity for fungal persistence in the animal.

Together, my dissertation establishes practices for the proper husbandry of *C. xamachana* to ensure quality in experimental data and utilizes those practices to explore the holobiont. Symbiont specificity is critically important to understand in all living organisms, as we know symbiosis is the basis of organic life. As more research is done on developmental symbiosis using this unique model organism, uncovering mechanisms underlying the life cycle of the upside-down jellyfish is necessary to relate this system to other marine organisms.

References

Ahyong, S., C.B. Boyko, J. Bernot, S.N. Brandão, M. Daly, S. De Grave, N.J. de Voogd, et al. "World Register of Marine Species (WoRMS)." WoRMS Editorial Board, September 28, 2024. https://www.marinespecies.org.

Ames, Cheryl L., Anna M. L. Klompen, Krishna Badhiwala, Kade Muffett, Abigail J. Reft, Mehr Kumar, Jennie D. Janssen, et al. "Cassiosomes Are Stinging-Cell Structures in the Mucus of the Upside-down Jellyfish Cassiopea Xamachana." Communications Biology 3, no. 1 (February 13, 2020): 1–15. doi:10.1038/s42003-020-0777-8.

AZA Aquatic Invertebrate TAG. 2021. Jellyfish (Cnidaria/Ctenophora) Care Manual (2nd ed.). Association of Zoos and Aquariums, Silver Spring, MD.

BALDERSTON, L. "STUDY OF SYMBIOTIC RELATIONSHIP BETWEEN SYMBIODINIUM MICROADRIATICUM FREUDENTHAL, ZOOXANTHELLA AND UPSIDE DOWN JELLYFISH, \ldots." NOVA HEDWIGIA 17, no. (1-4) (1969): 373–82.

Cary, L. R. "The Influence of the Marginal Sense Organs on Metabolic Activity in Cassiopea Xamachana Bigelow." Proceedings of the National Academy of Sciences of the United States of America 2, no. 12 (December 1916): 709. doi:10.1073/pnas.2.12.709.

Council, National Research, Division on Earth and Life Studies, Institute for Laboratory Animal Research, and Committee for the Update of the Guide for the Care and Use of Laboratory

Animals. Guide for the Care and Use of Laboratory Animals: Eighth Edition. National Academies Press, 2010.

Curtis, S. K., and R. R. Cowden. “Normal and Experimentally Modified Development of Buds in Cassiopea (Phylum Coelenterata; Class Scyphozoa).” Acta Embryologiae Experimentalis 3 (1971): 239–59.

Curtis, S. K., and R. R. Cowden. “Regenerative Capacities of the Scyphistoma of Cassiopea (Phylum, Coelenterata; Class, Scyphozoa).” Acta Embryologiae Experimentalis 1972 (1972): 429–54.

Curtis, Sherill K., and Ronald R. Cowden. “Some Aspects of Regeneration in the Scyphistoma of Cassiopea (Class Scyphozoa) as Revealed by the Use of Antimetabolites and Microspectrophotometry Author (s): Sherill K . Curtis and Ronald R . Cowden Stable URL : Http://Www.Jstor.Org/Stable/3882073 Y.” American Zoologist 14, no. 2 (1974): 851–66.

Dunn, Simon R., and Virginia M. Weis. “Apoptosis as a Post-Phagocytic Winnowing Mechanism in a Coral–Dinoflagellate Mutualism.” Environmental Microbiology 11, no. 1 (2009): 268–76. doi:10.1111/j.1462-2920.2008.01774.x.

Fitt, W. K., and R. K. Trench. “Endocytosis of the Symbiotic Dinoflagellate Symbiodinium Microadriaticum Freudenthal by Endodermal Cells of the Scyphistomae of Cassiopeia Xamachana and Resistance of the Algae to Host Digestion.” Journal of Cell Science 64 (1983): 195–212.

Fitt, William K., Dietrich K. Hofmann, Dustin W. Kemp, and Aki H. Ohdera. “Different Physiology in the Jellyfish Cassiopea Xamachana and C. Frondosa in Florida Bay.” Oceans 2, no. 4 (December 2021): 811–21. doi:10.3390/oceans2040046.

Freudenthal, Hugo D. “Observations on the Algal Cells (Zooxanthellae) Inhabiting the Anemone Cassiopea Sp.” J. Protozool 6 (1959): 12.

Gamero-Mora, Edgar, Allen G. Collins, Sheldon Rey Boco, Serafin Mendez Geson, André C. Morandini, and Nerida Wilson. “Revealing Hidden Diversity among Upside-down Jellyfishes (Cnidaria: Scyphozoa: Rhizostomeae: Cassiopea): Distinct Evidence Allows the Change of Status of a Neglected Variety and the Description of a New Species.” Invertebrate Systematics 36, no. 1 (January 31, 2022): 63–89. doi:10.1071/IS21002.

Gohar, H A F, and A M Eisawy. “The Biology of Cassiopea Andromeda (from the Red Sea) (with a Note on the Species Problem).” Publications of the Marine Biological Station, Ghardaqa 11 (1960): 3–39.

González-Pech, Raúl A., Vivian Y. Li, Vanessa Garcia, Elizabeth Boville, Marta Mammone, Hiroaki Kitano, Kim B. Ritchie, and Mónica Medina. “The Evolution, Assembly, and Dynamics of Marine Holobionts.” Annual Review of Marine Science 16, no. 1 (2024): null. doi:10.1146/annurev-marine-022123-104345.

Harvey-Clark, Chris. “IACUC Challenges in Invertebrate Research.” ILAR Journal 52, no. 2 (January 1, 2011): 213–20. doi:10.1093/ilar.52.2.213.

Hentschel, Ute, Jörn Piel, Sandie M. Degnan, and Michael W. Taylor. “Genomic Insights into the Marine Sponge Microbiome.” Nature Reviews Microbiology 10, no. 9 (September 2012): 641–54. doi:10.1038/nrmicro2839.

Hofmann, D. K., and M. G. Hadfield. “Hermaphroditism, Gonochorism, and Asexual Reproduction in Cassiopea Sp.—an Immigrant in the Islands of Hawai’i.” Invertebrate Reproduction and Development 41, no. 1–3 (2002): 215–21. doi:10.1080/07924259.2002.9652754.

Hofmann, D. K., R. Neumann, and K. Henne. “Strobilation, Budding and Initiation of Scyphistoma Morphogenesis in the Rhizostome Cassiopea Andromeda (Cnidaria: Scyphozoa).” Marine Biology 47, no. 2 (1978). doi:10.1007/BF00395637.

Hofmann, Dietrich K., and U Brand. “Induction of Metamorphosis in the Symbiotic Scyphozoan Cassiopea Andromeda: Role of Marine Bacteria and of Biochemicals.” Symbiosis 4 (1987): 99–116.

Holland, Brenden S., Michael N. Dawson, Gerald L. Crow, and Dietrich K. Hofmann. “Global Phylogeography of Cassiopea (Scyphozoa: Rhizostomeae): Molecular Evidence for Cryptic Species and Multiple Invasions of the Hawaiian Islands.” Marine Biology 145 (2004): 1119–28. doi:10.1007/s00227-004-1409-4.

LaJeunesse, Todd C. “Zooxanthellae.” Current Biology 30, no. 19 (October 2020): R1110–13. doi:10.1016/j.cub.2020.03.058.

LaJeunesse, Todd C., John Everett Parkinson, Paul W. Gabrielson, Hae Jin Jeong, James Davis Reimer, Christian R. Voolstra, and Scott R. Santos. “Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts.” Current Biology 28, no. 16 (August 2018): 2570–80. doi:10.1016/j.cub.2018.07.008.

Lehmann, Wade. “Coral Reef Aquarium Husbandry and Health.” In Invertebrate Medicine, 107–27. John Wiley & Sons, Ltd, 2022. doi:10.1002/9781119569831.ch6.

Mammone, Marta, Victoria Sharp, Michael Hewitt, and Mónica Medina. “The Influence of Photosymbiosis in Cassiopea Xamachana Regenerative Success.” Symbiosis 90, no. 1 (May 1, 2023): 61–70. doi:10.1007/s13199-023-00920-0.

“Marine Fungi: Their Ecology and Molecular Diversity | Annual Reviews.” Accessed March 25, 2024. https://www.annualreviews.org/content/journals/10.1146/annurev-marine-120710-100802.

Marques, Antonio C., and Allen G. Collins. “Cladistic Analysis of Medusozoa and Cnidarian Evolution.” Invertebrate Biology 123, no. 1 (2004): 23–42. doi:10.1111/j.1744-7410.2004.tb00139.x.

Mayer, Alfred G. Rhythmical Pulsation of the Medusae. 47th ed. Washington D.C., 1908.

Mayer, Alfred Goldsborough. Medusae of the World: Volume III The Scyphomedusae. Washington D.C.: Carnegie Institution of Washington, 1910.

McFall-Ngai, Margaret, Michael G. Hadfield, Thomas C. G. Bosch, Hannah V. Carey, Tomislav Domazet-Lošo, Angela E. Douglas, Nicole Dubilier, et al. “Animals in a Bacterial World, a New Imperative for the Life Sciences.” Proceedings of the National Academy of Sciences 110, no. 9 (February 26, 2013): 3229–36. doi:10.1073/pnas.1218525110.

Medina, Mónica, Victoria Sharp, Aki Ohdera, Anthony Bellantuono, Justin Dalrymple, Edgar Gamero-Mora, Bailey Steinworth, et al. “The Upside-Down Jellyfish Cassiopea Xamachana as an Emerging Model System to Study Cnidarian–Algal Symbiosis.” In Handbook of Marine Model Organisms in Experimental Biology. CRC Press, 2021.

Mellas, Rachel E., Shelby E. McIlroy, William K. Fitt, and Mary Alice Coffroth. “Variation in Symbiont Uptake in the Early Ontogeny of the Upside-down Jellyfish, Cassiopea Spp.” Journal of Experimental Marine Biology and Ecology 459 (2014): 38–44. doi:10.1016/j.jembe.2014.04.026.

Muffett, Kaden, Jessica Labonte, and Maria Pia Miglietta. “Bacterial Communities of Cassiopea in the Florida Keys Share Major Bacterial Taxa with Coral Microbiomes.” bioRxiv, January 31, 2024. doi:10.1101/2024.01.30.577655.

Muffett, Kaden McKenzie, Joleen Aulgur, and Maria Pia Miglietta. “Impacts of Light and Food Availability on Early Development of Cassiopea Medusae.” Frontiers in Marine Science 8 (January 25, 2022). doi:10.3389/fmars.2021.783876.

Muffett, Kaden, and Maria Pia Miglietta. “Demystifying Cassiopea Species Identity in the Florida Keys: Cassiopea Xamachana and Cassiopea Andromeda Coexist in Shallow Waters.” PLOS ONE 18, no. 3 (March 29, 2023): e0283441. doi:10.1371/journal.pone.0283441.

Muséum national d’histoire naturelle (France), and Muséum national d’histoire naturelle (France). Annales Du Muséum d’histoire Naturelle. Vol. v.14 (1809). Paris: G. Dufour, et Ed. d’Ocagne, 1809. https://www.biodiversitylibrary.org/item/23269.

Newkirk, Casandra R., Thomas K. Frazer, and Mark Q. Martindale. “Acquisition and Proliferation of Algal Symbionts in Bleached Polyps of the Upside-down Jellyfish, Cassiopea Xamachana.” Journal of Experimental Marine Biology and Ecology 508 (November 1, 2018): 44–51. doi:10.1016/j.jembe.2018.08.010.

Newkirk, Casandra R., Thomas K. Frazer, Mark Q. Martindale, and Christine E. Schnitzler. “Adaptation to Bleaching: Are Thermotolerant Symbiodiniaceae Strains More Successful Than Other Strains Under Elevated Temperatures in a Model Symbiotic Cnidarian?” Frontiers in Microbiology 11 (2020): 822. doi:10.3389/fmicb.2020.00822.

Nyholm, Spencer V., and Margaret McFall-Ngai. “The Winnowing: Establishing the Squid–Vibrio Symbiosis.” Nature Reviews Microbiology 2, no. 8 (August 2004): 632–42. doi:10.1038/nrmicro957.

Ohdera, Aki, Khushboo Attarwala, Victoria Wu, Rubain Henry, Henry Laird, Dietrich K. Hofmann, William K. Fitt, and Mónica Medina. “Comparative Genomic Insights into Bacterial

Induction of Larval Settlement and Metamorphosis in the Upside-Down Jellyfish Cassiopea." mSphere 8, no. 3 (June 22, 2023): e0031522. doi:10.1128/msphere.00315-22.

Ohdera, Aki H., Michael J. Abrams, Cheryl L. Ames, David M. Baker, Luis P. Suescún-Bolívar, Allen G. Collins, Christopher J. Freeman, et al. "Upside-down but Headed in the Right Direction: Review of the Highly Versatile Cassiopea Xamachana System." Frontiers in Ecology and Evolution 6 (April 2018): 35. doi:10.3389/fevo.2018.00035.

Reardon, Sara. "Octopuses Used in Research Could Receive Same Protections as Monkeys." Nature, September 15, 2023. doi:10.1038/d41586-023-02887-w.

Robinson, Nathan J., Jacopo Aguzzi, Sofia Arias, Christopher Gatto, Sophie K. Mills, Andrea Monte, Laura St.Andrews, Adam Yaney-Keller, and Pilar Santidrián Tomillo. "Global Trends in Sea Turtle Research and Conservation: Using Symposium Abstracts to Assess Past Biases and Future Opportunities." Global Ecology and Conservation 47 (November 1, 2023): e02587. doi:10.1016/j.gecco.2023.e02587.

Smith, H.G. "Contribution to the Anatomy and Physiology of Cassiopea Frondosa." Papers from the Tortugas Laboratory of the Carnegie Institution 31 (1936).

Smith, Jane A., Paul L. R. Andrews, Penny Hawkins, Susanna Louhimies, Giovanna Ponte, and Ludovic Dickel. "Cephalopod Research and EU Directive 2010/63/EU: Requirements, Impacts and Ethical Review." Journal of Experimental Marine Biology and Ecology, Cephalopod Biology a special issue compiled under the auspices of no-profit research organization CephRes, 447 (September 1, 2013): 31–45. doi:10.1016/j.jembe.2013.02.009.

Stockard, Charles R. “Studies of Tissue Growth.” Archiv Für Entwicklungsmechanik Der Organismen 29, no. 1 (March 1, 1910): 15–32. doi:10.1007/BF02297062.

Sunagawa, Shinichi, Silvia G. Acinas, Peer Bork, Chris Bowler, Damien Eveillard, Gabriel Gorsky, Lionel Guidi, et al. “Tara Oceans: Towards Global Ocean Ecosystems Biology.” Nature Reviews Microbiology 18, no. 8 (August 2020): 428–45. doi:10.1038/s41579-020-0364-5.

Thornhill, Daniel J., Michael W. Daniel, Todd C. LaJeunesse, Gregory W. Schmidt, and William K. Fitt. “Natural Infections of Aposymbiotic Cassiopea Xamachana Scyphistomae from Environmental Pools of Symbiodinium.” Journal of Experimental Marine Biology and Ecology 338, no. 1 (2006): 50–56.

Toyokawa, Masaya. “First Record of Wild Polyps of Chrysaora Pacifica (Goette, 1886) (Scyphozoa, Cnidaria).” Plankton and Benthos Research 6, no. 3 (2011): 175–77. doi:10.3800/pbr.6.175.

Trench, R. K. “The Cell Biology of Plant-Animal Symbiosis.” Annual Review of Plant Biology 30, no. Volume 30, 1979 (June 1, 1979): 485–531. doi:10.1146/annurev.pp.30.060179.002413.

Widmer, Chad L. How to Keep Jellyfish in Aquariums: An Introductory Guide for Maintaining Healthy Jellies. Wheatmark, 2008.

Wolfensohn, Sarah, and Maggie Lloyd. Handbook of Laboratory Animal Management and Welfare. John Wiley & Sons, 2008.

Wright, Anthony D., Claudia Osterhage, and Gabriele M. König. “Epicoccamide, a Novel Secondary Metabolite from a Jellyfish-Derived Culture of Epicoccum Purpurascens.” Organic & Biomolecular Chemistry 1, no. 3 (January 30, 2003): 507–10. doi:10.1039/B208588G.

Wright, Dynisty, and Stephen W. Schaeffer. “The Relevance of Chromatin Architecture to Genome Rearrangements in Drosophila.” Philosophical Transactions of the Royal Society B: Biological Sciences 377, no. 1856 (June 13, 2022): 20210206. doi:10.1098/rstb.2021.0206.

Yarden, Oded. “Fungal Association with Sessile Marine Invertebrates.” Frontiers in Microbiology 5 (May 15, 2014). doi:10.3389/fmicb.2014.00228.

Yue, Yang, Huahua Yu, Rongfeng Li, Ronge Xing, Song Liu, and Pengcheng Li. “Exploring the Antibacterial and Antifungal Potential of Jellyfish-Associated Marine Fungi by Cultivation-Dependent Approaches.” PLOS ONE 10, no. 12 (December 4, 2015): e0144394. doi:10.1371/journal.pone.0144394.

Chapter 2

Husbandry practices for care and proliferation of *Cassiopea xamach*ana in the context of experimental research

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Abstract

As the number of new model organisms increases, it is crucial to establish sound husbandry practices for these species. Standardized care requirements reduce variability in experimental research and ensure healthy lab populations. *Cassiopea xamachana,* the upside-down jellyfish, is a relatively new model organism, primarily used for studies on cnidarian-algal symbiosis. In the juvenile polyp stage, *C. xamachana* acquires its symbionts through horizontal transmission, and will only develop into sexually mature adults when symbiotic. Because polyps can be kept in dinoflagellate-free seawater, it is possible to keep polyps aposymbiotic indefinitely. Publicly available husbandry guides for scyphozoan jellyfish do not account for the need for aposymbiotic animals or explain methods for keeping each life stage of *C. xamachana*. For experimental research, it is critical to be able to successfully keep viable replicates of the organism and, as this model organism expands in use, the number of researchers beginning to work on this organism is also increasing. This husbandry manuscript presents the first comprehensive guide to *C. xamachana* care across all life stages. A literature review on articles that directly discuss *C. xamachana* husbandry is included along with small experimental procedures to validate ideal husbandry practices on *C. xamachana*. With this guide, it will be easier for new *C. xamachana* research laboratories and established laboratories alike to standardize their care parameters and ensure healthy organisms for data collection.

Introduction

Model organisms have been used for centuries to explore biological questions, and many are housed in a laboratory or clinical setting, such as *Drosophila* fruit flies which are used to

study inheritance (Matthews and Vosshall 2020). The quality of animal husbandry for these laboratory organisms is both an ethical concern and a reproducibility concern. The quality of data changes significantly if laboratory animals are kept in nebulous conditions. Stress affects an organism's reaction to experimental conditions, and results cannot be trusted if the organisms were experiencing active stress during data collection. Standardizing husbandry conditions is essential for animal welfare and accurate experimental proceedings (National Research Council 2010).

Cassiopea xamachana (Cnidaria; Scyphozoa) is a relatively new marine model organism and has been used in experiments ranging from fluid dynamics of its pulsations to its potential as a bioindicator species (Medina et al. 2021). *C. xamachana* also experiences the horizontal transmission of photosymbiont dinoflagellates in its polyp stage (Figure 2-1). It establishes symbiosis exclusively with species in the Family Symbiodiniaceae, and is commonly found associating with *Symbiodinium microadriaticum* in the wild. These photosymbionts become established endocellularly in the polyp tissue and induce the host's metamorphosis (i.e., strobilation) into adult medusa. The microalgae is ingested by polyps through their hypostome (mouth) and avoid host digestion within the lysosomes of endodermal cells. The lysosome membrane remains around the symbiont cell and is referred to as a symbiosome. Endodermal cells can migrate into the mesogleal tissue layer and move throughout the layer as mobile cells called amoebocytes (Fitt and Trench 1983). The symbionts are still able to undergo photosynthesis within the symbiosome (Iglesias-Prieto and Trench 1994). Laboratory experiments may focus on the symbiotic adults, the aposymbiotic (without dinoflagellate endosymbiont) juveniles, or strobilation. Because of the versatile animal's life cycle (Figure 2-1) and the wide array of questions being investigated with this organism, husbandry practices vary significantly across research groups and life stage. In particular, practices of raising the aposymbiotic polyps have been debated as established animal husbandry guides for jellyfish, such

as the American Zoological Association (AZA), do not have sections on aposymbiotic polyps. For instance, aposymbiotic polyps have often been raised in complete darkness to discourage the progression of photo-symbiosis if any contamination of the clean seawater with Symbiodiniaceae occurs (Fitt and Trench, 1983; Newkirk et al. 2018; Sharp et al. 2024), but, as we know *Cassiopea* spp. experience a circadian rhythm and prolonged dark exposure in adult *Cassiopea* spp. have decreased responses to stimuli (Nath et al. 2017). It is unclear if extensive dark conditions, even on polyps without photosynthetic symbionts, affect the animal's health. Another major obstacle in laboratory settings is acquiring enough replicates of a model organism for the statistically accurate execution of experiments. *C. xamachana* undergoes asexual reproduction at the polyp stage, a process called "budding." If an individual polyp is kept isolated and allowed to bud continuously, it can produce a colony of clonal polyps (Sharp et al. 2024). As buds are produced through localized cell division on the calyx of the polyp (Hofmann and Honegger 1990), sufficient energy is needed by the polyp to produce buds. For aposymbiotic polyps, this solely comes from heterotrophic feeding. The optimal feeding quantities to produce many buds rapidly in a laboratory setting are still being explored. Additionally, aquariums will typically feed jellyfish at the same time everyday, during light hours, but it is unclear if researchers only feeding polyps during a dark cycle will affect their abilities to survive and reproduce. Questions such as this may significantly impact the health of laboratory *C. xamachana*, and the potential stress responses to non-ideal conditions may impact reproducibility in data. It is critical to standardize care conditions of this rapidly expanding model organism to both facilitate inclusion of new researchers into the *Cassiopea* community, and ensure animals are receiving consistently good care for experimental procedures.

Here we report on established husbandry practices of *C. xamachana* in the context of laboratory research. Published papers that describe their husbandry techniques are included, and the guide is supplemented with the results from continuing husbandry efforts. Only papers and

experiments specifically utilizing *C. xamachana* are discussed here, as there are significant differences in physiology and ecology of various *Cassiopea* species (Fitt et al. 2021; Jarms and Morandini 2019). As use of this model organism increases, effective care and proliferation of the study organisms are critical for achieving accurate results. We summarize current knowledge on *C. xamachana* husbandry in the context of conducting scientific research.

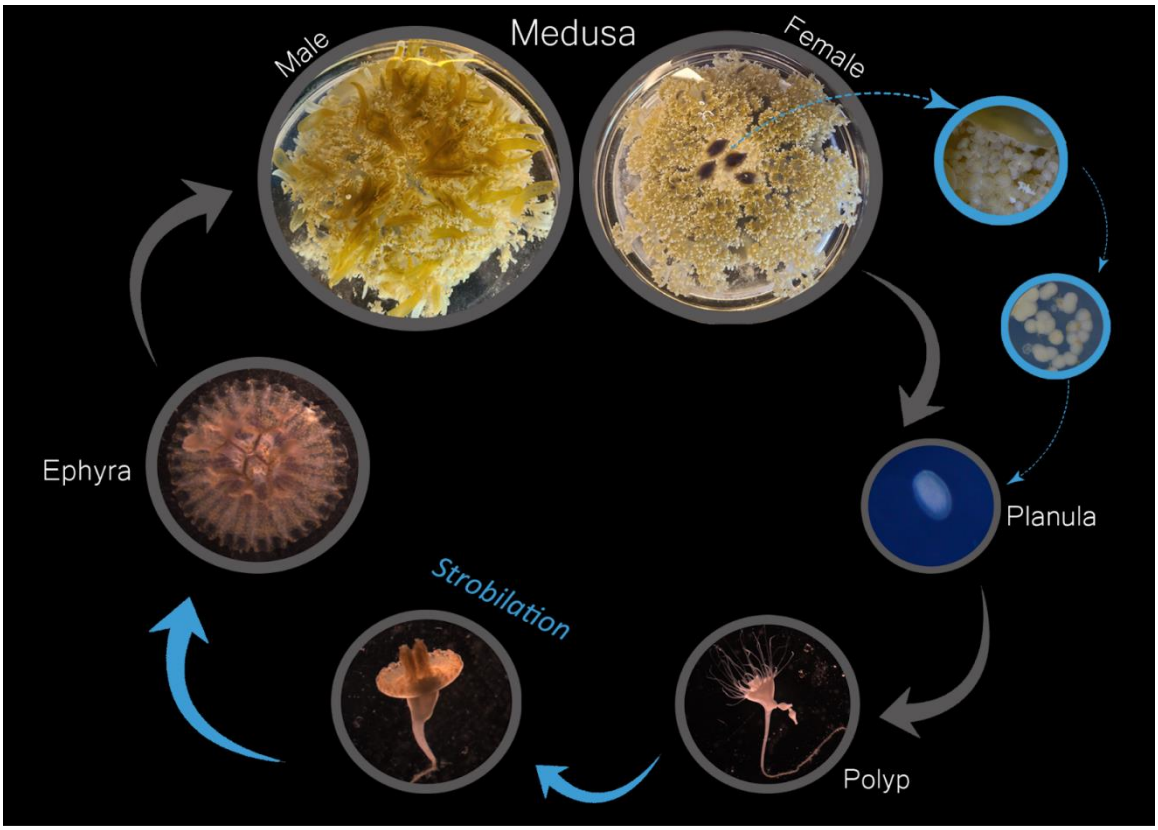

Figure 2-1. Life cycle of *C. xamachana*. Blue circles show a close-up of the white brooding appendages at the center of the female medusa bell (indicated by blue dotted arrow). The white cluster seen in the first close-up is shown in greater detail in the second blue circle. Planula separate from the egg cluster when developed. Solid blue arrows represent strobilation.

Cassiopea xamachana ecology and biology

Twelve *Cassiopea* species are currently recognized through morphological differences and genetic barcoding on the World Register of Marine Species (WoRMS). *C. xamachana* is found in the Caribbean and Gulf of Mexico, overlapping in region with *C. frondosa*. There has been substantial debate on species distinctions of *C. xamachana* and *C. andromeda*, which is native to the Red Sea, though it has been reported as alien in the Mediterranean Sea and in Hawai'i. Morphologically, *C. xamachana* and *C. andromeda* are identical, but genetic comparisons have found slight differences. It is currently unclear if the two species should be grouped, or if the difference in geographic location will eventually result in more distinctive species separations.

Adult medusa can be found bell side-down on sandy and muddy seafloor bottoms, in seagrass beds, and amongst mangrove roots (Bigelow 1900). They are often found in shallow waters between 1-2 meters in depth but have also been reported in deeper 3-4 meter waters. Adults have been recorded in temperatures ranging from 29 to 36°C (Fitt et al. 2021). While adult medusa are sexually dimorphic (Figure 2-1) and planula can be seen swimming away from fertile females in aquariums, male spawning has never been observed in the wild or in laboratory systems. Because of this, it is unclear how or where fertilization occurs in the adult medusa. While in the lab planula have been reported to settle on a variety of natural substrates such as crab shells and *Artemia* eggs (Ohdera et al. 2023), and settlement in the lab can be induced by certain peptides (Hofmann and Brand 1987), polyps have only been found in the wild on the underside of degraded mangrove leaves. Adult medusa have diameters ranging from 6.5 to 24 cm, but smaller medusa and ephyra have never been reported in the wild (Medina et al. 2021).

C. xamachana has a developmental symbiosis life cycle, where transition from polyp to adult only occurs with the establishment of symbiosis. Female medusa brood fertilized embryos and planula in their gastrovascular cavity and on the lapelets in the center of their bell (Figure 2-1). Planula are covered in cilia and once motile, swim away from the adult to settle into polyps. Planula settle aboral side down and develop a pedal disc for attachment to a substrate, after with a calyx and tentacles form. Once a fully functional mouth has developed, polyps are able to ingest dinoflagellates from the family Symbiodiniaceae and become symbiotic. This family of dinoflagellates is globally distributed and form symbioses with a variety of hosts, such as coral, clams, and foraminiferans. There are multiple species within this family that can establish symbiosis with polyps in a laboratory setting (Sharp et al. 2024; Chapter 3) however, adult medusa in the wild are overwhelmingly found associating with *Symbiodinium microadriaticum*. Once symbiotic, polyps begin to strobilate, which is the process in which scyphozoan jellyfish polyps produce adults. The head of the polyp will morph into a disc-shaped ephyra, which will eventually detach through its pulsing, causing lateral fission from the polyp head (Figure 2-1). The polyp will regrow a new head and repeat the production of ephyra. While some scyphozoans can produce multiple ephyra at once, *C. xamachana* is a monodisc strobilator, and only produces one ephyra at a time (Medina et al. 2021). Also, *Cassiopea* species are the only reported scyphozoans who only undergo this process once symbiotic (Jarms and Morandini 2019). If kept aposymbiotic, or without their dinoflagellate symbionts, *C. xamachana* polyps do not naturally undergo strobilation. Freshly-detached ephyra consist of 4 oral arms and rhopalia, the sense organs which control pulsation as part of their function. 1-2 days after detachment, the oral arms will bifurcate, forming 8 total oral arms which persist to adulthood. Laplets and colored vesicles will begin to form after this point (Medina et al. 2021). The timing of development of gonads is variable, but developed gonads have been reported on medusa as small as 7 cm (Hofmann and Hadfield 2002).

Cassiopea xamachana as a model organism

Following the initial description of *C. xamachana* medusa and polyps by Bigelow in 1892, several exploratory studies on the organism were performed in the early 1900s. General anatomy and behavior was of interest, including the influence of rhopalia (sense organs on the bell of the animal) on pulsation (Mayer 1908). Some initial studies on medusa regeneration were conducted during this time as well (Stockard 1910; Cary 1916), though regeneration studies would not be revisited until the 1970s (Curtis and Cowden 1972). Research on symbiosis and its role in the formation of the planet as we know it experienced a resurgence in the 1970s with work published by Lynn Margulis (Margulis 1971), and new interest sparked over the unique developmental symbiosis of *Cassiopea* (Balderston 1969). Studies explored the presence of dinoflagellate symbionts in *Cassiopea* tissue, and the progression of polyp development from planula through strobilation with their symbionts (Hofmann et al. 1978).

Reviews on *C. xamachana* as a model organism have been recently published, and only a brief summary is included here (Ohdera et al. 2018; Medina et al. 2021). There are many unanswered questions on *C. xamachana*, and several avenues of research that can be explored with this model organism. While we know *C. xamachana* cannot strobilate without its photosymbiont, the actual molecular process initiating strobilation is yet to be determined. Transcriptional work has suggested the retinoic acid pathway may be involved, but the role of the symbiont in triggering this pathway is still unclear (Ohdera et al. 2022). Additionally, in marine symbiosis with dinoflagellates, hosts frequently only associate with one or a few symbiont species, suggesting a mechanism for host identification of symbionts. Membrane ligands may be involved in host recognition of symbiont species but, again, this is yet to be determined. In *C. xamachana,* unlike other marine hosts, the jellyfish is able to associate with a wide assemblage of symbiont species from multiple genera (Sharp et al. 2024; Chapter 3). If a dinoflagellate was not able to persist in the tissue as a symbiont, strobilation did not occur. The lack of specificity in its endosymbiosis makes *C. xamachana* a unique model to study the patterns of symbiosis establishment in marine hosts.

C. xamachana is easy to find in the wild and relatively simple to culture in aquaria (Medina et al. 2021). This makes it an ideal model to answer cnidarian research questions that would otherwise be impeded. For instance, *C. xamachana* has been used to study venom and nematocyst structure which can be extrapolated to understand venom in related organisms like box jellyfish, which pose a significant threat to human health (Ames et al. 2020). *C. xamachana* has also been found to accumulate metals and other toxins in their tissue, and their global distribution makes it easy to sample these jellies for environmental studies on bioaccumulation of toxins (Templeman and Kingsford 2010). As research using this model organism increases globally, it is crucial to standardize husbandry practices. The following sections will cover the general parameters all scyphozoans need to thrive, before delving into *C. xamachana* specifically. Husbandry is divided into each life stage and sections are included for specific husbandry requirements researchers may need to know, such as preventing symbiosis of polyps and collecting planula from adult medusa.

General abiotic parameters and setup

All scyphozoans need a healthy bacterial community in the seawater system before animals are introduced (AZA Aquatic Invertebrate TAG 2021). This process, called "cycling" a tank, can be achieved easily through the use of commercial seawater biostarter kits. These liquid vials contain bacteria that once established, can facilitate nutrient cycling in your aquaria, and reduce the produced waste of your jellyfish. An aquarium is considered cycled if a living organism is introduced, such as *Artemia* shrimp, and the nitrate, nitrite, ammonia, and pH of the tank remains stable. Nitrates should be around 10 ppm or less, and pH should be between 8.0 and 8.4. Nitrites, ammonia, and dissolved metals should be close to zero (AZA Aquatic Invertebrate

TAG 2021). Data on the ranges of Total Ammonical Nitrogen (TAN), ammonium, nitrites, and nitrates of *C. xamachana* medusa and polyps can be found in Table 2-3.

When cultured in smaller water volumes, like a 6-well plate, seawater can be cycled by the addition of like *Artemia* shrimp and regular water changes. Up to 50-gallon aquaria have been successfully cycled with the bacteria accompanying live and deceased *Artemia* shrimp, but the process takes about a month of daily additions of shrimp and water changes (personal communication, Medina Lab). Commercial biostarters can take less time if used correctly, and this should be taken into consideration when preparing for housing *C. xamachana*.

The addition of commensals in *C. xamachana* aquaria is debated. The Medina Lab at Penn State has successfully kept adult *C. xamachana* with chitons without harm to the jellies, and aquarists have had success keeping algae-eating snails alongside adults as well (personal communication, JellyCamp 2023 conference). It is important to avoid keeping, in the same tank, organisms that can eat or harm jellies. While researchers are unlikely to keep *C. xamachana* with loggerhead turtles, a reported predator of these jellyfish, nudibranchs such as *Dondice* species have also been reported to eat medusa (Brandon and Cutress 1985). A breeding population of healthy medusa will regularly produce planula, and these settle into polyps on surfaces in the tank. If one desires to keep their planula, commensals that move along surfaces like snails and chitons, will carve paths through settled polyps and potentially kill them. While they may keep algae levels down, this is an important consideration. Additionally, *C. xamachana* produces a thick mucus of stinging cells, particularly when feeding (Ames et al. 2020). Commensals in aquaria are generally discouraged as they may get stung and eventually die by nematocyst exposure.

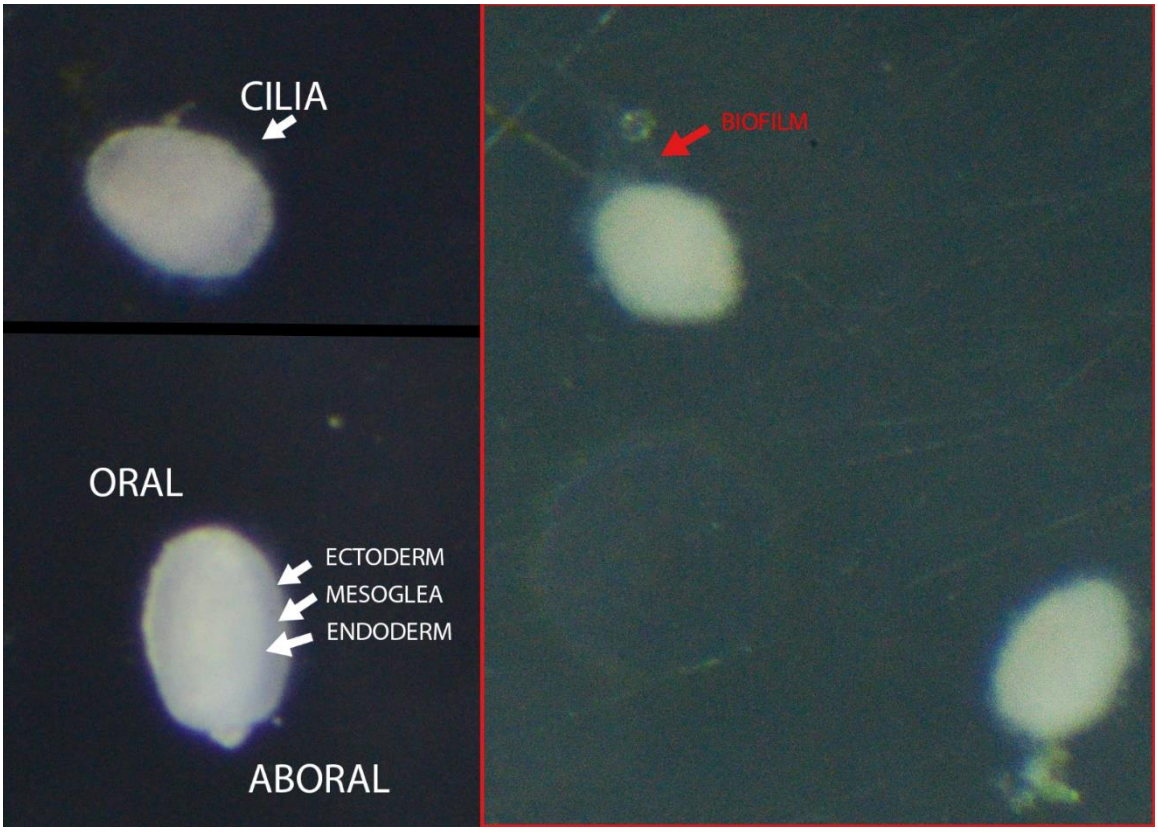

Figure 2-2. Diagram of healthy *C. xamachana* planula (left) and dying planula (right, outlined in red). Major anatomical features indicated by text and arrows. Unhealthy and deceased planula can be identified by reduced movement, biofilm formation, and degradation of tissue.

Planula

Anatomy

All stages of *Cassiopea xamachana* have three tissue layers; the epidermis, mesoglea, and endodermis. Planula range from 120 to 220 µm in length and 85 to 100 µm in width. They are ciliated and are do not host dinoflagellates at this time (Figure 2-2)(Medina et al. 2021).

Unhealthy planula will slow down or stop moving and develop a biofilm on their exterior. When deceased, the visible distinction between epidermis, mesoglea, and endoderm will disappear (Figure 2-2).

Collecting planula from medusa

Brooding females can be identified by a patch of densely clustered white oral appendages at the center of the bell (Figure 2-1)(Medina et al. 2021). If a fertile female and male are kept in the same body of water, fertilized embryos will appear on the brooding appendages within one day of co-habitation (Ohdera et al. 2023; personal communication, JellyCamp 2023). To date, the process of sperm release and subsequent fertilization of the embryos within the female animal has not been observed (Medina et al. 2021).

Fertilized embryos can be removed from the brooding appendages by gently pipetting seawater at the appendages (Fleck and Fitt 1999)(Ohdera et al. 2023). These embryos need to be housed in filtered seawater with antibiotics added to prevent water fouling and embryo mortality (see Ohdera et al. 2023 for antibiotics). Planula frequently escape from egg capsules and begin swimming upon immediate removal from the female, but some egg clusters remain unhatched (Fleck and Fitt 1999). Within 48 hours, these egg clusters will develop, and these planula can be

removed from the antibiotic seawater for subsequent development into polyps (Ohdera et al. 2023).

Rearing planula to polyps

Planula need a biofilm to settle, which can either be provided through natural substrates or by creating a biofilm in culture wells (protocol for the latter in Ohdera et al. 2023). Degrading mangrove leaves have previously been shown to induce settlement of *C. xamachana* larvae (Fleck and Fitt 1999), but other research found discarded mollusk shells to be more effective substrates (Ohdera et al. 2023). Of course, all naturally acquired substrates pose the risk of introducing Symbiodiniaceae into aquarium systems and should be considered only when there is no need for aposymbiotic polyps. Alternatively, the artificial inducer Z-Gly-Pro-Gly-Gly-Pro-Ala-OH can be purchased from several commercial websites, and a concentration of 1.2×10^{-5} M will induce bud settlement within 24 hours (Hofmann and Brand 1987).

Settlement of planula and buds have both been observed on the biofilms present on empty *Artemia* eggshells. Researchers can purchase flash-frozen *Artemia* eggs, which do not contain Symbiodiniaceae dinoflagellates on their exteriors, and hatch them to acquire empty eggshells for settlement (Curtis and Cowden 1971; Hofmann et al. 1996). For labs without the means to grow *Pseudoalteromonas* or *Vibrio* biofilms to induce settlement (Ohdera et al. 2023), this is an effective alternative for the production of aposymbiotic polyps from planula.

Once planula settle and develop into polyps, they are naturally aposymbiotic. until the mouth has developed and ingestion of the symbionts is possible (Medina et al. 2021). However, there is always a risk of dinoflagellates clinging to the outside surface of planula as the parent and surrounding water will have Symbiodiniaceae. If researchers have collected planula and want to

prevent symbiosis, it is advised to rinse planula as a precaution to remove dinoflagellates. The planula can be gently rinsed with sterile seawater through a mesh sieve several times and placed in dinoflagellate-free seawater for settlement. Leaving planula in the dark until they can be checked for symbiont presence under a fluorescent scope is also recommended. Emission wavelengths from Symbiodiniaceae are at approximately 680 nm (Hennige et al. 2008).

Planula can be kept in petri dishes or culture plates after settlement as they develop into polyps (Figure 5)(Ohdera et al. 2023). *C. andromeda* has been reported to develop a functioning hypostome after 24 hours (Curtis and Cowden 1971; Hofmann et al. 1978), but some settling planula have been observed to develop hypostomes over 24 hours later (Medina lab, unpublished). Researchers should carefully observe polyps to observe hypostome development.

Summary

Planula need a biofilm for settlement, but avoid fouling of the seawater. Petri dishes or culture plates are ideal for keeping planula as they settle. Planula do not eat and do not need light over than enough for a circadian rhythm (Table 2-5).

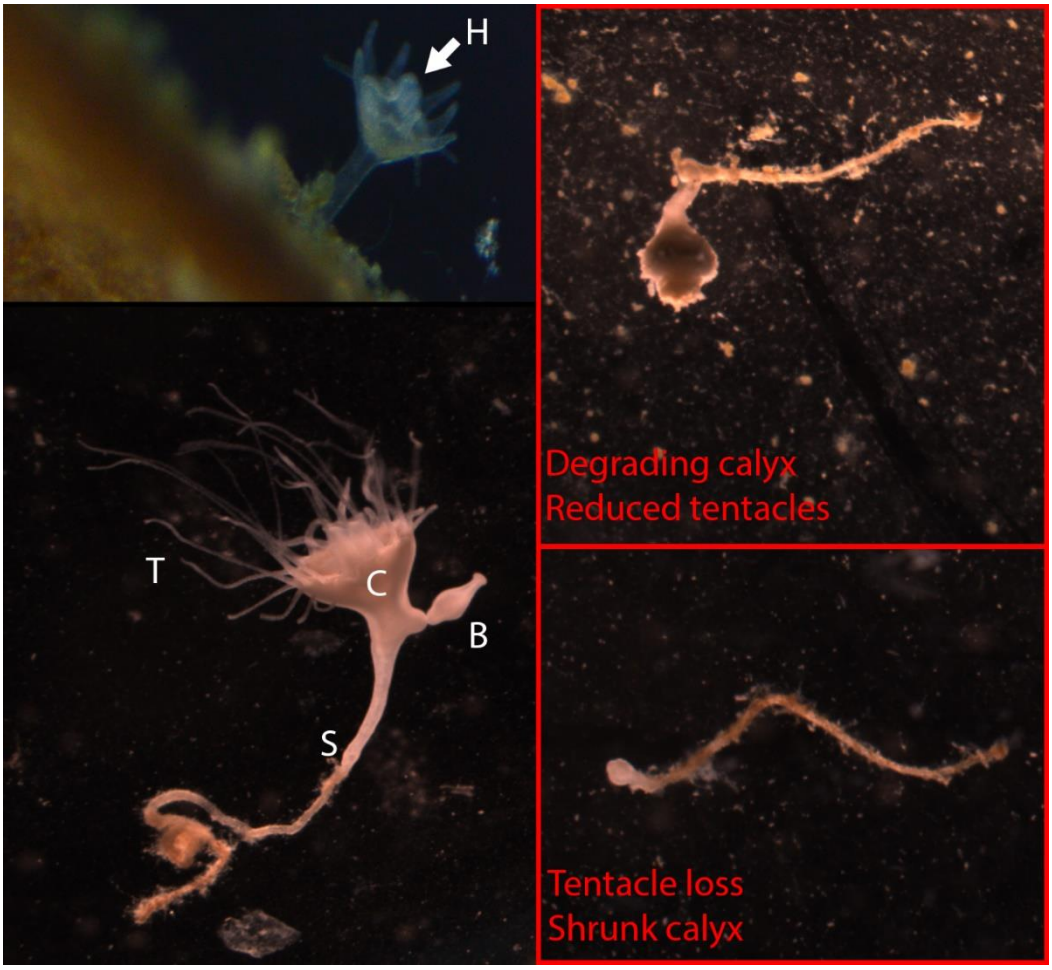

Figure 2-3. Diagram of healthy *C. xamachana* polyps (left) and dying polyps (right, outlined in red). Top left image shows a newly settled polyp (not to scale) and bottom left shows an older polyp forming a bud. Dying polyps can be identified by reduced/absent tentacles and a degrading calyx. Key to diagram: H, hypostome; T, tentacles; C, calyx; S, stalk; B, bud.

Polyps

Anatomy

Polyps are comprised of a stalk attached to a calyx (head) with 32 tentacles (Figure 2-3)(Medina et al. 2021). The calyx is around 1 mm in diameter, with body lengths around 3-4 mm long not including the tentacles, which are longer than the entire body when expanded. The calyx has 4 gastric pouches which can be seen through the translucent polyp head, and the pouches will turn the color of the polyps' prey once ingested. Once settled to a surface by the base of their stalk, the foot, they do not detach unless manually removed from the surface. Once detached, the foot heals, but they do not reattach to a surface again (Mammone, Sharp, et al. 2021). As seen in the experiments discussed in this paper (see Methods), polyps are still healthy and able to both strobilate and asexually bud without being attached to a surface. When detached polyps are present, animal caretakers should ensure to not remove loose polyps when doing water changes.

Budding occurs along the base of the calyx, and chains can form of 3-4 buds at a time (Figure 2-3). Buds will detach from the head or chain and are ciliated for independent swimming. Anatomically they are similar to planula (Medina et al. 2021).

Unhealthy polyps will have shrinking or nonexistent tentacles. The calyx will decrease in diameter, and tissue may appear more transclucent than before. There will be a decreased response to stimuli and prey. When deceased, the head will completely disintegrate. However, the stalk will not disappear, but all living tissue on it will disintegrate. The stalk will remain behind and become heavily covered in biofilm (Figure 2-3).

Aquaria conditions

Polyps are typically kept in culture plates with no water flow (Mammone, Sharp, et al 2021), or in glass aquaria (Newkirk et al. 2020, AZA Aquatic Invertebrate TAG 2021). In aquaria, it is common to use petri dishes suspended in the water for polyp settlement (Figure 2-9)(Pierce 2005). Suspending surfaces for settlement off the bottom of the aquarium provides more surface for buds and is thought to mimic the sideways or upside-down settlement patterns seen in scyphozoan polyps in the wild. Light water flow suitable for the benthic medusa is also suitable for polyps (AZA Aquatic Invertebrate TAG 2021). Polyps have been reported to tolerate a salinity range of 30 ppt (Fitt and Costley 1998) to 35 ppt (Muffet et al. 2022, AZA Aquatic Invertebrate TAG 2021). Polyps have also been kept from 21-40 ppt seawater at various periods in the Medina Lab, though polyps noticeably shrank in size above 38 ppt and died if kept in 40 ppt for long periods of time (personal observation). Polyps can survive at a wide range of temperatures, from 25°C to 36°C. Under 15°C halts feeding of the polyps and eventual death, and above 36°C results in loss of the tentacles (Fitt and Costley 1998). Polyps are typically kept from 22°C (Muffet et al., 2022) to 27°C (Sharp et al. 2024).

If polyps were kept aposymbiotic, artificial seawater will need to be used for husbandry. Natural seawater will contain Symbiodiniaceae, as that family has a very wide geographical range and is the most common symbiont in the oceans (LaJeunesse et al. 2018). If natural seawater is used, polyps will become symbiotic eventually, and there will be no way to control which symbiont they have or when they are introduced. When using an ocean salt mixture, like Instant Ocean, distilled or purified water should be used to mix to appropriate salinities. Tap water in most locations globally have excess minerals or other contaminants that may affect the health of the jellies (AZA Aquatic Invertebrate TAG 2021).

Like medusa, symbiotic polyps need sufficient light levels for proliferation of their endosymbiont. Without sufficient light, polyps will still strobilate, but it will be slow and inconsistent (Medina Lab, unpublished). With no light, strobilation will not occur (Jinkerson et al. 2022). However, endosymbionts will persist in the polyp tissue for up to 6 months after induction of symbiosis, even with no light (Jinkerson et al. 2022; Newkirk et al. 2018). Aposymbiotic polyps have traditionally been kept in dark environments (Fitt and Trench 1983), however, several authors of this review investigated the effectiveness of this method (Figure 2-4). Aposymbiotic *C. xamachana* polyps were kept in either exclusively dark ("Dark") conditions or kept with a 12:12 day/night cycle with light at approximately 100 μm/m^2/s ("Light"). Polyps kept in dark exclusively experience higher mortality than polyps with a day/night cycle (Figure 2-4). While it does discourage the establishment of symbiosis in the event of accidental Symbiodiniaceae introduction to aposymbiotic polyps, it does not encourage the health of the animal. Light levels should remain low to ensure minimal heating of the water (see Methods) but have wavelengths between 400-500 μm (AZA Aquatic Invertebrate TAG 2021).

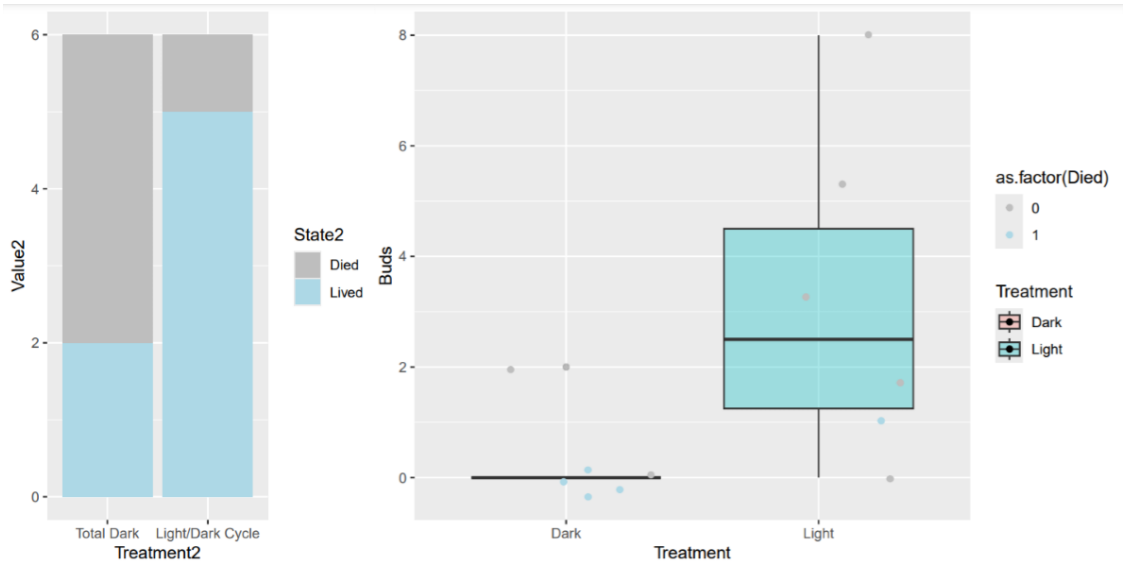

Figure 2-4. Number of buds produced by polyps kept only in the dark (Dark) or with a 12:12 day/night cycle (Light). Left shows number of polyps that died (gray) and lived (blue). Right shows box plot of number of buds produced by treatment with each dot representing an individual polyp. Dots colored by whether the polyp lived (blue) or died (gray).

Feeding and nutrition

Polyps are commonly fed recently-hatched *Artemia* shrimp or rotifers (Ohdera et al. 2018), both of which are popular aquarium foods. These organisms are easily hatched, and small in size for easy predation to ensure smaller, still-developing polyps can still feed (Léger et al. 1986). Newly settled polyps should be fed rotifers or ground up *Artemia* until they are large enough to eat bigger prey (approximately 1 month after planula settle). However, planuloid buds settle into larger initial polyps, and they can be fed larger prey anywhere from 3-4 weeks after settlement.

Frequency of feeding is an essential practice to consider when considering *C. xamachana* husbandry. A short experiment was conducted by several of the authors of this guide where

symbiotic polyps were fed once a week, three times a week, and everyday. Polyps were kept on a 12:12 day/night cycle, and either fed 30 minutes after the day cycle begun, or 30 minutes after the night cycle begun. Polyp mortality and time to strobilation was recorded to test feeding frequency and timing on inducing successful reproduction of *C. xamachana* (Table 2-1). Feeding only once per week will result in polyp death of a proportion of the population, causing the health of the colony to drastically decrease. Feeding everyday appears to lower the amount of time it takes to induce strobilation, which increases animal reproductive success and the production of animals for laboratory work. Feeding frequency significantly affects the reproductive success of polyps (Table 2-1) and feeding regimes should be developed based on the labs' needs. Meanwhile, time of feeding did not significantly impact polyp mortality or time to strobilation, and polyps appear able to feed even during their "sleep" period (Table 2-1).

Table 2-1. Average time to strobilation with varying polyp feeding schedules. Asterisks indicate treatments where one or more polyps died.

	Feeding Regime	# strobilating polyps	Average # Days to Strobilation
Fed during daytime	Everyday	6/6	16
	3x/week	6/6	16.833
	1x/week	1/6	21*
Fed during nighttime	Everyday	6/6	16.667
	3x/week	6/6	18
	1x/week	0/6	N/A

Many aquaculture facilities feed *Artemia* to scyphozoans (AZA Aquatic Invertebrate TAG 2021), but the *Artemia* will be fed blends of phytoplankton or other supplements to provide additional nutrients for the jellyfish. By feeding these supplements to *Artemia*, it ensures bioaccumulation of nutrients in the jellyfish while minimizing waste accumulation in the jellyfish

water (Watanabe et al. 1982). While *Artemia* by themselves have successfully been used to feed *C. xamachana* and led to bud production and strobilation (Sharp et al. 2024), changing the diet of *Artemia* prior to feeding jellies can increase the available lipids, proteins, caratenoids, and fatty acids. In a study with larval fish fed *Artemia* on a diet of algae and Selco, larvae had increased survivorship and overall fatty acid composition. Even fed one type of phytoplankton, there was a slight increase in proteins, lipids, and ash of *Artemia* nauplii (El-Sayed et al. 2022).

Several of the authors on this paper conducted an experiment where *A. fransiscana* nauplii were fed different supplements, and then fed to *C. xamachana* polyps. Nauplii less than 24 hours old were collected and transferred into seawater solutions of various nutrient supplements as described in Table 2-2. *A. fransiscana* nauplii were left to feed for 4 hours before being filtered, resuspended in fresh 35 ppt seawater, and given to polyps. Each polyp received 3-10 nauplii. The mortality and number of buds produced by polyps were recorded (Figure 2-5). The average number of buds produced per polyp was highest for the 'Algemac' treatment (6.333 buds) compared to the control of 0.8333 buds, and this treatment produced 55 total buds compared to 8 total for the control. The nutrient treatment of *'Spirulina'* produced the fewest number of buds for all treatments (21 total, 3.5 per polyp)(Figure 2-5). These results show that the addition of nutrient supplements to *Artemia* prior to feeding to *C. xamachana* results in an uptick in bud production. We then explored if these nutrient supplements would decrease the time to produce ephyra, another form of asexual reproduction. After one month of observation, any deceased polyps were replaced, the polyps were fed the diet for another 2 weeks. These polyps were then given *Symbiodinium microadriaticum* cells at a concentration of ~150,000 cells/mL following the protocol in Sharp et al. 2024 to induce symbiosis. The time to strobilation and mortality was then recorded (Figure 2-6).

As shown in Figures 2-5 and 2-6, nutritional supplements fed to *Artemia* individuals can boost both budding and strobilation reproduction of the polyps. It appears the "All" treatment had a consistent benefit to polyp reproduction, as it produced a large number of buds and induced strobilation faster than the control (Figures 2-5, 2-6). Additionally, the "All" nutrient combination had zero polyp mortality upon introduction of the symbionts, while the other treatments had polyp death (Figure 2-6). There were multiple organic compounds in the nutrient supplements that are not found in *Artemia* nauplii (Table 2-3), and it appears the addition of caratenoids, vitamins, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) may be necessary compounds for increased *C. xamachana* asexual reproduction. We saw an increase in survivorship and reproductive success in *C. xamachana* polyps, showing that the addition of nutrients to *Artemia* cultures aid researchers achieve abundant jelly populations. In the wild, *C. xamachana* have been reported to eat a variety of zooplankton including copepods and ostracods (Larson et al. 1997). The addition of nutrients to a strictly *Artemia* diet may help balance the lack of dietary diversity laboratory kept polyps experience. These positive effects on polyps suggest *Artemia* nutritional supplements will have beneficial effects on all heterotroph stages of *C. xamachana* and, while the authors of this guide did not test these supplements on medusa, there is potential for this diet to lead to healthier jellies across all life stages.

Table 2-2. Nutrient supplements given to *Artemia fransiscana* nauplii prior to feeding to *Cassiopea xamachana* polyps. The names of treatments correspond to Figures 2-7 and 2-8.

Name	Ingredient	Quantity	Concentration	Source
Control	*Artemia fransiscana*	3-10 nauplii	NA	Brine Shrimp Direct

Spirulina	Spirulina Powder (*Arthrispira platensis*)	~0.02%	0.02%	Brine Shrimp Direct
Spresso	S.presso	½ drop / 50 mL SW	0.0006%	INVE Aquaculture
Algemac	Liquid Red Algamac-DHA30	1.5 drops/50 mL	0.002%	Aquafauna Bio-Marine
	S.presso	0.5 drops/50 mL	0.0006%	INVE Aquaculture
	Easy DHA Selco	0.5 drops/50 mL	0.0006%	INVE Aquaculture
All	Spirulina Powder (*Arthrispira platensis*)	5 mL of each treatment	0.02%	Brine Shrimp Direct
	Pavlova sp. Cryo-Preserved Algae Paste	1 drop	0.006%	Brine Shrimp Direct
	Liquid Red Algamac-DHA30	5 mL of each treatment	0.002%	Aquafauna Bio-Marine
	S.presso	5 mL of each treatment	0.0006%	INVE Aquaculture
	Easy DHA Selco	5 mL of each treatment	0.0006%	INVE Aquaculture

Table 2-3. Composition of nutrient supplements given to *Artemia fransiscana* nauplii prior to feeding to *Cassiopea xamachana* polyps. Information came from the ingredients lists of manufacturer website which can be found in Table 2-2.

Artemia fransiscana	Spirulina Powder *(Arthrospir*	S. presso	Liquid Red Algamac-	Easy DHA Selco	*Pavlova sp*. Cryo-Preserved Algae Paste

		a platensis)		DHA30		
Ash	9.7 +/- 4.6	7.2	0.20%		0.30%	<8%
BHA			72 mg/kg		27 mg/kg	
BHT			72 mg/kg		27 mg/kg	
Calcium			0.10%		0.10%	
Caratenoids		3000-4870 ppm				
Carbohydrates	14.8 +/ 4.8			>15%		>1.7%
DHA Content			260 mg/g dry weight	28-30%	150 mg/g dry weight	
EPA			25 mg/g dry weight		50 mg/g dry weight	
Fat		5.74%	35%	>68%	66%	
Fibre			0.10%		0.10%	
Lipid	18.9 +/ 4.5					>1.5%
Phosporus			0.20%		0.10%	
Propyl Gallate			100 mg/kg		100 mg/kg	
Protein	52.2 +/- 8.8	59.20%		>6%	0.50%	>4%
Sodium			0.20%		0.02%	
Sum 3 HUFA						
Vitamin A			110,000 IU/kg		1,6000,000 IU/kg	
Vitamin C						
Vitamin D3			10,000 IU/kg		150,000 IU/kg	
Vitamin E						

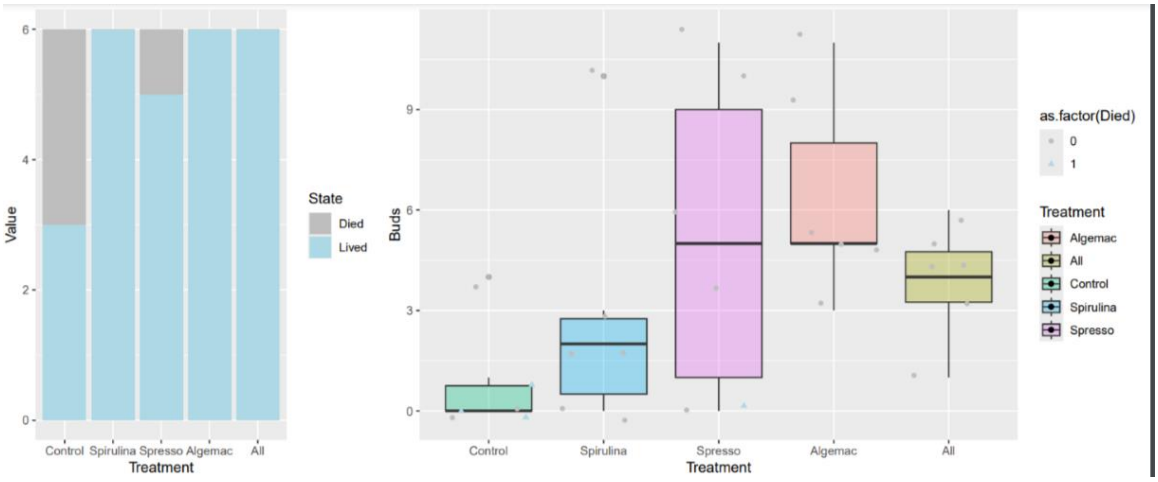

Figure 2-5. Number of buds produced per nutrient treatment. Left graph shows the number of polyps that lived (blue) and died (gray) per nutrient treatment. Right shows the number of buds produced by nutrient treatment. Dots represent individual polyps, with dots colored whether the polyp lived (blue) or died (gray) by the end of the experiment.

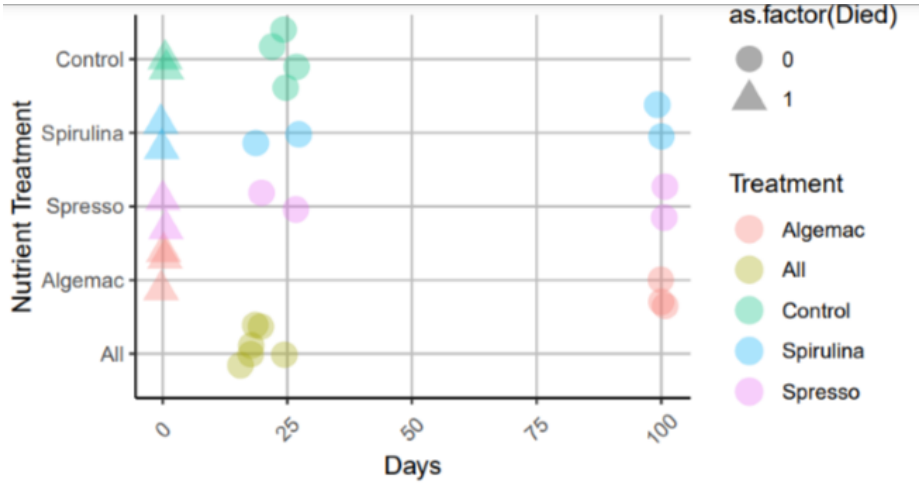

Figure 2-6. Number of days to strobilation for polyps fed nutrient treatments. The polyps had been fed nutrient treatments for a minimum of 2 weeks prior to being given symbionts. Values of 100 denote polyps that never strobilated.

Preventing symbiosis

If researchers have symbiotic polyps and wish to acquire aposymbiotic polyps, there are several methods that take various amounts of time. If ample time is available, polyps can be fed abundantly and planuloid buds collected. As seen in Figure 2-4, polyps just fed *Artemia* 3 times a week will produce anywhere from 1-3 buds in a month timespan, and this can be increased by increasing feeding frequency (Hofmann et al 1978) and providing nutrient supplements to *Artemia* (Figure 2-5). These buds can be observed under a fluorescent scope, as buds sometimes have symbionts within them or not based on the cells they divide from (Hofmann and Honegger 1990), and if free of internal symbionts, these buds can be reared into aposymbiotic polyps. The buds can be rinsed several times in artificial seawater to minimize symbionts sticking to their exterior and reared in a dark environment until polyps. Once fully developed with mouths, researchers can check the polyps using fluorescence again to see if they are properly symbiotic. The authors of this paper have successfully used this method to isolate aposymbiotic polyps of *C. andromeda* (unpublished).

An alternate method takes approximately 6-7 months. If the polyps are moved to a dark environment for this length of time and given routine water changes with only artificial seawater, eventually the photosymbionts will no longer be in the water or animal tissue (Newkirk et al. 2020). However, this makes lab experiments difficult to perform in the meantime, as the animals will not be as healthy in the dark as in the light (Figure 2-4).

Asexual reproduction and clonal lines

As seen in the results described in this paper, polyps will naturally produce buds without prompting from the researcher. Budding chains have been recorded of as many as 3 buds (Hofmann et al. 1978), though chains of 5 buds was seen during the experiments described in Sharp et al. 2024. Buds once detached will begin to swim and settle on a biofilm for subsequent development into a polyp. If no alternate biofilm is provided, buds have been seen attaching to the base of the stalk of other polyps in the same water (personal observations). If experiments are being performed on individual polyps, it is highly recommended any detached buds to avoid later confusion on which polyp was the original experimental animal. When individual polyps are kept genetically isolated from other polyps and allowed to continuously bud, a clonal line will result. While individual variation of polyps produced from buds has not been studied genetically or otherwise, all buds originate from cell division of the same precursor polyp, and these can be used as biological replicates in experiments. The Medina Lab at Penn State has kept clonal lines for over 8 years without the lines dying as long as regular water changes and feeding is performed.

Inducing strobilation to produce ephyra

Once polyps are symbiotic, they will strobilate between 14 to 38 days depending on the symbiont species (Sharp et al. 2024). Once strobilation has begun, it is a cascade effect that does not halt for approximately 3 days. The strobilating polyp will not need to eat during this time. However, the polyp head regrows shortly before detachment of the ephyra and can begin eating again as soon as strobilation ends. They do not need any additional stimuli to strobilate, as shown through the experiments discussed in this guide. However, Lugol's iodine solution has been reported to induce strobilation in symbiotic polyps within one week of raising seawater levels to 0.06 ppm (Pierce 2005). *C. xamachana* is a monodisc strobilator, meaning the polyp develops one ephyra at a time and releases it completely before producing another (Medina et al. 2021). Once released, polyps take approximately 1-2 weeks to produce a second ephyra (Sharp et al. 2024). Polyps have been shown to produce from 3-5 ephyra from a single individual. Although aposymbiotic polyp can't strobilate in the wild, strobilation can be induced in the laboratory through chemical inducers such as indomethacin (Cabrales-Arellano et al. 2017), but ephyra produced through indole compounds have not been kept alive longer than 2 weeks (Medina Lab, unpublished). In *C. andromeda*, similar indole compounds have been used to induce strobilation, but developmental abnormalities results (protocols for inducement found in Deng et al. 2023).

Summary

Both aposymbiotic and symbiotic polyps need a day/night cycle to ensure healthy animals. Light parameters for symbiotic polyps should have wavelengths between 400-500 μm and levels between 70-150 μm /photons. Polyps need low to no flow and can be housed in culture plates or aquaria with suspended petri dishes. Polyps can be fed live rotifers or *Artemia* nauplii (Table 2-5).

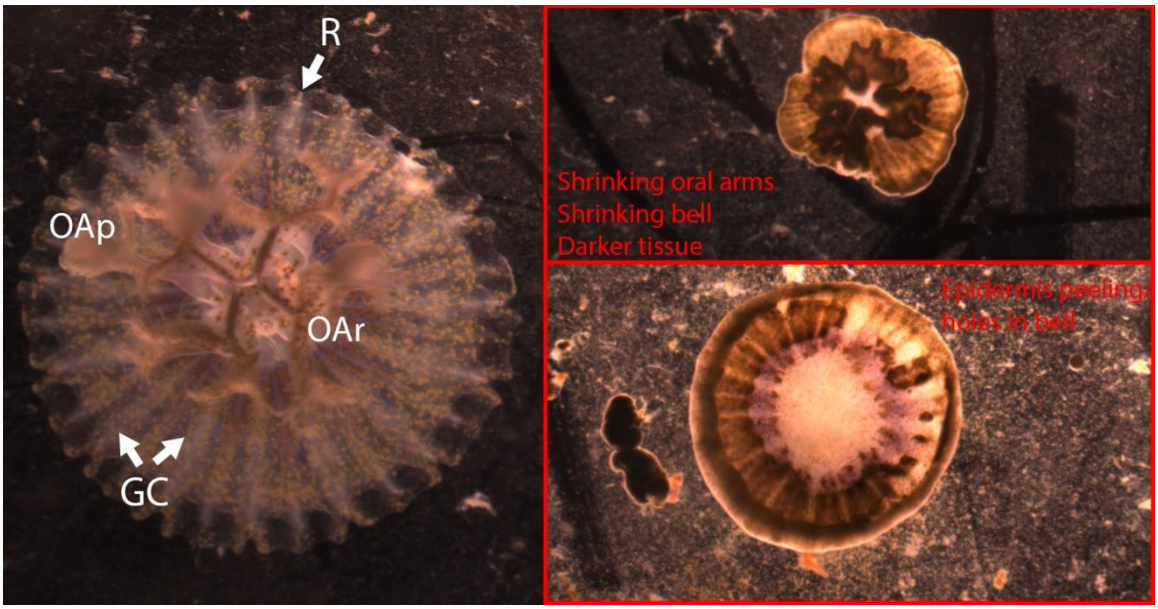

Figure 2-7. Diagram of healthy *C. xamachana* ephyra (left) and dying ephyra (right, outlined in red). Dying ephyra can be identified by shrinking oral arms and bell, darkening of tissue, and degradation of tissue. Key to diagram: R, rhopalia; OAp, oral appendages; OAr, oral arms; GC, gastrovascular canals.

Ephyra

Anatomy

The ephyra will not eat for 2 days as it continues to develop its gastrovascular canals (Medina Lab, unpublished). When newly detached, ephyra have 4 oral arms and their full set of rhopalia, typically 16 for this species (Figure 2-7). Their bell margin will have one lappet for each rhopalia. As its gastrovascular canals develop, the oral arms will split in two, forming 8 total oral arms. These will eventually develop the oral appendages that give the "fluffy" appearance of medusa (Medina et al. 2021). Ephyra may form with slight coloration in their bell, like the blue commonly seen in adults, or may develop coloration later (Muffet et al. 2022). Ephyra are typically considered adult medusa when gonads have fully developed, and this depends on size of the animal. Gonads have been reported in medusa as small as 7 cm, but it can vary (Hofmann and Hadfield 2022).

Unhealthy ephyra will have oral arms that shrink in size. This typically happens before the bell diameter decreases, however, if the bell becomes damaged it may start to shrink. Ephyra tissue is translucent and most of the coloration comes from their symbionts or colored pigment. When dying, ephyra tissue will shrink but the symbionts will remain inside the host, and dying ephyra can appear "darker" or more brown in color as the ratio of symbiont to host tissue increases (Figure 2-7). Deceased ephyra completely disintegrate.

Ephyra husbandry

Aquariums can be designed with a capture system for retrieving ephyra. In a flow-through polyp system, if a separate chamber is kept around the outflow system, detached ephyra will get swept through the outflow and trapped in the chamber. Retrieval is then possible for moving the ephyra to a designated setup (Pierce 2005).

Ephyra can be fed many saltwater animal foods, such as live *Artemia* and rotifers. Typically live food is fed, but food blends of dead *Artemia* or other shrimp have been effective as well. Unlike other jellyfish ephyra, they do not need decapsulated *Artemia* for feeding, as there have been no adverse effects on *C. xamachana* ephyra seen with untreated *Artemia* eggs (Sharp et al. 2024).

If ephyra are produced in an aquarium system, they can be housed in a floating nursery. Their small size and weaker pulsations compared to medusa make them easily swept away and lost in an aquarium system. Floating nurseries can be made by hand very easily using plastic containers with holes added and mesh glued over the holes, and a flotation foam material around the perimeter so the container does not sink. Many aquariums have creative systems to house ephyra, including trays with a mesh outflow weighed down by rocks (Pierce 2005). However, if ephyrae are kept floating at the top of the aquarium system, light levels should be carefully monitored. Like medusa, ephyrae do well with light levels at a maximum of 200 μm /photons. The light at the surface of an aquarium housing medusa may be much higher than this, and it is recommended ephyra be kept in a separate aquarium until large enough (> 0.6 cm) to survive in a larger setup without being buffeted by the flow (Pierce 2005). The higher light at the surface will also raise seawater temperature if the ephyra are kept in a shallow container, and ephyra need to be kept around 27°C.

Ephyrae have been successfully housed in 6-well culture plates, approximately 1 inch in diameter and 0.5 inches deep, for 3 months (Sharp et al. 2024). While this is functional for short term care, if a researcher aims to grow ephyra into adult medusa, a larger system is needed.

Summary

Ephyra should be housed in separate containers where they cannot be swept away or lost in an aquarium system until > 0.6 cm in diameter. Ephyrae need temperatures between 22 and 27°C and light at a maximum of 200 μm /m^2/s like medusa. They can be fed live plankton like rotifers or *Artemia* (Table 2-5).

Figure 2-8. Diagram of healthy *C. xamachana* medusa (left) and dying medusa (right, outlined in red). Degrading medusa can be identified by reduction/absence of oral arms, degradation of bell tissue, excess mucus production, and reduced activity. Key to diagram: OAp, oral appendages; OAr, oral arms; B, bell.

Medusa

Anatomy

Medusa rest bell-side down on surfaces. The bell is slightly concave in the center, allowing suction to a surface. Bell diameters have been reported at a maximum of 32.6 cm in the wild (Morejón-Arrojo & Rodríguez-Viera, 2023). Bells have a layer of muscle over the oral surface (i.e., subumbrella), and several gastrovascular canals running through the bell. Dinoflagellate symbionts will be visible in clusters in the bell, but there are fewer in the center of the bell where the gastric cavity is. Rhopalia line the edge of the bell and typically number 16 in *C. xamachana*. Eight oral arms face upwards and these may be covered in any number of oral appendages and colored laplets (Figure 2-8).

Medusa release cnidocysts (stinging cells) in the form of cassiosomes; small bundles of stinging cells and symbionts contained within their mucus. Oral arms also contain cnidocysts, and touching the oral arms can result in pain to humans. *C. xamachana* release their mucus primarily during feeding or after forms of agitation such as a water change, but there is a chance for mucus to be present in the water at all times. Human reaction to the stings varies, but many report increased sensitivity to stings over time, and care should be taken to avoid being stung when possible (Ames et al. 2020).

Unhealthy medusa shrink in size and oral appendages decrease. Oral arms will decrease in size as well. Pulsations become less frequent along with reactions to stimuli. Occasionally holes will form in the bell margin as well. When stressed, adults produce more mucus (Figure 2-8). When deceased, medusa disintegrate and there will be a lump of mucus covered tissue where the jellyfish last was.

Medusa husbandry

As benthic jellies, *C. xamachana* do not require the kreisel aquariums typically required for other scyphozoans (AZA Aquatic Invertebrate TAG 2021), but aquaria still need several components for healthy adult jellies. A sump aquarium system is ideal to provide light flow through the aquaria, while still maintaining space for culture media and filters (Figure 2-9). Medusa should be kept in a tank with an inflow and outflow, with a weak but consistent current. Direct, strong water flow should not be on the adults themselves as this can cause stress and potentially push the animals against the outflow pipe. Likewise, dividers are essential to keep the medusa away from the outflow. At least 3 inches of space is recommended, as less than this can cause the animals to be pressed against the outflow divider, which will eventually lead to death. If a researcher is keeping male and female medusa together, dividers should have holes small enough to not remove produced polyps and ephyra (< 1 μm for small polyps; 1-2 μm for newly detached ephyra (Medina Lab, unpublished)). Along with a pump for water flow, a filter is critical for lowering dissolved inorganic compound levels in the water and keeping the system's algae levels low. Protein skimmers may also be used for a flow-through sump system, but are not essential. Oxygenation of the water needs to occur, either through a waterfall system with the flow-through, or extra bubblers. For larger tanks, bubblers are recommended (Figure 2-9). Medusa have also successfully been kept in a single aquarium without a flow-through system and only a pump and waterfall-style filter for oxygen exchange, but this simpler type of system does not easily encourage animal reproduction (Medina lab, personal communication).

C. xamachana medusae expel algae from their tissue consistently, though the rate of expulsion has not been measured. Researchers will find algal biofilms growing on all surfaces in the aquarium within a few weeks of housing medusa. While this is normal, excess algal growth can spike nitrate levels as the algae naturally dies, and regular scraping of surfaces and water

changes should occur. However, it has been reported by numerous aquarists that if *C. xamachana* medusa are kept in sterile or near-sterile water, they will die (personal communications with aquarists at Newport Aquarium, and at the conference JellyCamp). Essentially, the natural expulsion of algae is natural and keeps the medusa healthy, and only routine cleaning of the aquariums is needed to ensure healthy animals.

Like most tropical jellies, adult *C. xamachana* do well at temperatures between 22 and 28 °C (AZA Aquatic Invertebrate TAG 2021). Most commercial saltwater aquarium heaters get to this range and can be used for the appropriately-sized tank. While *C. xamachana* can survive at higher temperatures for prolonged periods, they typically shrink in size after a few days, and this is not recommended for healthy lab animals. The Sea Life Facility at Texas A&M at Galveston reported a lack of growth and eventual mortality when medusa were kept at higher temperatures (26-28°C) and salinities (35-37 ppt). When adults were kept between 25-26°C and 30-33 ppt, medusa experienced significant growth. Raising the temperature to 29°C resulted in a large increase in strobilation of polyps in the system (Katie St. Clair, personal communication) (Table 2-4).

Light levels are essential for the health of the holobiont, and light measurements should be taken both at the surface level of the tank and the bottom where the jellies will predominantly reside. Wavelengths of 400-500 μm are required in any light system, so light bulbs should be acquired accordingly. Light level at the bottom of the aquarium should be kept at 200 μmol/photons for medusa to primarily use autotrophy instead of heterotrophy for nutrient acquisition (Mammone and Galindo-Martinez, in prep). While lower light levels will still keep the symbionts photosynthetically active, daily or twice daily feeding is needed to increase medusa size (Medina lab, unpublished). Lights commercially sold for the growth of coral in aquariums will work well for *C. xamachana* (Gamero-Mora et al. 2019), but frequent cleaning should be

performed to reduce the proliferation of phytoplankton growth (Medina lab, personal communication). Additional temperature measurements of the water should be performed once the lighting is installed to ensure the jellies do not overheat (AZA Aquatic Invertebrate TAG 2021).

Scyphozoan jellies are highly sensitive to dissolved metals in the water, and no excess trace metals should be in the seawater. Metal testing kits for seawater can be used to check levels, and dissolved metals can be avoided by only using clean aquariums and plumbing with no rust.

Summary

Adults need a cycled tank with low flow and no sharp objects. Light is critical and ideally at 200 μmol/photons with wavelengths of 400-500 μm. Feeding of live plankton such as *Artemia* is suitable for adults (Table 2-5).

Table 2-4. Ranges of abiotic conditions of adult medusa and polyps at the Sea Life Facility, Texas A&M at Galveston.

RANGE	**25 - 26 C**	**30 - 33 ppt**	**8.0 - 8.4**	**< 0.05 mg/L**	**< 0.02 mg/L**	**< 0.02 mg/L**	**< 10 mg/L**

	Temp	Salinity	pH	TAN	NH3	Nitrite-N	Nitrate-N
Lower	24.9	30	7.9	0.05	0.003	0.001	0.7
Upper	27.6	35	8.4	0.07	0.0271	0.025	3.2

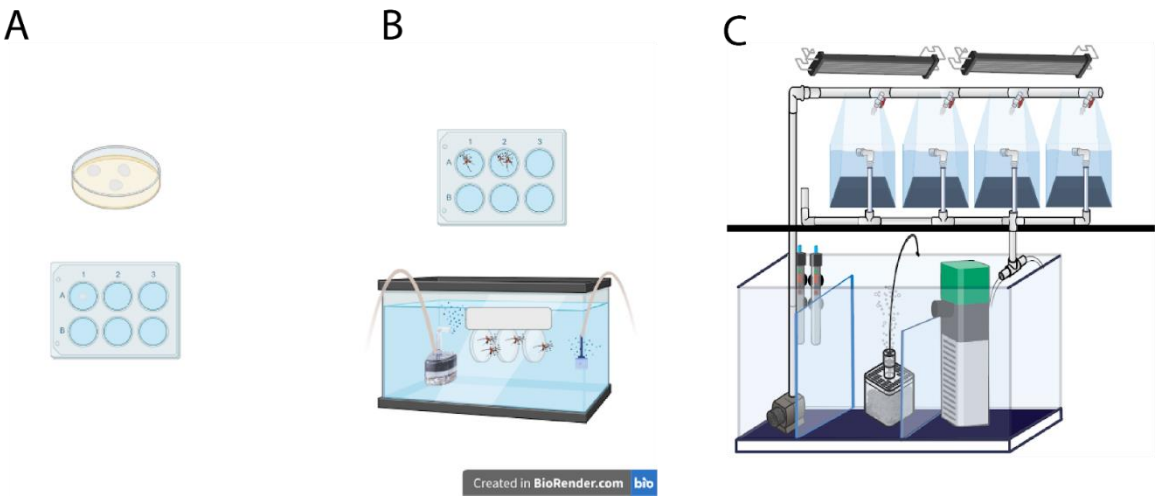

Figure 2-9. Recommended aquarium setup for *Cassiopea xamachana*. (A) Planula should be kept in petri dishes or culture plates with seawater and a biofilm for settlement. (B). Polyps can be kept in culture plates for smaller setups, or aquaria with a filter and bubbler. (C) Medusa should be kept in a sump, flow-through system with dividers between jellies and inflow/outflow, sufficient lighting for symbiosis, and filter media.

Table 2-5. Summary of basic husbandry conditions for each life stage of *C. xamachana*.

Life stage	Optimal salinity	Optimal temperature	Optimal light	Feeding regime	Tank set up
Planula	32-36 ppt	22 - 28 °C	N/A, just day/night cycle needed	No feeding until settlement and formation of mouth	Petri dishes or culture plates
Polyp / *Scyphistomae*	32-36 ppt	22 - 28 °C	Aposymbiotic: Just day/night cycle needed Symbiotic: 70-150 µm /photons	Rotifers or other small plankton such as *Artemia fransiscana*	Culture plates or aquaria with suspended petri dishes for settlement and low flow
Ephyra	32-36 ppt	22 - 28 °C	200 µm /photons	Live plankton such as *Artemia*	Mesh-lined contained in a larger aquaria with low flow Eventual flow-through aquarium setup when larger than 0.6 cm
Medusa	32-36 ppt	22 - 28 °C	200 µm /photons	Live plankton such as *Artemia*	Flow-through aquaria with low flow and no sharp objects

Conclusions

As new model organisms popularize, establishing sound husbandry practices is crucial for both animal welfare and scientific enterprise. Working with a new system poses many challenges for researchers, and this guide hopes to help standardize laboratory husbandry of *C. xamachana* and aid the expansion of this unique model system. It is the authors' hope that this framework leads to many new discoveries on the upside-down jellyfish *C. xamachana.*

References

Ames, Cheryl L., Anna M. L. Klompen, Krishna Badhiwala, Kade Muffett, Abigail J. Reft, Mehr Kumar, Jennie D. Janssen, et al. “Cassiosomes Are Stinging-Cell Structures in the Mucus of the Upside-down Jellyfish Cassiopea Xamachana.” Communications Biology 3, no. 1 (February 13, 2020): 1–15. https://doi.org/10.1038/s42003-020-0777-8.

Ankeny, Rachel A., and Sabina Leonelli. “Model Organisms.” Elements in the Philosophy of Biology, November 2020. https://doi.org/10.1017/9781108593014.

AZA Aquatic Invertebrate TAG. 2021. Jellyfish (Cnidaria/Ctenophora) Care Manual (2nd ed.). Association of Zoos and Aquariums, Silver Spring, MD.

BALDERSTON, L. “STUDY OF SYMBIOTIC RELATIONSHIP BETWEEN SYMBIODINIUM MICROADRIATICUM FREUDENTHAL, ZOOXANTHELLA AND UPSIDE DOWN JELLYFISH, \ldots.” NOVA HEDWIGIA 17, no. (1-4) (1969): 373–82.

Bengtson, David A., Philippe Léger, and Patrick Sorgeloos. “Use of Artemia As a Food Source for Aquaculture.” In Artemia Biology. CRC Press, 1991.

Bigelow RP. “The Anatomy and Development of Cassiopea Xamachana.” Boston Society of Natural History, 1900.

Cabrales-Arellano, Patricia, Tania Islas-Flores, Patricia E. Thomé, and Marco A. Villanueva. “Indomethacin Reproducibly Induces Metamorphosis in Cassiopea Xamachana Scyphistomae.” PeerJ 5 (March 1, 2017): e2979. https://doi.org/10.7717/peerj.2979.

Cary, Lewis R. “The Influence of the Marginal Sense Organs on the Rate of Regeneration in Cassiopea Xamachana.” Journal of Experimental Zoology 21, no. 1 (July 1916): 1–32. https://doi.org/10.1002/jez.1400210102.

Council, National Research, Division on Earth and Life Studies, Institute for Laboratory Animal Research, and Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals: Eighth Edition. National Academies Press, 2010.

Curtis, S. K., and R. R. Cowden. “Normal and Experimentally Modified Development of Buds in Cassiopea (Phylum Coelenterata; Class Scyphozoa).” Acta Embryologiae Experimentalis 3 (1971): 239–59.

———. “Regenerative Capacities of the Scyphistoma of Cassiopea (Phylum, Coelenterata; Class, Scyphozoa).” Acta Embryologiae Experimentalis 1972 (1972): 429–54.

Deng, Liqiu, Shuhong Wang, Ting Wang, Yuxuan Zhao, and Qi Luo. “Indoles Can Induce Strobilation in Aposymbiotic Cassiopea Andromeda Polyps but Are Associated with Developmental Abnormalities.” Hydrobiologia 849, no. 15 (September 1, 2022): 3275–85. https://doi.org/10.1007/s10750-022-04883-z.

El-Sayed, Heba S., Amal Z. Ghonim, Gihan M. El-Khodary, Mohamed A. El-Sheikh, and Hanan M. Khairy. “Application of Enriched Cyclops Abyssorum Divergens with Mixed Algal Diet Compared to Artemia Franciscana for Improving Larval Growth and Body Composition of

Dicentrarchus Labrax.” Aquaculture Reports 20 (July 1, 2021): 100715. https://doi.org/10.1016/j.aqrep.2021.100715.

Fitt, W. K., and R. K. Trench. “Endocytosis of the Symbiotic Dinoflagellate Symbiodinium Microadriaticum Freudenthal by Endodermal Cells of the Scyphistomae of Cassiopeia Xamachana and Resistance of the Algae to Host Digestion.” Journal of Cell Science 64 (1983): 195–212.

Fleck, J, and WK Fitt. “Degrading Mangrove Leaves of Rhizophora Mangle Linne Provide a Natural Cue for Settlement and Metamorphosis of the Upside down Jellyfish Cassiopea Xamachana.” Journal of Experimental Marine Biology and Ecology 234 (1999): 83–94.

Gallagher, Margie., and W. Duane. Brown. “Composition of San Francisco Bay Brine Shrimp (Artemia Salina).” Journal of Agricultural and Food Chemistry 23, no. 4 (July 1, 1975): 630–32. https://doi.org/10.1021/jf60200a008.

Hofmann, D. K., and M. Gottlieb. “Bud Formation in the Scyphozoan Cassiopea Andromeda: Epithelial Dynamics and Fate Map.” Hydrobiologia 216, no. 1 (1991): 53–59. https://doi.org/10.1007/BF00026443.

Hofmann, D. K., and M. G. Hadfield. “Hermaphroditism, Gonochorism, and Asexual Reproduction in Cassiopea Sp.—an Immigrant in the Islands of Hawai’i.” Invertebrate Reproduction and Development 41, no. 1–3 (2002): 215–21. https://doi.org/10.1080/07924259.2002.9652754.

Hofmann, D. K., and T. G. Honegger. “Bud Formation and Metamorphosis in Cassiopea Andromeda (Cnidaria: Scyphozoa): A Developmental and Ultrastructural Study.” Marine Biology 105, no. 3 (1990). https://doi.org/10.1007/BF01316322.

Hofmann, D K, R Neumann, and K Henne. “Strobilation, Budding and Initiation of Scyphistoma Morphogenesis in the Rhizostome Cassiopea Andromeda (Cnidaria: Scyphozoa).” Marine Biology. Vol. 47, 1978.

Hofmann, D. K., R. Neumann, and K. Henne. “Strobilation, Budding and Initiation of Scyphistoma Morphogenesis in the Rhizostome Cassiopea Andromeda (Cnidaria: Scyphozoa).” Marine Biology 47, no. 2 (1978). https://doi.org/10.1007/BF00395637.

Hofmann, Dietrich K., and U Brand. “Induction of Metamorphosis in the Symbiotic Scyphozoan Cassiopea Andromeda: Role of Marine Bacteria and of Biochemicals.” Symbiosis 4 (1987): 99–116.

Hofmann, Dietrich K., William K. Fitt, and Jürgen Fleck. “Checkpoints in the Life-Cycle of Cassiopea Spp.: Control of Metagenesis and Metamorphosis in a Tropical Jellyfish.” International Journal of Developmental Biology 40 (1996): 331–38. https://doi.org/10.1387/ijdb.8735945.

Iglesias-Prieto, R, and Rk Trench. “Acclimation and Adaptation to Irradiance in Symbiotic Dinoflagellates. I. Responses of the Photosynthetic Unit to Changes in Photon Flux Density.” Marine Ecology Progress Series 113 (1994): 163–75. https://doi.org/10.3354/meps113163.

Jarms, Gerhard, and André C. Morandini. World Atlas of Jellyfish. Dölling und Galitz Verlag, 2019.

Jensch, F, and Dietrich K. Hofmann. “The Cnidomes of Cassiopea Andromeda Forsk\aal, 1775, and Cassiopea Xamachana Bigelow, 1882 (Cnidaria: Scyphozoa).” In Proceedings of the 6th International Conference on Coelenterate Biology, 279–85, 1995.

Larson, R. J. “Feeding Behaviour of Caribbean Scyphomedusae: Cassiopea Frondosa (Pallas) and Cassiopea Xamachana Bigelow.” Studies on the Natural History of the Caribbean Region 73, no. 1 (January 1, 1997): 43–54.

Léger, P., D. Bengtson, K. Simpson, and P. Sorgeloos. “The Use and Nutritional Value of Artemia as a Food Source.” Oceanography and Marine Biology, 1986. https://www.semanticscholar.org/paper/The-use-and-nutritional-value-of-Artemia-as-a-food-L%C3%A9ger-Bengtson/96e5ea038a942a263c9ec5b1b13d1cf0db437d9a.

Mammone, Marta, Victoria Sharp, Michael Hewitt, and Mónica Medina. “The Influence of Photosymbiosis in Cassiopea Xamachana Regenerative Success.” Symbiosis 90, no. 1 (May 1, 2023): 61–70. https://doi.org/10.1007/s13199-023-00920-0.

Margulis, Lynn. “The Origin of Plant and Animal Cells: The Serial Symbiosis View of the Origin of Higher Cells Suggests That the Customary Division of Living Things into Two Kingdoms Should Be Reconsidered.” American Scientist 59, no. 2 (1971): 230–35.

MARTIN, VICKI J., and FU-SHIANG CHIA. “FINE STRUCTURE OF A SCYPHOZOAN PLANULA, CASSIOPEIA XAMACHANA.” The Biological Bulletin 163, no. 2 (1982). https://doi.org/10.2307/1541269.

Matthews, Benjamin J., and Leslie B. Vosshall. “How to Turn an Organism into a Model Organism in 10 ‘easy’ Steps.” Journal of Experimental Biology 223 (2020): 223. https://doi.org/10.1242/jeb.218198.

Mayer, Alfred G. Rhythmical Pulsation of the Medusae. 47th ed. Washington D.C., 1908.

Medina, Mónica, Victoria Sharp, Aki Ohdera, Anthony Bellantuono, Justin Dalrymple, Edgar Gamero-Mora, Bailey Steinworth, et al. “The Upside-Down Jellyfish Cassiopea Xamachana as an Emerging Model System to Study Cnidarian–Algal Symbiosis.” In Handbook of Marine Model Organisms in Experimental Biology. CRC Press, 2021.

Morejón-Arrojo, Ramón Damián, and Leandro Rodriguez-Viera. “Characterization of the Populations of Upside-down Jellyfish in Jardines de La Reina National Park, Cuba.” PeerJ 11 (April 25, 2023): e15254. https://doi.org/10.7717/peerj.15254.

Muffett, Kaden McKenzie, Joleen Aulgur, and Maria Pia Miglietta. “Impacts of Light and Food Availability on Early Development of Cassiopea Medusae.” Frontiers in Marine Science 8 (January 25, 2022). https://doi.org/10.3389/fmars.2021.783876.

Ohdera, Aki, Khushboo Attarwala, Victoria Wu, Rubain Henry, Henry Laird, Dietrich K. Hofmann, William K. Fitt, and Mónica Medina. “Comparative Genomic Insights into Bacterial

Induction of Larval Settlement and Metamorphosis in the Upside-Down Jellyfish Cassiopea." mSphere 8, no. 3 (June 22, 2023): e0031522. https://doi.org/10.1128/msphere.00315-22.

Ohdera, Aki H., Viridiana Avila-Magaña, Victoria Sharp, Kelly Watson, Angela Z. Poole, Bailey Steinworth, Erika Diaz-Almeyda, et al. "Modulation of Gene Expression Driven by Symbiosis: Strobilation Mechanism in the Upside-down Jellyfish.," In prep (b).

Pierce, J. "A System for Mass Culture of Upside-down Jellyfish Cassiopea Spp as a Potential Food Item for Medusivores in Captivity." International Zoo Yearbook 39, no. 1 (2005): 62–69. https://doi.org/10.1111/j.1748-1090.2005.tb00005.x.

Stockard, Charles R. "Studies of Tissue Growth." Archiv Für Entwicklungsmechanik Der Organismen 29, no. 1 (March 1, 1910): 15–32. https://doi.org/10.1007/BF02297062.

Templeman, Michelle A., and Michael J. Kingsford. "Trace Element Accumulation in Cassiopea Sp. (Scyphozoa) from Urban Marine Environments in Australia." Marine Environmental Research 69, no. 2 (March 1, 2010): 63–72. https://doi.org/10.1016/j.marenvres.2009.08.001.

Thornhill, Daniel J., Michael W. Daniel, Todd C. LaJeunesse, Gregory W. Schmidt, and William K. Fitt. "Natural Infections of Aposymbiotic Cassiopea Xamachana Scyphistomae from Environmental Pools of Symbiodinium." Journal of Experimental Marine Biology and Ecology 338, no. 1 (2006): 50–56.

Watanabe, Takeshi, Masahiro Ohta, Chikara Kitajima, and Shrio Fujita. "Improvement of Dietary Value of Bring Shrimp Artemia Salina for Fish Larvae by Feeding Them on Ω3 Highly

Unsaturated Fatty Acids.” Nippon Suisan Gakkaishi 48, no. 12 (1982): 1775–82. https://doi.org/10.2331/suisan.48.1775.

Chapter 3

Host-symbiont plasticity in the upside-down jellyfish *Cassiopea xamachana*: strobilation across symbiont genera

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Abstract

In the upside-down jellyfish, Cassiopea xamachana (Cnidaria: Scyphozoa), the establishment of photosymbiosis with dinoflagellates (family Symbiodiniaceae) is necessary for the sessile polyp to undergo metamorphosis (strobilation) into a free-swimming adult. C. xamachana has the capacity to associate with a wide variety of dinoflagellate species and representatives of divergent genera. While some studies have looked at the successful induction of symbiosis, none to date have examined the lasting effect of diverse symbiont taxa on host survivorship and development, which is needed to assess the fitness costs of such symbioses. Our study exposes C. xamachana polyps to 22 different cultured Symbiodinaceae strains representing 13 species from 5 genera. We analyzed the time to strobilation, the number of ephyra (juvenile medusa) produced, and the proportion of ephyra that died prematurely. Here we show that C. xamachana strobilation can be induced by nearly each symbiodinacean strain we tested, with the exception of free-living species (i.e., unknown to establish symbiosis with any other marine host). Additionally, ephyrae did not display morphological variation or survivorship differences with varying symbionts. However, we observed intraspecific variation in time to induce strobilation with different cultured dinoflagellate strains. This work expands the known symbiont species that can form stable mutualisms with C. xamachana, primarily in the genera Symbiodinium and Breviolum. Additionally, we provide evidence of differences in ability of cultured symbiodiniaceans to establish symbiosis with a host, which suggests population-level differences in dinoflagellate cultures impact their symbiosis success. By utilizing an animal like C. xamachana with flexible symbiont uptake, we are able to explore how symbiont diversity can influence the timing and success of symbiosis-driven development.

Introduction

Numerous marine animals receive nutritional benefits from mutualistic microbes. The presence of certain microorganisms is also critical for the animal during the early stages of development (McFall-Ngai et al., 2013). It has become increasingly evident that most, if not all, marine organisms rely on the presence of symbionts throughout their life cycles, as reviewed by González-Pech et al. (2024). For example, many invertebrate larvae rely on the presence of a bacterial biofilm to settle and develop into adults and require the presence of specific bacteria for settlement (Freckelton et al., 2017; Cavalcanti et al., 2020). In the Hawaiian bobtail squid *Euprymna scolopes* (Mollusca : Cephalopoda), symbiosis with diverse bacteria is necessary for the proper development of the female reproductive system (McAnulty et al., 2023). Many cnidarians, including reef-building corals, sea anemones, sea fans, and jellies, depend upon mutualisms with endocellular dinoflagellate photosymbionts (Dinophyceae: Suessialess: Symbiodinaceae) (LaJeunesse, 2020). Newly metamorphosed polyps of these animals will fail to survive without a compatible algal symbiont (McIlroy et al., 2019). Symbiont acquisition is therefore critical for survivorship.

In animal–dinoflagellate mutualisms, most hosts acquire their symbionts horizontally from environmental sources during the early stages of development (Harii et al., 2009; Fransolet et al., 2012; Baird et al., 2021). Initial infections may not be representative of the symbiont species found in adult animals, indicating the potential for ontogenetic flexibility in early symbiont uptake (Thornhill et al., 2006; Mellas et al., 2014). Despite this lack of symbiont specificity in early development, a winnowing process occurs, and the “host-specific,” or homologous, symbiont becomes rapidly prevalent (Coffroth et al., 2001; McIlroy et al., 2019). While this sorting process occurs in nature, little is known about the long-term compatibility of heterologous symbionts and how these mutualisms would perform if the homologous symbiont were absent. This and larger questions about factors governing host–symbiont specificity, such as

developmental phenotype trajectories, may be addressed under controlled experimental conditions.

The upside-down jellyfish *Cassiopea xamachana* (Cnidaria: Scyphozoa) is an established experimental system, useful for investigating animal–dinoflagellate interactions (Lampert, 2016; Ohdera et al., 2018; Medina et al., 2021). In the wild, this jellyfish establishes a nutritional and developmental symbiosis with *Symbiodinium microadriaticum*, a photosynthetic dinoflagellate in the family Symbiodiniaceae (Hofmann et al., 1978; Thornhill et al., 2006; Mellas et al., 2014; LaJeunesse, 2017; Medina et al., 2021). Unique to *Cassiopea* spp., the host experiences halted sexual development until symbiosis is established (Jarms and Morandini, 2019; Medina et al., 2021). Soon after, *C. xamachana* undergoes a metamorphic process termed strobilation, during which the polyp head develops into a pre-adult swimming ephyra. These ephyrae detach from the polyp through lateral fission and develop into adult medusae (Figure 3-1A). The polyp remains behind and regenerates a new head, undergoing strobilation two to three more times (Hofmann et al., 1978; Hofmann and Brandt, 1987; Hofmann et al., 1996; Medina et al., 2021). Without the presence of a symbiont, *C. xamachana* remains in the polyp stage indefinitely, though when fed, it will propagate itself through asexual budding (Figure 3-1A). The ability to maintain these animals aposymbiotically and infect them with individual strains representative of different symbiont species makes this animal ideal for assessing symbiont specificity and the relative compatibility and performance of different algal partner combinations (Medina et al., 2021).

Although *S. microadriaticum* is the homologous symbiont of adult *C. xamachana* (Thornhill et al., 2006; LaJeunesse, 2017), juvenile jelly polyps have been shown to uptake symbionts across several Symbiodiniaceae genera (Mellas et al., 2014; Newkirk et al., 2020; Medina et al., 2021). While *C. xamachana* polyps have been shown to associate with phylogenetically diverse symbiodiniaceans up until strobilation (reviewed in LaJeunesse, 2001), this symbiosis has not been monitored ontogenetically through the resulting ephyrae. It is

currently unknown whether heterologous symbioses in these cnidarians are maintained after strobilation and how they may affect host phenotype and fitness. To observe how a cnidarian host responds to heterologous algal symbionts, we introduced numerous species and strains from five symbiodiniacean genera to clonal aposymbiotic polyps. We measured developmental phenotypes, such as time to strobilation, growth, and survivorship, of both polyps and ephyra. Here, we report wide phenotypic plasticity in host developmental response to a broad range of algal symbionts up to approximately 3 months after symbiont uptake.

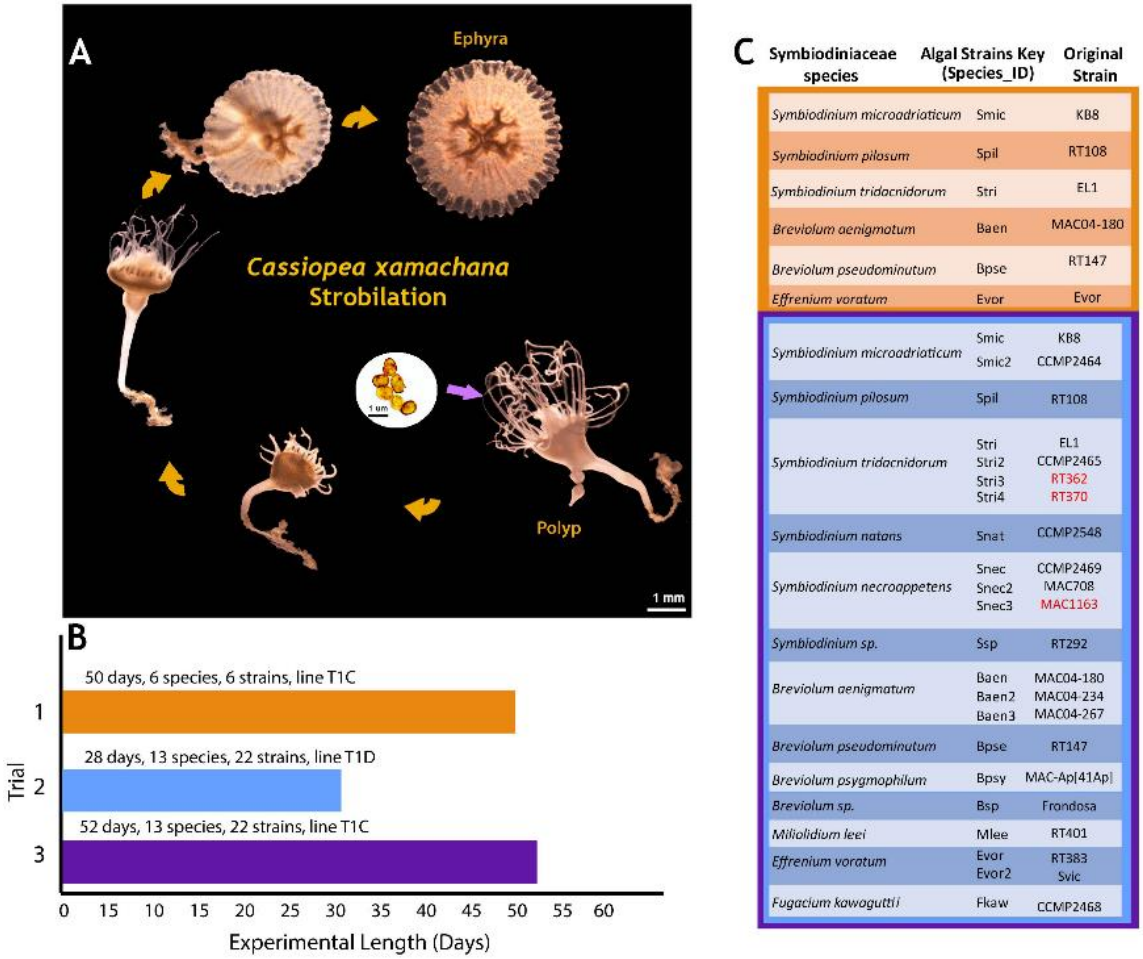

Figure 3-1: *Cassiopea xamachana* life cycle and experimental design. (A) *C. xamachana* strobilation. Algal ingestion and subsequent symbiosis establishment occurred at day 0. Scale bar for algae in black (1 mm); scale bar for host in white (1 mm). (B) Number of days each trial was run, number of species, and number of algal strains. Symbionts were introduced on day 0. (C) List of symbiont species, algal strains, and average number of days to strobilation. Trial 1's strains and species shown in orange, while trials 2 and 3 used the same strains and species, outlined in blue and purple, respectively.

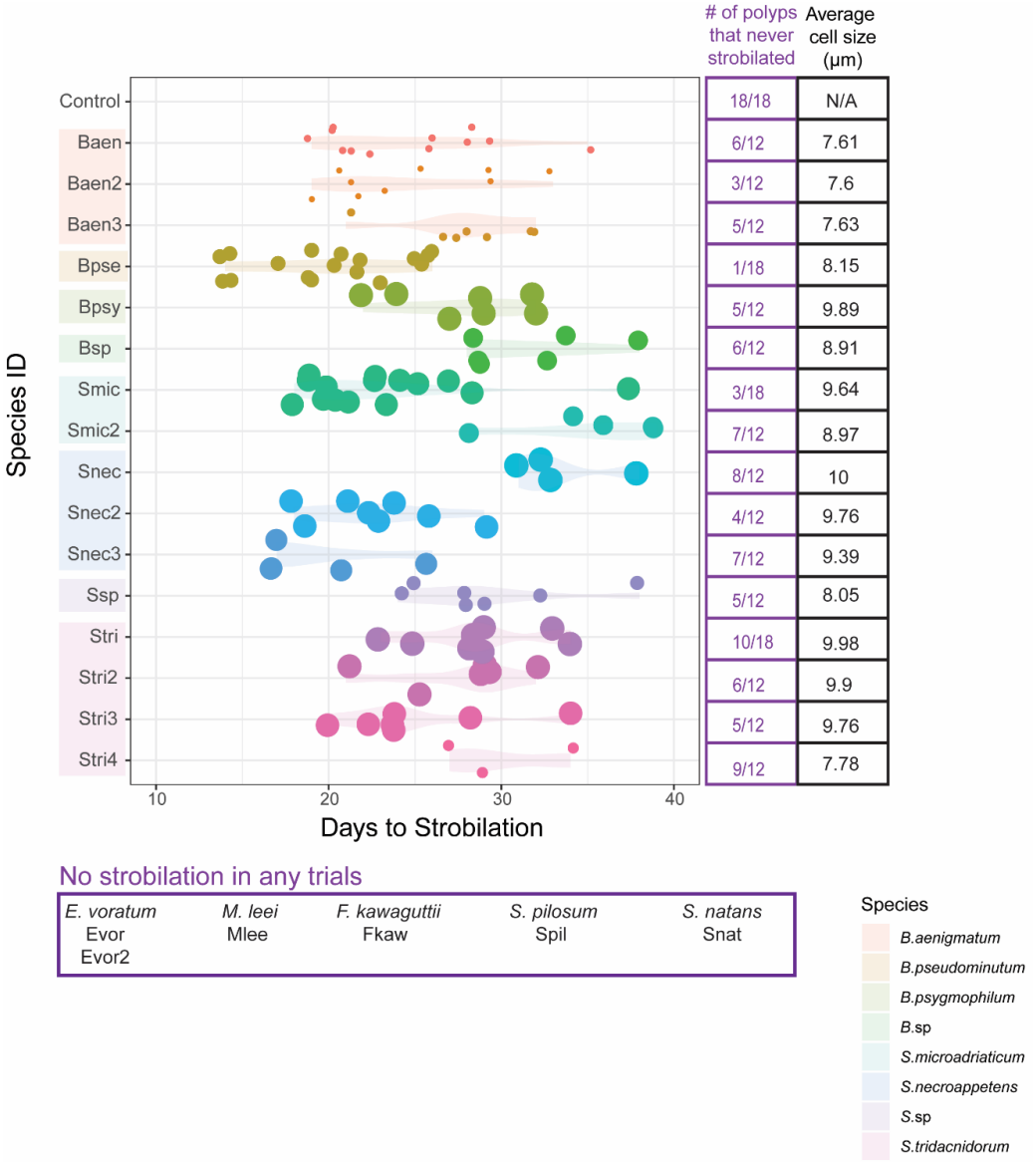

Figure 3-2: Number of days to strobilation pooled across 3 trials. Y-axis groups Species_ID by genera and is color-coded by species. Each point represents one polyp inoculated with that Species_ID. Column on right side of figure lists how many polyps did not strobilate out of the total inoculated across all 3 trials (column 1) and average algae cell size (column 2). Point size corresponds to average algal cell size. Species_ID which never induced strobilation or showed any signs of symbiosis in any trial is listed in "No strobilation in any trials" box at bottom.

Materials and Methods

Aposymbiotic *C. xamachana* polyps were obtained from a clonal line kept by the Medina Lab (Pennsylvania State University). Each line represents one originating aposymbiotic polyp collected from the Florida Keys, USA, that was allowed to bud continuously, forming a clonal colony. Line T1C was used for trials 1 and 3, and T1D was used for trial 2 (Figures 3-1B, C).

One polyp was placed in each well of a six-well CellStar cell culture plate (Thomas Scientific, Swedesboro, NJ, USA) with approximately 7–9 mL of 35 parts per thousand (ppt) Instant Ocean (Spectrum Brands, Inc., Miramar, FL, USA) seawater (n = 6 per algal treatment). Polyps were fed daily with two to fifteen 1-day-old *Artemia franciscana* hatched from eggs purchased from Brine Shrimp Direct, Inc. (Ogden, UT, USA), and water changes were performed three times a week. Aposymbiotic polyps were kept in an incubator with a 12:12 day/night cycle (27.5°C day, 25.5°C night) for 2 weeks prior to the start of the experiment to acclimate them to the light cycle. Light levels ranged between 70 and 150 μmol·m−2·s−1 depending on proximity to the fluorescent lights. Therefore, plates were rotated daily to ensure even light exposure over the course of the experiment.

Symbiodiniaceae cultures (Supplementary Table 3-1) cultured and/or maintained by the LaJeunesse lab (Pennsylvania State University) were grown in Asp-8A media (Blank, 1987) for 2–4 months prior to infections. They were grown at ~180 μmol·m−2·s−1 lighting in a 12:12 day:night cycle of 27.5°C during the day and 26°C at night. Prior to the start of the experiment, culture cell density was calculated by counting live algal cells on a hemacytometer. Culture aliquots were pelleted at 0.8 rcf, and the media were removed before resuspension with an equal volume of 35 ppt seawater. Symbionts were added at a concentration of ~150,000 cells/mL per polyp to each 9-mL well of a six-well sterile tissue culture plate along with live *A. franciscana* to encourage the immediate ingestion of algae. A control plate with aposymbiotic polyps was kept in the same incubator and fed and cleaned on the same schedule as plates with symbiotic polyps.

The identity of algal cultures was confirmed by Sanger sequencing of the 28S (LSU) ribosomal DNA, and a conserved marker was used to identify Symbiodiniaceae genera (forward: 5′-CCCGCTGAATTTAAGCATATAAGTAAGCGG-3′; reverse: 5′-GTTAGACTCCTTGGTCCGTGTTTCAAGA-3′) (LaJeunesse et al., 2018). DNA extraction and polymerase chain reaction followed the methods described in LaJeunesse et al. (2018). Sequencing was performed at the Pennsylvania State Genomic Core Facility. Sequences were imported into Geneious Prime® 2023.0.4 (https://www.geneious.com), and BLAST was performed against the LSU rRNA sequences from LaJeunesse et al. (2018). Geneious Tree Builder was used to generate a neighbor-joining phylogenetic tree using the Tamura–Nei genetic distance model, and the outgroup was set to an LSU sequence of *Biecheleria halophila*, a dinoflagellate from a different family. Photographs were taken of each culture using a Leica MZ16 F fluorescence stereo microscope. The length (longest dimension) of ~20 cells per culture was measured using ImageJ (Schneider et al., 2012) to calculate average cell size. Only cells not actively dividing were used for this analysis.

Out of 22 Symbiodiniaceae cultures sequenced, three LSU sequences did not match their recorded species; RT362 and RT370 were reported as *S. microadriaticum* but matched to *Symbiodinium tridacnidorum*, and MAC1163 was reported as *Breviolum minutum* and matched to *Symbiodinium necroappetens* (Supplementary Table 1, bolded and underlined). While these new identifications do not affect our results, we note this information for anyone looking for a particular culture used in this study. Unique labels termed “Species ID” were created for each algal culture and were used herein for figures and discussion (Figure 1C, Supplementary Table 1). Species IDs use the first letter of the genus and the first three letters of the species, followed by a number if it is a different culture of the same microalgal species. For example, two cultures of *S. microadriaticum*, the homologous symbiont, were used in this experiment, labeled as KB8 and

CCMP2464 by initial cultivists. KB8 received the Species ID “Smic”, while CCMP2464 was labeled “Smic2.” All results were compared between algal species and algal cultures.

An initial experiment, run from January to March 2020 (50 days, Figure 1B), introduced six algal species to aposymbiotic *C. xamachana* polyps (clonal line T1C, Supplementary Table 1). The rate of strobilation and morphological variation of the host was recorded visually. Pictures were taken of the ephyrae once a day to measure the growth rate by calculating the change in host diameter over time. Anesthesia was not used for this; instead, photo bursts were used to ensure a photo was taken of the ephyra at maximum diameter while pulsing. Ephyra diameter was measured using ImageJ. Some ephyrae perished within 1 day of detachment and were only measured once. These deaths made it difficult to accurately compare growth rates, as there was no measurement of ephyra decline in size leading to death in these samples. The algal species that had colonized these ephyra included the homologous symbiont *S. microadriaticum* (Thornhill et al., 2006) as well as symbiont species historically used in Medina Lab experiments (see Supplementary Table 1).

A second experiment, run from January to February 2021 (28 days, Figure 1B), introduced 22 algal strains (complete list in Supplementary Table 1) to polyps (clonal line T1D). Clonal polyp lines T1C and T1D, with different genotypes, were previously colonized with various symbionts without noticeable differences in symbiosis establishment. Polyps were imaged three times a week to measure growth, time to strobilation, and to record potential morphological differences (i.e., tentacle numbers, strobilation progress, and mortality). When ephyrae detached, they were kept in the same well as the polyp they originated from and experienced the same conditions. Ephyrae were imaged one to two times a day to measure growth and mortality. As ephyra growth measurements were taken twice a day, the approximate number of hours since the time of infection was recorded for each time point and used to calculate the

average ephyra growth rate. Ephyra diameter across the bell margin was measured using ImageJ for each time point to calculate the average change in diameter.

A third experiment, run from July to August 2021 (52 days, Figure 1B), introduced the same 22 algal strains to the polyp clonal line T1C. Data collection was identical to the second experimental run. All three experiments were pooled to compare host response to algal species and days to strobilation (see trial summary in Figure 1C). Analyses were performed on pooled results and separated by experiment to account for any experimental effects.

A Zero-Inflated Poisson Regression was chosen to analyze the number of days to strobilation, as it accounts for excess zeros in a dataset, e.g., polyps that were visibly symbiotic but did not strobilate in trial 2. Data analysis and figure generation were performed using the ggplot2, dplyr, emmeans, and ggfortify packages in R version 4.1.2 (Searle et al., 1980; Tang et al., 2016; Wickham, 2016; R Core Team, 2018; Wickham et al., 2022). The cladogram was generated using FigTree (http://tree.bio.ed.ac.uk/software/Figtree/) and edited using Adobe Illustrator (Adobe Inc.). A Bonferroni p-value correction was applied to all tests that had multiple comparisons by dividing the p-value cutoff ($p < 0.05$) by the number of comparisons (n) (Tables 1, 2).

Table 3-1: Results of zero-inflated Poisson regressions on significance of factors on days to strobilation.

Days to Strobilation	Algal Species	Algae strain (Species_ID)	Trial	Average Algal Cell Size (µm)
Species *p < 0.05*	**p=0.0387**	–	–	-
Species_ID *p < 0.05*	–	p=0.73203	–	-
Species+Species_ID *p < 0.025*	p=0.0521	p=0.5680	–	-
Species+Species_ID+Trial *p < 0.017*	p=0.018817	p=0.573088	**p=0.000301**	-
Species+Average Algal Cell Size *p < 0.025*	**p=0.017**	–	–	p=0.184
Species_ID+Average Algal Cell Size *p < 0.025*	–	p=0.763	–	p=0.684
Species+Trial+Average Algal Cell Size *p < 0.017*	**p=0.003838**	–	**p=0.000278**	p=0.197774
Species_ID+Trial+Average Algal Cell Size *p < 0.017*	–	p=0.83719	**p=0.00138**	p=0.80796
Species+Species_ID+Trial+Average Algal Cell Size *p < 0.0125*	**p=0.010206**	p=0.580803	**p=0.000319**	p=0.211123

"~" after "Days to strobilation" indicates that this value is being compared to the factors listed in the first column. A Bonferroni correction for multiple comparisons was applied to a p-value cutoff of $p < 0.05/n$ for all tests, and the cutoff is listed in the first column in italics. Bonferroni-corrected p-values that achieved significance are in bold. The "-" represents factors that were not compared for that analysis.

Table 3-2: One-way ANOVA comparisons (rows 1-4) and chi-squared comparisons (rows 5-6).

	Algal Species	Algal strain (Species_ID)
Average ephyra growth rate	p = 0.5742	p = 0.05804
# days ephyrae survived	p = 0.8331	p = 0.614
# ephyrae died/total produced	p = 0.1088	**p = 0.01533**
Total produced ephyrae	p = 0.3838	p = 0.5464
Ephyrae lived 14 days or less	p = 0.6047	p = 0.6084
Proportion ephyrae died in 14 days/total produced	p = 0.2745	-

The "-" represents factors that were not compared for that analysis. p-Values <0.05 are in bold.

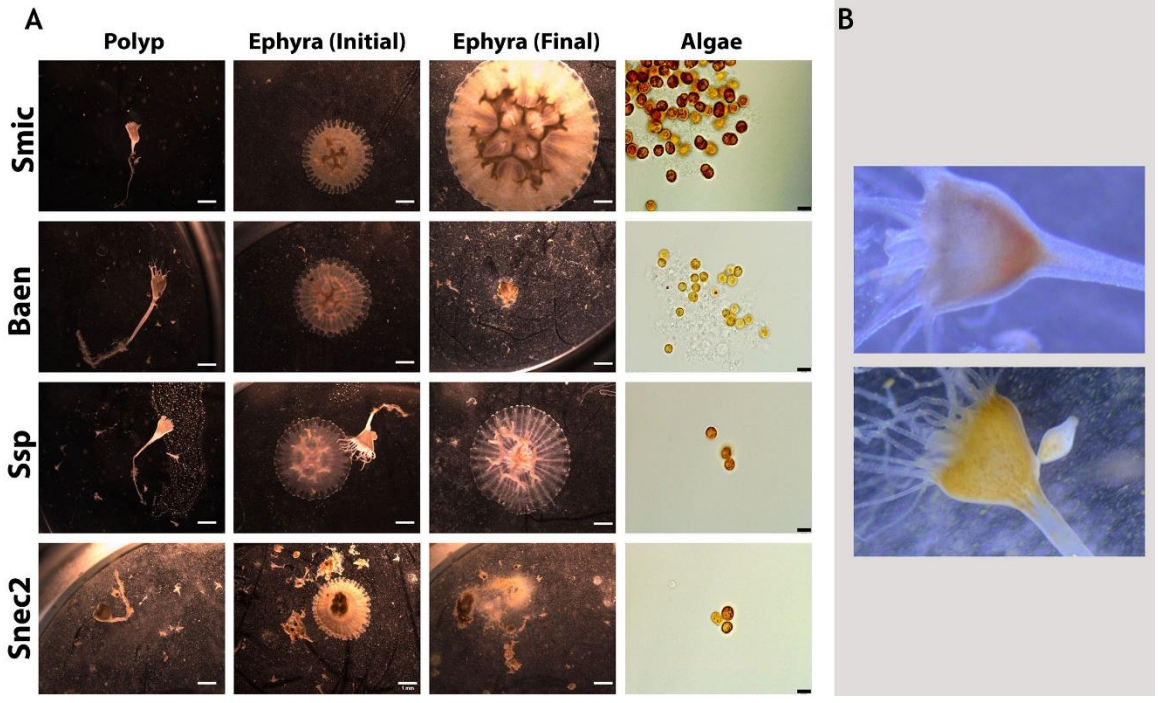

Figure 3-3: Representative images of *Cassiopea xamachana* morphology when associating with different symbionts. (A) Polyps (column 1) and their produced ephyra (column 2) along with final appearance of ephyrae (column 3) and algal cultures (right). Black scale bars represent 10 mm. Each row represents a different algal culture. Rows 2 and 4 show ephyrae that were deceased by the final day (day 52 during trial 3). (B) Aposymbiotic polyp (top) and symbiotic polyp (bottom) with an arrow indicating the visible symbionts inside the polyp head. Images approximately 50 mm in width.

Results

Sixteen algal strains from eight species induced strobilation in at least one trial (Figure 3-2). Six algal strains representing four species did not induce strobilation in all three experiments (Spil, Snat, Evor, Evor2, Mlee, and Fkaw; Figure 3-2, Supplementary Table 3-1). These six strains did not visibly colonize polyps, as determined by the absence of visible symbiont cells in the translucent head of the polyp (Figure 3-3B). In another study conducted by several of the authors, symbionts were visible inside the polyp to the human eye and were verified by fluorescent microscopy, supporting the unofficial metric of symbiosis (Mammone et al., 2023). Visible symbiont cells were observed in the polyp head of all strains that induced strobilation (Figure 3-3B). Ten out of the 22 strains failed to induce strobilation during trial 2, despite confirmation of the successful acquisition of the symbiont cells by the polyps (Smic2, Stri, Stri2, Stri3, Stri4, Snec, Snec2, Snec3, Ssp, and Baen2). All 10 strains induced strobilation during trial 3. These strains were binned by “Fast” (0–20 days), “Medium” (20–30 days), and “Slow” (30 or more days) and mapped onto a phylogenetic tree of our LSU sequences (Figure 3-4, outgroup

taken from LaJeunesse et al., 2018, dataset). There was no pattern observed for phylogenetic distance and bin, but strains that did not induce strobilation clustered together (Figure 3-4).

No statistically significant differences in time to strobilation (measured in number of days post-dinoflagellate introduction) were observed between algal strains when the three experimental results were grouped (zero-inflated Poisson regression; $p = 0.73203$) (Table 3-1). When the results were grouped by species, we found that Breviolum pseudominutum decreased the number of days it took to strobilate compared to the averages of other species (average of 20 days, zero-inflated Poisson regression, $p = 0.0475$), while S. tridacnidorum increased the number of days it took to strobilate (average of 28 days, zero-inflated Poisson regression, $p = 0.0318$) (Supplementary Table 3-1). Bonferroni corrections for multiple comparisons did not change any p-value significance except when algal species, strain, and trial were compared against the number of days to strobilation, which resulted in only the trial achieving significance after the p-value correction (Table 3-1).

The average algal cell size is recorded in Supplementary Table 3-1. The strain sizes ranged from 7.6 to 12.01 µm. There were five species with multiple strains, where Breviolum aenigmatum had the lowest standard deviation in average algal cell size between strains (7.6–7.63 µm, SD = 0.011). Effrenium voratum (11.28–12.01 µm, SD = 0.334), S. microadriaticum (8.97–9.64 µm, SD = 0.335), and S. necroappetens (9.39–10 µm, SD = 0.25) had similar standard deviations. S. tridacnidorum had the largest range in algal cell size between strains (7.78–9.9 µm, SD = 0.85).

Using only the data from species that induced strobilation, a zero-inflated Poisson regression tested the correlation between average dinoflagellate length and time to strobilation. When algal cell size and species or algal cell size and strain were considered, we found no significant effect on time to strobilation (Table 3-1). When species, algal strains, trial, and average algal cell diameter were considered together, we found significant differences ($p =$

0.010206) in the number of days to strobilation. Algal strain and size were not associated with time to strobilation in any comparison. Bonferroni corrections for multiple comparisons were conducted on all tests, and the p-value cutoff ($p < 0.05$) was divided by the number of comparisons (n) to establish a new p-value cutoff for those tests (Table 3-1).

No quantitative measurements were taken for polyp and ephyra morphology beyond ephyra diameter, only visual assessment. Polyp and ephyra shape, tentacles, bell margin, and coloration appeared consistent across association with different symbiont species (Figure 3-3A). The total number of ephyra produced by algal treatments that induced strobilation was compared against algal species/culture for significance (Table 3-2). The total number of days the ephyrae survived, the total number of produced ephyrae, and the proportion of deceased ephyrae out of the total produced were compared. Species and algal culture were not significantly associated with these outcomes except for algal strain compared against a proportion of deceased ephyra (one-way ANOVA; Table 3-2). A post-hoc test found that no strain drove this significance. A chi-squared test was performed to determine if species or algal culture played a significant role in whether or not ephyra lived 14 days or less and the proportion of ephyra that survived at least 14 days. The total number of produced ephyra for this analysis excluded any ephyra produced within 14 days of the end of the experiment. No significant association was found between algal species or algal culture and either comparison (whether ephyrae lived 14 days or less or the proportion that survived 14 days or more) (Figure 3-5, Table 3-1). When the ephyra growth rate was calculated, average diameter change was not affected by algal species or algal culture (one-way ANOVA, $p = 0.5742$ and $p = 0.05804$, respectively; Table 3-2). As none of the ANOVA or chi-squared tests achieved significance, no post-hoc tests were performed.

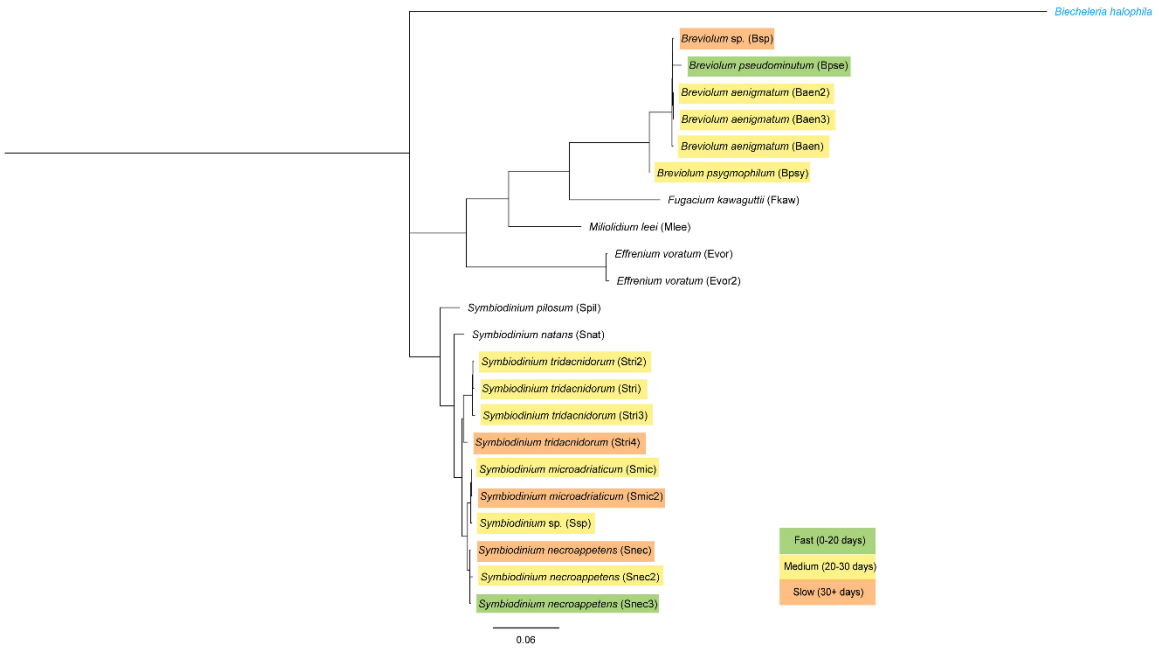

Figure 3-4: Phylogeny of the 28S (LSU) ribosomal RNA gene sequenced from algal cultures used. Genetic distance scale bar at bottom. Outgroup in blue font. Each culture is color-coded by the average number of days it took to induce strobilation (see Supplementary Table 3-1).

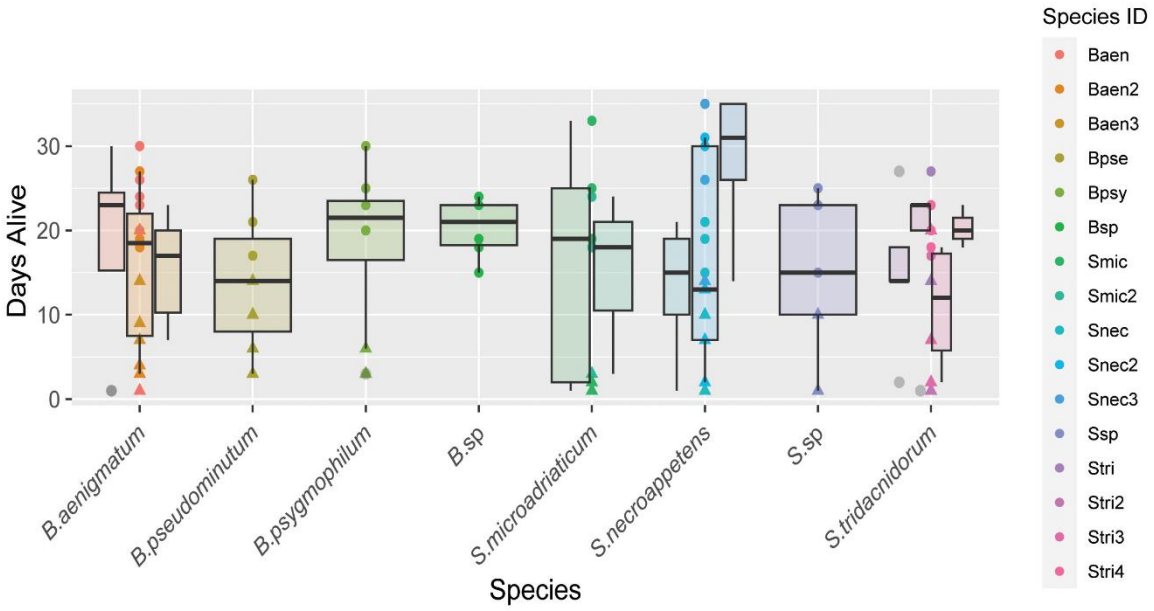

Figure 3-5: Box plot of the number of days ephyra survived (y-axis) sorted by algal species (x-axis). Boxplots of strains of the same species are clustered and color coded according to the key.

Discussion

Our results support the flexibility of *C. xamachana* to form symbiosis with a variety of Symbiodiniaceae species from the *Breviolum* and *Symbiodinium* genera. In fact, the homologous *S. microadriaticum* did not induce strobilation faster on average than other species (Figures 3-2, 3-4). This lack of correlation between algal species and host metamorphosis suggests that despite *C. xamachana* being predominantly associated with *S. microadriaticum* in the wild (Thornhill et al., 2006), strobilation does not require the acquisition of the homologous symbiont, and the developmental transition can be initiated by a wide range of symbiodiniacean taxa. The species that did not establish symbiosis have been predominantly recorded as non-symbiotic and solely free-living (Jeong et al., 2014; LaJeunesse et al., 2018; Pochon and LaJeunesse, 2021) (Figure 3-

2). While these two symbiotic dinoflagellates cannot associate with *C. xamachana*, they present interesting subjects for future studies on the specificity of host–symbiont interactions. Otherwise, most symbiodiniaceans capable of forming symbiosis with a marine host appear to be able to associate with *C. xamachana*. While we did not utilize them in this study, other wild Cassiopea species have been reported to host symbionts in the genera *Cladocopium* and *Durisdinium* (Winstead, 2019; Mammone et al., 2021).

C. xamachana has differing abilities to establish symbiosis depending on the symbiodiniacean algal strain, including the time from aposymbiotic host exposure to dinoflagellate cells, to strobilation. While algal strain did not significantly affect time to strobilation across the full dataset, not divided by trial (Table 3-1), if algal strains within the same species are compared, a noticeable difference in average number of days to strobilation is visible (Figures 3-2, 3-4, Supplementary Table 3-1). Whether these differences are due to the origins of the different cultures (i.e., the algal cultures were isolated from specific hosts), or from the effect of algal generation time, is yet to be determined. The dinoflagellates used in this study have been kept in isolated cultures for multiple years and may have experienced microevolutionary events, possibly dividing monoclonal cultures into subpopulations among culture flasks. As seen in the 2021 study by Gonzalez-Pech et al., Symbiodiniaceae cultures show varying levels of divergence depending on the culture origin. As many cnidarian studies use lab-reared algal cultures passed between research groups (Rahat and Reich, 1986; Abrego et al., 2009; Newkirk et al., 2020), it is crucial to begin to understand whether different cultures of the same algal species function differently to establish the significance for future studies on artificial symbiosis induction.

When looking further into the number of days to strobilation among different strains of symbiont, this effect was greater when results from individual experiments were separated (Table 3-1). Due to the different periods of time that each experiment was run (Figures 3-1B, C), it is clear that the lack of strobilation in trial 2 was due to the shorter time frame (visualized in

Supplementary Figure 3-1) and unlikely due to other differences such as clonal polyp line (T1C in trials 1 and 3 and T1D in trial 2). This is further supported by algal species having a significant association with time to strobilation when all data were grouped, but not when divided by trial (Table 3-1). Additionally, when species, trial, and average cell size were compared, species achieved significance (Table 3-1). After running a post-hoc test, this significance was driven by the differences in the algal cell sizes of species that induced strobilation in trial 3, but not trial 2. The post-hoc test (run using the R-function "emmeans") indicated that it expected that no species with cells larger than 8.77 µm should have induced strobilation. As an example, culture Snec, with an average cell size of 10 µm, did not induce strobilation in trial 2; however, when the length of the experiment was extended for trial 3, this culture induced strobilation. This difference serves to further highlight the differences between algal strains and timelines to induce strobilation, and this range is useful for researchers to keep in mind when observing hosts interacting with different symbiont strains.

Strobilation in *C. xamachana* can be induced by a chemical stimulant (Cabrales-Arellano et al., 2017), indicating that once polyps receive the signal for strobilation, the metamorphic pathway will continue independent of colonization by a symbiont. Our results support this with natural symbiont infections; the strobilation cascade proceeds along a designated developmental trajectory. Considering the host relies on the presence of the symbiont in the wild to undergo a morphogenetic transition, we hypothesized that different symbiont genotypes may influence host phenotype. However, no morphological differences were observed between polyps and ephyra colonized by different species, nor any visual differences in animal coloration, tentacle/oral arm development or abundance, or shape of the polyps (Figure 3-3). This reveals that animal morphogenesis during strobilation proceeds independently of the algal species. The average number of days to strobilation for each strain fell within a range of 19–36 days (Supplementary Table 3-1), and polyps inoculated with the same strain did not always strobilate within the time

window (Figure 3-2). Strobilation timing was highly variable and dependent on symbiont, uncovering broad host phenotypic plasticity despite the single clonal nature of the initial experimental polyps. Plasticity in the initiation of strobilation has been seen in other scyphozoans when temperatures and feeding regimes fluctuated, but this is the first example of this temporal shift involving the presence of other symbionts (Prieto et al., 2010; Treible and Condon, 2019). The monodisc strobilation style of *C. xamachana* is thought to be an evolutionarily derived strategy (Marques and Collins, 2004), but there appears to be a similar pattern in polydisc strobilating scyphozoans; strobilation timing can change depending on the environment proceeding in a cascade after initiation.

Biquand et al. (2017) speculated that algal cell size would be positively correlated with the time to establish symbiosis with a cnidarian host. However, our data regarding the timing of strobilation, which is triggered after the establishment of symbiosis, do not support this theory. Several symbiont strains led to symbiosis, characterized by visible "spots" in the animals in the upper part of the calyx (Figure 3-3B), but did not induce strobilation in every polyp. These symbiont strains covered a wide range of cell diameters (Smic2, Stri, Stri2, Stri3, Stri4, Snec, Snec2, Snec3, Ssp, and Baen2, 7.6 to 9.9 μm; Supplementary Table 3-1), suggesting in this scyphozoan host that symbiont cell size is not correlated with time to symbiosis establishment.

B. pseudominutum induced strobilation the fastest in all three trials (Figure 3-2). This species is found in sub-tropical environments (Parkinson et al., 2015) and is expected to co-occur in the same water as wild *C. xamachana*. Conversely, *B. aenigmatum*, which fell within the medium range of days to induce strobilation (Figure 3-4), has only been isolated and cultured as a background fraction of symbionts for the Caribbean coral *Porites astreoides* (Parkinson et al., 2015). *B. aenigmatum*'s ability to grow and persist despite occurring as a rare member of the photosymbiont community in these hosts may indicate an increased capacity to infiltrate and reproduce once inside a host. This capacity could explain its success in inducing *C. xamachana*

strobilation, which we can further explore in the future. The species *S. tridacnidorum*, which is naturally found within tridacnid clams (Lee et al., 2015), was associated with a longer time to strobilation independent of cell size, which varied across strains of this species. This result suggests that the successful establishment of symbiosis depends on other factors than cell size. In addition, the free-living symbiont species that did not establish symbiosis with *C. xamachana* had a variety of cell sizes (Supplementary Table 3-1).

The survivorship of ephyrae produced by any one photosymbiont strain appears to be highly variable, and we found no significant association between algal species or cultures and the total number of produced ephyrae or their survivorship (Figure 3-5, Table 3-2). The exception to this was when the proportion of deceased ephyra was compared against the algal strain (Table 3-2). However, no individual strain drove this significance in the post-hoc test. Given the small sample size of ephyrae and the variability in numbers between strains (Figure 3-5), this may be a false positive. Further experiments would need to be conducted with a higher sample size. In our experiments, ephyrae with the homologous symbiont did not have a greater likelihood of survival, so it does not appear that the persistence of ephyrae to adulthood is reliant on initial symbiont association (Table 3-2, Figure 3-5). It has been observed in other marine invertebrates that early developmental stages tend to be generalists in symbiont uptake, but as the symbiosis matures and the juvenile individual acquires its homologous (coevolved) symbiont, there is a winnowing of the heterologous symbionts when reaching adult stages (Coffroth et al., 2001; McIlroy et al., 2019). This is also the case of *C. xamachana* (Thornhill et al., 2006), and because the morphogenetic transition from polyp to ephyra is so critical for lifecycle completion, it may be that this early symbiosis promiscuity aids the survivorship of this host and may be an adaptive trait that is shared among photosymbiotic marine invertebrates.

We saw no cases of loss of symbiosis, showing that *C. xamachana* symbiosis is highly stable in laboratory conditions once established. Several species and strains of algae produced

more ephyrae over the course of the experiments due to faster strobilation induction (Figures 3-2, 3-5), but there was no significant association between any algal species and strain and ephyra survivorship (Table 3-2). Symbionts in the genus *Breviolum* did seem to consistently induce strobilation the fastest, but we found no pattern for the genus *Symbiodinium* and the number of days to strobilation. The two algal strains of the homologous symbiont, *S. microadriaticum*, also had different average times to strobilation, showing a clear difference in strains of the same species, and failing to support the hypothesis that the homologous symbiont always results in a higher production rate of ephyra (Figure 3-4). Long-term observation of developing *C. xamachana* as they grow into medusae with different symbionts would need to be performed to conclusively state that there is no change in host fitness. It is also possible that while we observed large phenotypic plasticity at the morphological level with no apparent fitness costs, there may be distinct molecular processes that can have downstream fitness effects on host survival that could not be captured in our study due to the length of the experiments. The associated algal microbiomes appear to have a potential nutritional role to the assembled holobiont that could also have a key role in fitness and survival (Medina Lab, unpublished).

Overall, we found that life history, animal host origin, and ecological niche do not affect the success of mutualistic Symbiodiniaceae species in forming a symbiosis with this marine host in a lab environment. The results of this study suggest that symbiosis in *C. xamachana* is highly flexible regardless of symbiont host origin or phylogenetic relatedness to the homologous symbiont, providing valuable information for symbiosis researchers on compatible symbionts in this system, the timing of strobilation, and survivorship of ephyrae. Symbiodiniaceae contains a large number of symbionts to marine hosts, and many of these comprise obligate symbioses with cnidarians (LaJeunesse et al., 2018). For hosts that acquire their photosymbionts from the environment, the timing of the acquisition is crucial for survivorship in the wild. If we project the results for *C. xamachana* on coral, which is increasingly at risk of bleaching due to climate

change, the timing of a few weeks between symbiont introduction and asexual reproduction may influence the survivorship of an animal. Understanding how plastic symbiont acquisition and host development is in a marine host aids predictions of changing ecosystems. The factors involved in the successful establishment of symbiosis with a cnidarian marine host are still unclear, but this study elucidates how the process of establishing symbiosis is highly stable if the host and symbionts are compatible yet how flexible it is in early ontogenetic stages. Other systems that appear to have high host–symbiont specificity in adults will need to be studied to examine patterns of phenotypic plasticity during the establishment of developmental symbiosis. Considering how diverse the response to exposure of different symbiont species is in *C. xamachana* polyps, it is clear that the early establishment of marine host–symbiont relationships is a complex process. Molecular approaches that examine symbiosis-driven development will shed light on the underlying mechanisms of such vast developmental phenotypic plasticity in marine holobionts.

References

Abrego, D., Oppen, M. J. H. V., and Willis, B. L. (2009). Onset of algal endosymbiont specificity varies among closely related species of Acropora corals during early ontogeny. Mol. Ecol. 18, 3532–3543. doi: 10.1111/j.1365-294X.2009.04276.x.

Baird, A. H., Yakoleva, I. M., Harii, S., Sinniger, F., and Hidaka, M. (2021). Environmental constraints on the mode of symbiont transmission in corals. J. Exp.

Mar. Biol. Ecol. 538, 151499. doi: 10.1016/j.jembe.2020.151499.

Biquand, E., Okubo, N., Aihara, Y., Rolland, V., Hayward, D. C., Hatta, M., et al. (2017). Acceptable symbiont cell size differs among cnidarian species and may limit symbiont diversity. ISME J. 11, 1702–1712. doi: 10.1038/ismej.2017.17.

Blank, R. J. (1987). Cell architecture of the dinoflagellate Symbiodinium sp. inhabiting the Hawaiian stony coral Montipora verrucosa. Mar. Biol. 94, 143–155. doi: 10.1007/BF00392906.

Cabrales-Arellano, P., Islas-Flores, T., Thomé , P. E., and Villanueva, M. A. (2017). Indomethacin reproducibly induces metamorphosis in Cassiopea xamachana scyphistomae. PeerJ 5, e2979. doi: 10.7717/peerj.2979.

Cavalcanti, G. S., Alker, A. T., Delherbe, N., Malter, K. E., and Shikuma, N. J. (2020). The influence of bacteria on animal metamorphosis. Annu. Rev. Microbiol. 74, 137–158. doi: 10.1146/annurev-micro-011320-012753.

Coffroth, M. A., Santos, S. R., and Goulet, T. L. (2001). Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. Mar. Ecol. Prog. Ser. 222, 85–96. doi: 10.3354/meps222085.

Fransolet, D., Roberty, S., and Plumier, J. C. (2012). Establishment of endosymbiosis: The case of cnidarians and Symbiodinium. J. Exp. Mar. Biol. Ecol. 420–421, 1–7. doi: 10.1016/j.jembe.2012.03.015.

Freckelton, M. L., Nedved, B. T., and Hadfield, M. G. (2017). Induction of invertebrate larval settlement; different bacteria, different mechanisms? Sci. Rep. 7, 42557. doi: 10.1038/srep42557.

González-Pech, R.A., Li, V. Y., Garcia, V., Boville, E., Mammone, M., Kitano, H., et al. (2024). The Evolution, Assembly, and Dynamics of Marine Holobionts. Ann Rev Mar Sci. 16 (1), 443–466. doi: 10.1146/annurev-marine-022123-104345.

Harii, S., Yasuda, N., Rodriguez-Lanetty, M., Irie, T., and Hidaka, M. (2009). Onset of symbiosis and distribution patterns of symbiotic dinoflagellates in the larvae of scleractinian corals. Mar. Biol. 156, 1203–1212. doi: 10.1007/s00227-009-1162-9.

Hofmann, D. K., and Brandt, U. (1987). Induction of metamorphosis in the symbiotic scyphozoan Cassiopea andromeda: role of marine bacteria and of biochemicals. Symbiosis 4, 99–116.

Hofmann, D. K., Fitt, W. K., and Fleck, J. (1996). Checkpoints in the life-cycle of Cassiopea spp.: Control of metagenesis and metamorphosis in a tropical jellyfish. Int. J. Dev. Biol. 40, 331–338. doi: 10.1387/ijdb.8735945.

Hofmann, D. K., Neumann, R., and Henne, K. (1978). Strobilation, budding and initiation of scyphistoma morphogenesis in the rhizostome cassiopea andromeda (Cnidaria: scyphozoa). Mar. Biol. 47, 161–176. doi: 10.1007/BF00395637.

Jarms, G., and Morandini, A. C. (2019). World atlas of jellyfish (Hamburg, Germany: Dölling und Galitz Verlag).

Jeong, H. J., Lee, S. Y., Kang, N. S., Yoo, Y. D., Lim, A. S., Lee, M. J., et al. (2014). Genetics and Morphology Characterize the Dinoflagellate Symbiodinium voratum, n. sp., (Dinophyceae) as the Sole Representative of Symbiodinium Clade E. J. Eukaryotic Microbiol. 61, 75–94. doi: 10.1111/jeu.12088.

LaJeunesse, T. C. (2001). Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus symbiodinium using the its region: in search of a "Species" Level marker. J. Phycology 37, 866–880. doi: 10.1046/j.1529-8817.2001.01031.x.

LaJeunesse, T. C. (2017). Validation and description of Symbiodinium microadriaticum, the type species of Symbiodinium (Dinophyta). J. Phycology 53, 1109–1114. doi: 10.1111/jpy.12570.

LaJeunesse, T. C. (2020). Zooxanthellae. Curr. Biol. 30, R1110–R1113. doi: 10.1016/j.cub.2020.03.058.

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., et al. (2018). Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr. Biol. 28, 2570–2580. doi: 10.1016/j.cub.2018.07.008.

Lampert, K. P. (2016). "Cassiopea and its zooxanthellae," in The Cnidaria, Past,

Present and Future: The world of Medusa and her sisters. Eds. S. Goffredo and Z. Dubinsky (New York, USA: Springer International Publishing). doi: 10.1007/978-3-319-31305-4.

Lee, S. Y., Jeong, H. J., Kang, N. S., Jang, T. Y., Jang, S. H., and LaJeunesse, T. C. (2015). Symbiodinium tridacnidorum sp. nov., a dinoflagellate common to Indo-Pacific giant clams, and a revised morphological description of Symbiodinium microadriaticum Freudenthal, emended Trench & Blank. Eur. J. Phycology 50, 155–172. doi: 10.1080/09670262.2015.1018336.

Mammone, M., Ferrier-Pagé s, C., Lavorano, S., Rizzo, L., Piraino, S., Rossi, S., et al. (2021). High photosynthetic plasticity may reinforce invasiveness of upside-down zooxanthellate jellyfish in Mediterranean coastal waters. PloS One 16, e0248814. doi: 10.1371/journal.pone.0248814.

Mammone, M., Sharp, V., Hewitt, M., and Medina, M.. (2023). The influence of photosymbiosis in Cassiopea xamachana regenerative success. Symbiosis 90, 61–70. doi: 10.1007/s13199-023-00920-0.

Marques, A. C., and Collins, A. G. (2004). Cladistic analysis of Medusozoa and cnidarian evolution. Invertebrate Biol. 123, 23–42. doi: 10.1111/j.1744-7410.2004.tb00139.x.

McAnulty, S. J., Kerwin, A. H., Koch, E., Nuttall, B., Suria, A. M., Collins, A. J., et al. (2023). Failure to launch": development of a reproductive organ linked to symbiotic bacteria. mBio 14, e02131–e02122. doi: 10.1128/mbio.02131-22.

McFall-Ngai, M. J., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Dougles, A. E., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl Acad Sci. U S A. 110 (9), 3229–36. doi: 10.1073/pnas.1218525110.

McIlroy, S. E., Cunning, R., Baker, A. C., and Coffroth, M. A. (2019). Competition and succession among coral endosymbionts. Ecol. Evol. 9, 12767–12778. doi: 10.1002/ece3.5749.

Medina, M., Sharp, V., Ohdera, A. H., Bellantuono, A., Dalrymple, J., Gamero-Mora, E., et al. (2021). "The upside-down jellyfish cassiopea xamachana as an emerging model system to study cnidarian–algal symbiosis," in Handbook of Marine Model Organisms in Experimental Biology (Florida, USA: CRC Press).

Mellas, R. E., McIlroy, S. E., Fitt, W. K., and Coffroth, M. A. (2014). Variation in symbiont uptake in the early ontogeny of the upside-down jellyfish, Cassiopea spp. J. Exp. Mar. Biol. Ecol. 459, 38–44. doi: 10.1016/j.jembe.2014.04.026.

Newkirk, C. R., Frazer, T. K., Martindale, M. Q., and Schnitzler, C. E. (2020). Adaptation to bleaching: are thermotolerant symbiodiniaceae strains more successful than other strains under elevated temperatures in a model symbiotic cnidarian? Front. Microbiol. 11. doi: 10.3389/fmicb.2020.00822.

Ohdera, A. H., Abrams, M. J., Ames, C. L., Baker, D. M., Suescú n-Bolívar, L. P., Collins, A. G., et al. (2018). Upside-down but headed in the right direction: Review of the highly versatile Cassiopea xamachana system. Front. Ecol. Evol. 6. doi: 10.3389/

Fevo.2018.00035.

Parkinson, J. E., Coffroth, M. A., and LaJeunesse, T. C. (2015). New species of Clade B Symbiodinium (Dinophyceae) from the greater Caribbean belong to different functional guilds: S. aenigmaticum sp. nov., S. antillogorgium sp. nov., S. endomadracis sp. nov. and S. pseudominutum sp. nov. J. Phycology 51, 850–858. doi: 10.1111/jpy.12340.

Pochon, X., and LaJeunesse, T. C. (2021). Miliolidium n. gen, a New Symbiodiniacean Genus Whose Members Associate with Soritid Foraminifera or Are Free-Living. J. Eukaryotic Microbiol. 68, e12856. doi: 10.1111/jeu.12856.

Prieto, L., Astorga, D., Navarro, G., and Ruiz, J. (2010). Environmental control of phase transition and polyp survival of a massive-outbreaker jellyfish. PloS One 5, e13793. doi: 10.1371/journal.pone.0013793.

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: https://www.R-Project.org/.

Rahat, M., and Reich, V. (1986). Algal endosymbiosis in brown hydra: host/symbiont specificity. J. Cell Sci. 86, 273–286. doi: 10.1242/jcs.86.1.273.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. doi: 10.1038/nmeth.2089.

Searle, S. R., Speed, F. M., and Milliken, G. A. (1980). Population marginal means in the linear model: an alternative to least squares means. Am. Statistician 34, 216–221. doi: 10.1080/00031305.1980.10483031.

Tang, Y., Horikoshi, M., and Li, W. (2016). ggfortify: unified interface to visualize statistical result of popular R packages. The R Journal 8, 474–485. doi: 10.32614/RJ-2016-060

Thornhill, D. J., Daniel, M. W., LaJeunesse, T. C., Schmidt, G. W., and Fitt, W. K. (2006). Natural infections of aposymbiotic Cassiopea xamachana scyphistomae from environmental pools of Symbiodinium. J. Exp. Mar. Biol. Ecol. 338, 50–56. doi: 10.1016/j.jembe.2006.06.032.

Treible, L. M., and Condon, R. H. (2019). Temperature-driven asexual reproduction and strobilation in three scyphozoan jellyfish polyps. J. Exp. Mar. Biol. Ecol. 520, 151204. doi: 10.1016/j.jembe.2019.151204.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis eBook (New York: Springer-Verlag). doi: 10.1007/978-3-319-24277-4.

Wickham, H., Francois, R., and Muller, K. (2022)dplyr: A grammar of data manipulation. Available online at: https://dplyr.tidyverse.org/ (Accessed 1 December 2022).

Winstead, D. (2019). THE EFFECT OF SYMBIONT COMPETITION ON HOST-SYMBIONT SPECIFICITY IN ANIMAL-ALGAL CASSIOPEA MUTUALISMS. State College, Pennsylvania, USA: Pennsylvania State University.

Chapter 4

In vivo fungal diversity of Cassiopea xamachana revealed through isolate sequencing

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Abstract

Marine fungi are highly understudied, as historical literature has predominantly focused on culturable fungus from the sediment and water column. Only in recent decades has awareness increased of a major hub of marine fungus; marine multicellular organisms. Oceanic plants and animals are found associating in higher frequencies with marine fungi than the sediment or water column, and many records of marine fungi originate from sampling of eukaryotic organisms. Coral and sponges have diverse endomycotal communities, and both additionally experience fungal pathogens, showing evidence of marine host-symbiont parasitic relationships. However, the existence and diversity of fungus in a benthic jellyfish has yet to be studied. *Cassiopea xamachana*, the upside-down jellyfish, is a scyphozoan with a multi-ocean distribution. Unlike other scyphozoan jellies, which have a relatively low quantity of culturable isolates, *C. xamachana* exists in shallow environments on the bottom of the seafloor. This gives it a close association with the air-water interface, human interactions, and the fungal communities known to exist in marine sediment. The existence of an endomycotal community within *C. xamachana* has yet to be established. We sampled male and female *C. xamachana* medusa from three wild locations in Key Largo, FL, USA with varying environments and abiotic parameters. An additional location was lab-reared medusa from Carnegie Institute of Science, where the animals are kept in artificial seawater. Tissue was rinsed and plated in petri dishes, isolated, and sequenced to genus-level to identify isolates. This study is the first record of internal fungi presence in a benthic jellyfish. It reveals that while environment influences fungi diversity in *C. xamachana*, certain fungal isolates can be found exclusively in jelly tissue or environmental samples. There appears to be a level of specificity in *C. xamachana* influencing the existence and persistence of certain fungal species within its tissue.

Introduction

The kingdom Fungi is one of the most diverse and ubiquitous taxa in the world, and its individuals are found in all areas of life. In general, fungi can be split into two groups; filamentous fungi and unicellular yeasts. Filamentous fungi have long thread-like hyphae used to disseminate spores and for structural support, while yeasts reproduce by budding or fission. Additionally dimorphic fungi may take a filamentous or yeast form depending on the environmental conditions (Hoboken 2011). In laboratory conditions, fungi grown on agar exhibits a vast array of morphology. Hyphae can grow almost indefinitely given continous and consistent nutrients, and these fungi will often cover the entire surface of an agar plate. However, in nature, fungal growth is limited by nutrient availability. Fungi need fixed forms of carbon such as sugar or other organic compounds, and fungi cannot fix nitrogen and need to obtain nitrogen compounds from their environment (Hoboken 2011). Consequently, fungi are often found on decaying material or as symbionts (including parasites) in order to receive nitrogenous and organic compounds. Much of our knowledge on fungal diversity originated from terrestrial studies of culturable fungi, which presents a very narrow view of global distributions of fungi. In particular, despite knowing fungi are present in oceans and estuarine environments for decades (Kohlmeyer and Kohlmeyer 1979), marine fungi are highly understudied (Richards et al. 2012).

Definitions on marine fungi have varied over time, but the definitions by Kohlmeyer and Kohlmeyer (1979) have been widely accepted by mycologists (Richards et al. 2012). They define two groups of marine fungi; obligate, where the fungus can only grow and reproduce in a marine or estuarine environment, and facultative, where the fungus can grow in terrestrial, marine, or freshwater. Kohlmeyer and Kohlmeyer do not specify that facultative marine fungi need to be able to reproduce in a marine environment, which has led to controversy over their definition. Wider definitions have been proposed, such as any fungus with the capacity to grow and reproduce in a marine environment or within the body of a marine host animal, or able to be

metabolically active in a saline environment (Gonçalves et al. 2022). The debate over the delineation of terrestrial and marine fungus primarily comes from sampling efforts, and the question if a sampled fungus from a marine environment was growing there, or simply a contaminant of the air-water interface (Richards et al. 2012). Many of these questions have been remedied by an increase in genetic sequencing. Several marine-sourced fungi form novel clades in the overall fungal lineage, but branch closely to terrestrial or freshwater fungal sequences, indicating both an evolutionary relationship between fungi of different environments and diversification of these species (Richards et al. 2012). However, the knowledge of terrestrial-marine transition events and vice versa, as well as the adaptations marine fungi developed to cope with a saline environment, is hindered by the lack of marine fungi sampling.

Marine fungi are highly understudied, with an estimated 0.6% of all taxa having been cultured (Richards et al. 2012). Environmental DNA (eDNA) sampling has increased our understanding of marine fungus presence, but many of these studies sample from sediment and water columns. As stated previously, fungi rely on nutrient-rich environments and a substrate to grow and proliferate (Richards et al. 2012). These requirements are easier to come by in sediment than water columns, and this reflects in the species diversity cultured and sequenced from these environments. Sediment provides abundant surface area for hyphae and yeasts, and deceased organic matter sinks to the floor in marine environments. The decaying matter provides the organic and nitrogenous compounds that fungi need to grow and reproduce. However, the floating matter in water columns is mostly composed of phytoplankton and other planktonic organisms. Plankton are typically in low concentrations in water columns and do not have abundant volume or surface area for fungal growth. Additionally, the low concentrations make sampling of water column fungus difficult, and studies on water column fungi skewed perspectives on marine fungi abundance for years (Richards et al. 2012). More recent research

has found a high diversity and abundance of marine fungi in the afformentioned sediment but also within marine organisms (Amend et al. 2019).

Fungal growth requirements of needing a substrate and organic and nitrogenous compounds make marine organisms an ideal ecosystem for fungi to thrive in the ocean. This theory is supported by fungus culture experiments, predominantly in sponges and coral (Richards et al. 2012; Amend et al. 2019). Sponges have a well-established internal fungal community, and there is evidence for host selectivity in certain sponge species. For example, *Gelliodes fibrosa* collected from two geographically isolated locations in Hawai'i had similar host-specific fungal communities (Li and Wang, 2009). This suggests not only are marine hosts preferential environments for fungi growth, but one or both of the organisms are driving specificity in their interactions. Coral as well have several well-studied fungal interactions dating back as far as the 1990s (Raghukumar and Ravindran 2012). *Aspergillus sydowii* interferes with reproduction in the octocoral *Gorgonia ventalina*, necrosis of the tissue, and eventual death, but *A. sydowii* has also been isolated from apparently healthy coral and sponges (Soler-Hurtado et al. 2016; Yarden 2014). This shows that fungi presence does not necessarily correlate with a phenotype in a marine host and fungi may be present in most marine organisms. It is suggested that all marine eukaryotes have a rich assemblage of microbes and studying the holobiont, as in the assemblage of a host with its symbionts, is necessary for understanding an organism's ecology (Gonzalez-Pech et al. 2024). Only by sampling a wider variety of marine organisms can we expand our knowledge of marine fungal diversity, and additionally study the specificity of fungal community composition with marine hosts.

While coral have documented interactions with pathogenic and benign internal fungal communities, fungi has not been as extensively studied in other cnidarians. Scyphozoan jellyfish have a global distribution (Jarms and Morandini 2019) and, unlike their fellow cnidarians (coral), a notable lack of diseases aside from bell rot, which arises when existing lesions on jelly tissue

start to corrode away (Steers et al. 2003). The exact cause of bell rot is unknown however, the initial lesions are caused by physical damage to the animal and not from a parasitic infection. Biopsy of rotting tissue saw either bacterial growth or ciliates on the lesion, and were successfully treated with antibiotics, suggesting fungus is not the cause of bell rot (Steers et al. 2003). To date, no fungal pathogen of a scyphozoan jellyfish has been recorded. However, fungal isolates have been cultured from jellyfish, supporting the evidence that fungal presence in marine organisms is not strictly limited to pathogens. Scyphozoan abundance and apparent lack of pathogens had led to the rise of jellyfish in bioactive compound research for human pharmaceutics (Leone et al. 2015; De Domenico et al. 2023). Frequently, fungal spores are swabbed from jellyfish and grown in a petri dish to test antimicrobial properties. Compounds derived from *Paecilomyces variotii*, a fungus isolated from the exterior bell of the scyphozoan *Nemopilema nomurai*, have been extensively studied for years for antimicrobial properties and natural product production (Liu et al. 2011; Fields et al. 1996). However, most studies on jellyfish have isolated fungus species from the external surfaces of the animals, those most in contact with the outside water columns. Very few studies have looked at endomycotal presence. One study exploring marine-derived metabolites isolated the fungus *Epicoccum purpurascens* from the "inner tissue" of an *Aurelia aurita* jelly, though the study does not specify which part of the animal was cut or if it was rinsed before being plated (Wright et al. 2002). Additionally, an *N. nomurai* study isolated *in situ* fungus from the bell, tentacles, and gonads from two adult medusa, and only 5 isolates were recovered from the bell and tentacles (Yue et al. 2015). In contrast, sampling of 12 sponges using the same culture-based techniques yielded over 200 culturable isolates, a stark increase from *N. nomurai* (Li and Wang 2009). Like many adult scyphozoans, *N. nomurai* is pelagic and rarely comes in direct physical contact with other organisms or sediment (Yue et al. 2015; Jarms and Morandini 2019), two of the most common pools of marine fungi (Richards et al. 2012). It is currently

unknown what fungi is associated with a benthic scyphozoan, such as the upside-down jellyfish *Cassiopea xamachana*.

C. xamachana medusae lay umbrella-side down on the sandy bottoms of shallow mangrove environments. While able to swim for brief periods, they are unique from other scyphozoans by predominantly existing in direct contact with the marine seafloor (Medina et al. 2021; AZA Aquatic Invertebrate TAG 2021). Additionally, it has been proven pulsations from *Cassiopea* spp. pump water through the sediment below them, enriching the water column with nutrients (Jantzen et al, 2010; Durieux et al. 2023). As marine fungi is known to exist in high abundances in sediment, it can be theorized *C. xamachana* medusa come into frequent contact with fungi through their regular pulsations. However, while the *C. xamachana* holobiont is known to include bacteria and dinoflagellates, an internal community of fungus has never been established (Muffet et al. 2024, preprint; Medina et al. 2021). Only one fungal species has been recorded from *C. xamachana*, but only cultured from the exterior surface of the bell for to analyze for bioactive compounds (Trischman et al. 1993). The existence of *in situ* fungi, the diversity of this fungus, and the potential existence of *Cassiopea*-specific fungal communities has yet to be explored. In order to investigate *C. xamachana* fungi, jellies were collected from three wild locations and one lab aquarium system. Samples were taken from a variety of tissue compartments and environmental samples and isolated through culturing. This study represents the first record of the *C. xamachana* fungal community and reveals the diversity of fungi found in a single scyphozoan species from a variety of environments.

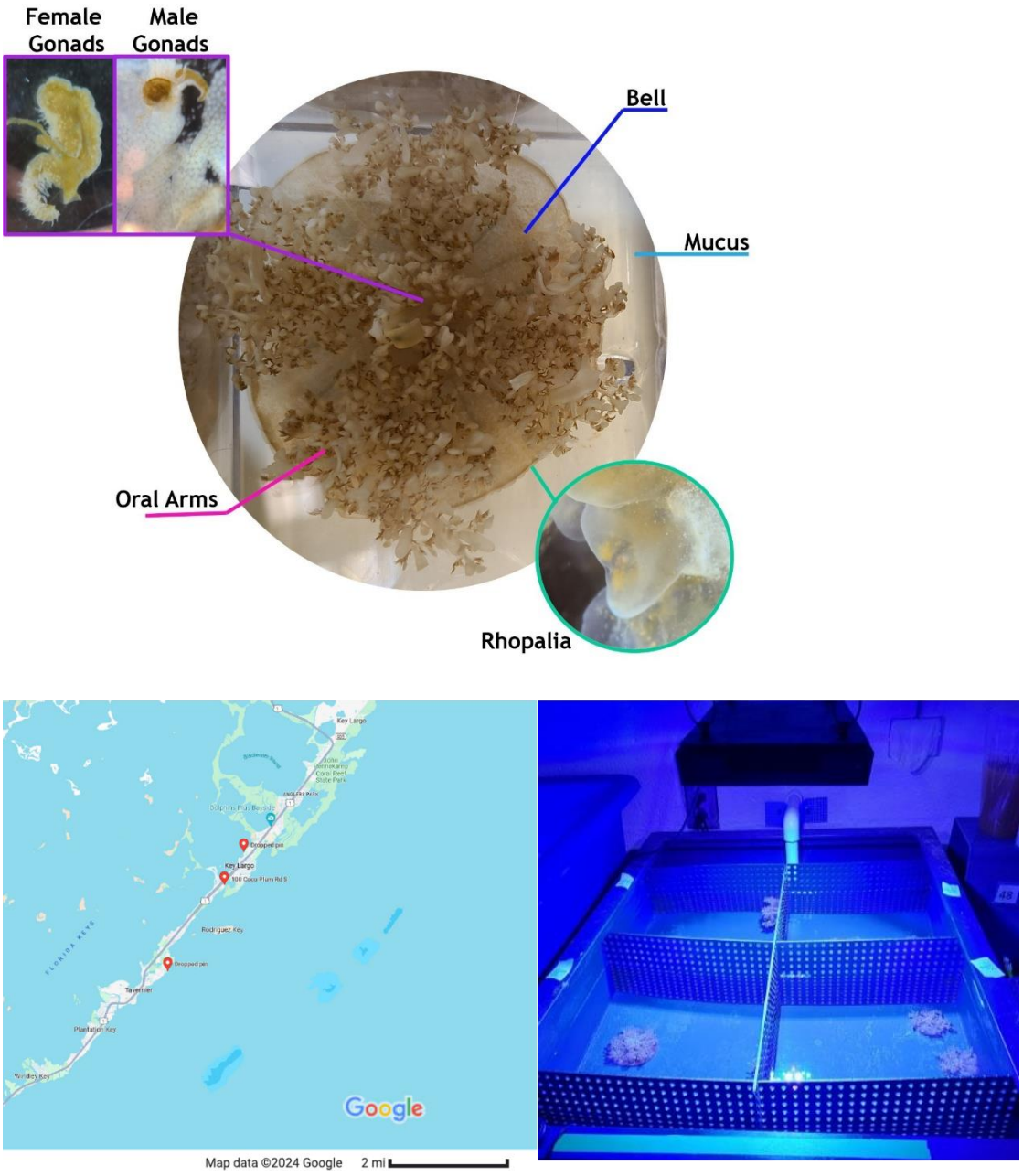


Figure 4-1: Sampling locations of *C. xamachana*. A) Locations of tissue compartments collected for plating. B) Map with markers indicating wild-locations in Key Largo, FL, USA (left) and an image of the aquaculture system at Carnegie (right).

Table 4-1: Sampling locations of the lab-reared (row 1) and wild-caught (rows 2-4) C. xamachana medusa. Coordinates of wild locations, dates of sampling, and environmental parameters are recorded. The number of plated replicates of each tissue type is shown in columns 6-13.

Location	Coordinates	Date of Sampling	Temperature	Salinity		Bell	Oral Arms	Rhopalia	Gonads	Mucus	Seawater	Outside Invertebrates/Algae
Carnegie Institute of Science					# initial replicates	6	6	6	6	6	6	0
Baltimore, MD, USA		14-Apr-23	26°C	35 ppt	# cultures grown	5	12	0	0	7	3	N/A
Dock					# initial replicates	7	7	7	7	7	3	2
Key Largo, FL, USA	25.1014846, -80.4388100	8-May-23	28.1°C	35 ppt	# cultures grown	3	9	3	5	9	3	2
Coco Plum					# initial replicates	6	6	6	6	6	3	2
Key Largo, FL, USA	25.079474, -80.452842	14-May-23	30°C	39 ppt	# cultures grown	5	3	3	7	20	9	1
Harry Harris					# initial replicates	6	6	6	6	6	3	1
Key Largo, FL, USA	25.0233582, -80.4941312	15-May-23	34°C	39.5 ppt	# cultures grown	3	5	2	3	11	5	0

Materials and Methods

Culturing conditions and plating

Culture media was made of potato dextrose agar (PDA) with 250 mg/L of chloramphenicol (to discourage the growth of bacteria, Ben-Dor et al. 2021) and poured into standard-sized petri dishes. Each petri dish corresponded to one tissue region for an individual animal.

The tissue collection process was as follows for each jellyfish. The animal was removed from aquarium/collection containers and placed in a bowl with sterile seawater. Once in the bowl, produced mucus was collected with flame-sterilized forceps, and rinsed 3 times in clean dish with sterile seawater. The mucus was immediately spread onto a petri dish and left to dry right-side up for 24 hours. After 24 hours, the plates were flipped upside-down to discourage condensation on

the agar. Mucus was always collected first to ensure any trapped fungal spores did not come from the jelly tissue after dissection.

Tissue was collected from 4 jellyfish compartment (Figure 4-1). Tissue from the bell (n=3) and oral arms (n=3) was sliced away with a sterile scalpel and rinsed three times with fresh sterile seawater prior to plating. Tissue was embedded into the agar manually with sterile forceps to prevent the tissue from falling off the agar. One rhopalia (sensory organ) (n=1) was dissected from the animal under a microscope and plated. To collect gonad tissue, a cross was cut into the center of the animal from the oral side into the gastrovascular cavity. Gonad tissue (n=3) was removed with flame-sterilized forceps and confirmed to lack gastric tissue under a microscope. At this point, the sex of the animal was also confirmed. Petri dishes were left to dry for 24 hours right-side up before being flipped.

Seawater from each location was collected and spun down at approximately 3,000 rpm on an IEC Clinical Centrifuge to concentrate fungal spores. Seawater from the bottom of the centrifuged tubes was collected with a sterile pipette and plated.

All animals were left in isolated aquariums with seawater for 24 hours, at which point the cross-section through their center had healed, and regeneration of the lost tissue was underway, before being returned to their habitats.

Petri dishes were grown at 25°C and checked daily for fungal growth. Growths with unique border shapes, color, and texture were isolated on fresh plates using sterile inoculating loops. Final plates used for DNA extraction consisted of one morphologically distinct culture.

Specimen collection: lab-reared

Adult *C. xamachana* were acquired from the Cleves Lab at Carnegie Institute of Science. These animals are F2 generation, raised from a cross between two initial male and female adults

obtained from Florida, USA (aquarium setup in Figure 4-1). Once crossed, the resulting polyps were genetically isolated and allowed to bud, creating genetic lines. The sex of each line was confirmed by the Cleves Lab by rearing produced ephyra from polyps to medusa. Each ephyra from one genetic line developed into the same sex, confirming sexual determination happens before strobilation and ensuring even replicates of our samples (Cleves Lab, personal communication). Genetic lines JB3 (males) and JB4 (females) were utilized. Three individuals of each line were used. Both lines were kept in the same flow-through system, but 6 total replicates of seawater were taken and plated.

Specimen collection: wild locations

3 adult males and 3 adult females were collected from 3 wild locations (Figure 4-1) for plating. The sexes of individuals were determined visually, as *C. xamachana* displays sexual dimorphism (Medina et al. 2021) and confirmed during the collection of the gonadal tissue. The location named "Dock" was the dock at the Key Largo Marine Station, Key Largo, Florida, USA. The location "Coco Plum" was a lagoon at the end of Coco Plum Road, Key Largo, Florida, USA. The location "Harry Harris" was the Harry Harris Marine Park, Key Largo, Florida, USA (Table 4-1).

Animals were collected through snorkeling into plastic bags and buckets and brought to shore for sampling. Animals were removed entirely from the water and placed into bowls with sterile seawater to remove the mucus produced during sampling. Tissue sampling proceeded as described above, with a few exceptions. Only one seawater plate was made for the 3 wild locations. An extra female was collected from "Dock" as it was initially ID'ed as male until gonad collection. Also, a randomly sampled invertebrate was collected from each location and plated. From "Dock," an *Exaiptasia* anemone was embedded whole into the plate after rinsing

with sterile seawater. From “Coco Plum,” an individual ascidian from an orange cluster of unknown species was sliced in half and plated. From “Harry Harris,” a piece of a lettuce sea slug, *Elysia clarki,* was dissected with a sterile scalpel, rinsed, and plated. Collection and plating was attempted of a common cup algae, *Acetabularia crenulata,* from each location as well, but the algae could not be found at “Harry Harris” (Table 4-1).

DNA extractions and Sequencing

Pieces of oral arms were additionally cut from each animal and preserved in either 100% ethanol (Carnegie samples) or a DMSO-salt solution (wild samples) for DNA extraction to confirm host species and dinoflagellate species. The DMSO-salt solution was comprised of 20% di-methyl sulfoxid, 0.25 M EDTA (ph=8) in water supersaturated with NaCl.

A flame-sterilized scalpel was used to cut a small piece of fungal isolate off an agar dish, and the piece was placed in a screw-top tube with sterile forceps. The tube was immediately placed in liquid nitrogen to flash freeze it and stored at –80°C until DNA extraction.

Fungal isolate DNA extraction was performed using the Qiagen DNeasy Plant Mini Kit following the provided procotol, with one exception. In step 2, 100 uL of 0.5 mm silicon beads were added to a tube with the Buffer AP1 and RNase A. This was run on a Biospec Mini-Beadbeater-16 at max speed (3450 oscillations/min) for 2 minutes. The tubes then proceeded to the incubation step with vortexing 3 times during the incubation. Additionally, step 4 was included, and step 12 was not. DNA extractions were stored at -20°C. The polymerase chain reaction (PCR) was done for the ITS gene using the primers ITS1 and ITS4 (Ben-Dor et al. 2021).

DNA extraction for jellyfish and dinoflagellate species identification followed the procedure in LaJeunesse et al. (2018). For dinoflagellate symbiont identification, primers of the

28S (LSU) ribosomal DNA were used (forward: 5 ′-CCCGCTGAATTTAAGCATATAAGTAAGCGG-3′; reverse: 5′-GTTAGACTCCTTGGTCCGTGTTTCAAGA-3′) (LaJeunesse et al. 2018). The PCR followed the methods described in LaJeunesse et al. 2018. For *Cassiopea* species identification, primers of the eukaryotic 16S ribosomal gene were used (forward (med-rnl-F): 5'-GACTGTTTACCAAAGACATAGC-3'; reverse (med-rnl-R): 5'-AAGATAGAAACCTTCCTGTC-3') and followed the methods described in Lawley et al. 2016.

Sequencing was performed at the Pennsylvania State Genomic Core Facility. Sequences were imported into Geneious Prime® for manual curation. They were globally aligned using the Geneious alignment option of the software. IqTree was used with the in-built JModelTest feature (flag "-m TESTNEW") to construct phylogenetic trees. A boostrap of 1000 replicates was used for tree generation.

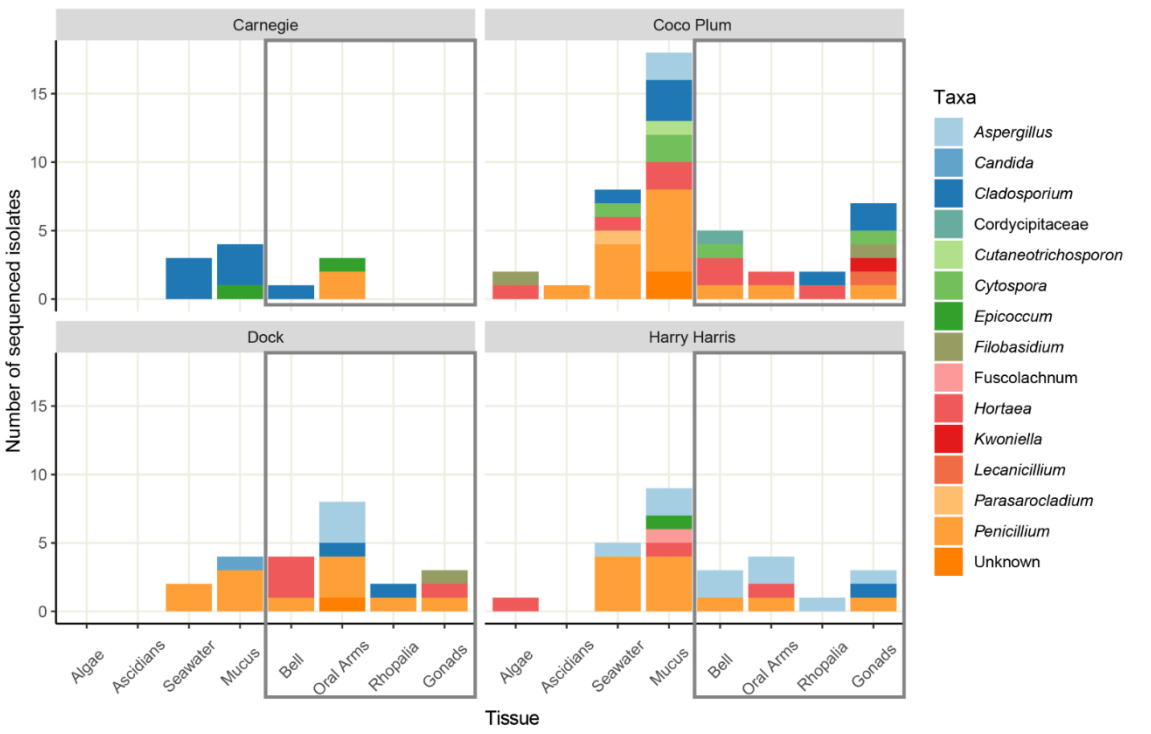


Figure 4-2: Number of fungi isolates sequenced and identified with the ITS gene. Grouped by

location (location names in gray headers). Genera in taxa legend shown in italicized font. Black boxes on each graph show the columns representing isolates from jellyfish tissue, not environment.

Results

At least one fungal isolate grew from each tissue replicate except for gonad and rhopalia tissue collected from Carnegie, the lab-reared jellies (Table 4-1). A Fisher's Exact Test on the correlation between the average number of cultures that grew by location achieved significance with a cutoff of $p < 0.05$ ($p=0.04494$), but the average number of cultures compared to sex of jelly did not achieve significance. Across all locations, mucus grew the most morphologically distinct cultures (n=72), while rhopalia and seawater grew the least (n=33 and 35 respectively).

BLAST results were recorded down to genus level, as the primers used cannot get to species level (Ben-Dor et al. 2021). A Fisher's Exact Test was run on average number of isolates by genera against location, tissue, and sex. Only location achieved significance ($p=0.02208$), with tissue getting a p-value of 0.4465 and sex getting a p-value of 0.9897. Post-hoc analysis showed the significance of location was driven by the presence of *Candida* only in the Dock location, and by the abundance of *Cladosporium* and *Epicoccum* from Carnegie jellies. Additionally, significance was driven by the abundance of *Aspergillus* from Harry Harris. Genera diversity by location is recorded in Figure 4-4. For the outside invertebrates, algae collected from Coco Plum (n=2) and Harry Harris (n=1) grew cultures from the genus *Hortaea* exclusively (Figure 4-2). The *Aiptasia* anemone from Dock and the ophistobranch from Harry Harris both did not grow any cultures (Table 4-1), but the ascidians from Coco Plum grew one culture from *Penicillium* (Figure 4-2).

JModelTest generated a TIMe tree, shown in Figure 4-3. Phylogeny overall confirmed BLAST identification, with a few exceptions. Samples 38A, 11A, 12A, 16A, 42A, and 27A were identified as *Cytospora* spp., yet the tree placed *Cryphonectria neoparasitica* in between the haplotypes and *Cytospora* (see sample names and metadata in Supplemental Table 4-1). This may be a case of a mislabeled sequence in GenBank, or paraphyly. Samples 7A, 19A, and 28A were identified as *Lecanicillium* spp. by BLAST (Supplemental Table 4-1), but are neighboring branches on the tree (Figure 4-4 B). All of these samples were from Coco Plum, but a mix of tissue type and female/male origin (Supplemental Table 4-1).

There were several isolates only found within jelly tissue and not environmental samples (i.e. seawater, non-*Cassiopea* invertebrates, and mucus). These were identified through a combination of BLAST against the NCBI nucleotide database and the clustering of sequences in the produced phylogenetic tree (Figures 4-4, 4-5) (Supplemental Table 4-1). 29A, 30A, and 33A were all identified as *Cladosporium* spp. (Supplemental Table 4-1), and clustered independently from other *Cladosporium* species on the tree (Figure 4-4 A) However, 30A and 33A were both isolates from the same gonad replicate of a Coco Plum female and are assumed to be identical (Supplemental Table 4-1). 19A was identified as a *Lecanicillium* sp. (Supplemental Table 4-1), but clustered with 28A which was identified as from the family Cordycepticeae (Figure 4-4 B). Both were only found in jelly tissue. 2A was identified as a *Penicillium* sp. (Supplemental Table 4-1), which was the most abundant genus in the sampled community, but clustered as an independent branch on the tree (Figure 4-4 C). Samples 80A and 126A clustered together as a clade within *Aspergillus*, and 64A and 128A were both identified as *Aspergillus* spp. uniquely found in jelly tissue (Figure 4-4 D) (Supplemental Table 4-1). 135A was identified through BLAST as *Epicoccum italicum*, while 76A was identified as *E. nigrum* (Supplemental Table 4-1), however they cluster together on the tree with a 96% bootstrap value. 135A was found in jelly tissue from Carnegie, while 76A was only found in an environmental sample from Harry Harris

(Figure 4-4 D). Finally from class Basidomycota, 21A (*Kwoniella* sp.) and 125A (unknown genus) were only found in jelly tissue samples (Figure 4-5).

Along with sample 76A mentioned above, several samples were only found in environmental samples. 58A was identified as a unique *Aspergillus*, and 3A and 6A clustered together under *Cladosporium.* 88A was a unique *Cladosporium* from the mucus of a lab reared male, while the *Cladosporium* 8A was isolated from a Coco Plum female. 122A was the only *Candida* species identified, isolates from the mucus of a lab-reared male. 45A was the only *Cutaneotrichosporon* isolated. 42A was identified as a *Cytospora* sp. 76A and 92A were identified as two separate *Epicoccum* lineages, both found in a mucus sample from Harry Harris and the lab-reared Carnegie jellies respectively. 20A was the only *Parasarocladium* identified. 78A was a unique *Penicillium.* 36A and 40A also fell within *Penicillium,* but as both samples came from subsequent streaks from the same initial Coco Plum seawater sample, these are likely duplicate strains. Likewise, 9A and 48A, which fell within *Aspergillus*, were streaked from the same Coco Plum female mucus sample, and are likely duplicates. 7A and 38A both were isolates whose species could not be identified, though they clustered separately within Ascomycota. Finally 79A was unique to environmental samples, but the sequence was low quality. The identification may not be accurate.

All sampled jellyfish were identified as *C. xamachana* and all photosymbionts were identified as *Symbiodinium microadriaticum* (Supplemental Figure 1, 2).

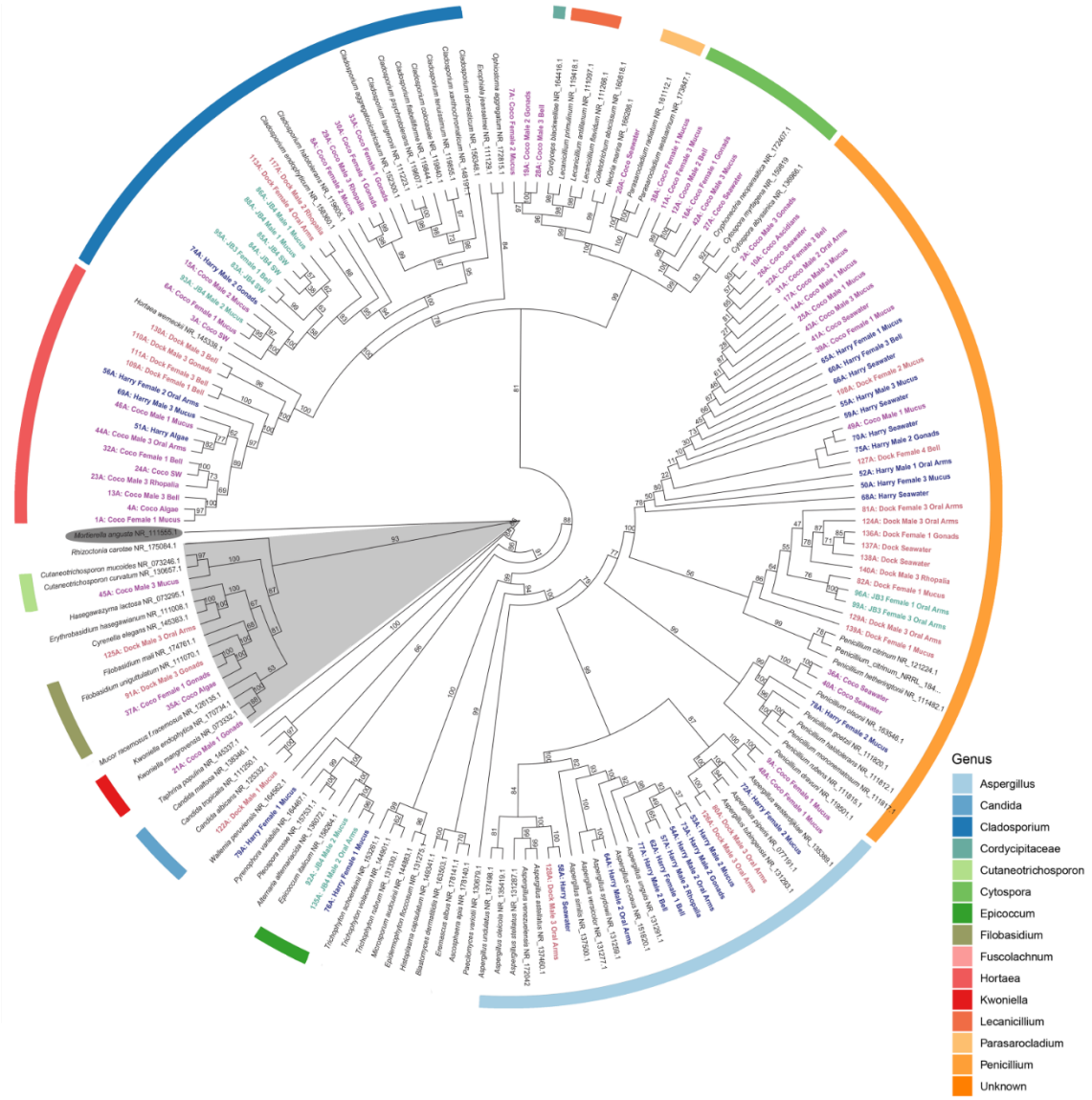


Figure 4-3: Cladogram of fungal isolates (in colored text) aligned against NCBI Genbank fungal sequences (in black). Color blocks represent major taxa clades. Branches with white background are from Class Ascomycota; gray background if Class Basidomycota. Outgroup of *Mortierella angusta* (NCBI ID NR_111555.1) shown in dark gray circle. Bootstrap values on each node (1000 replicates).

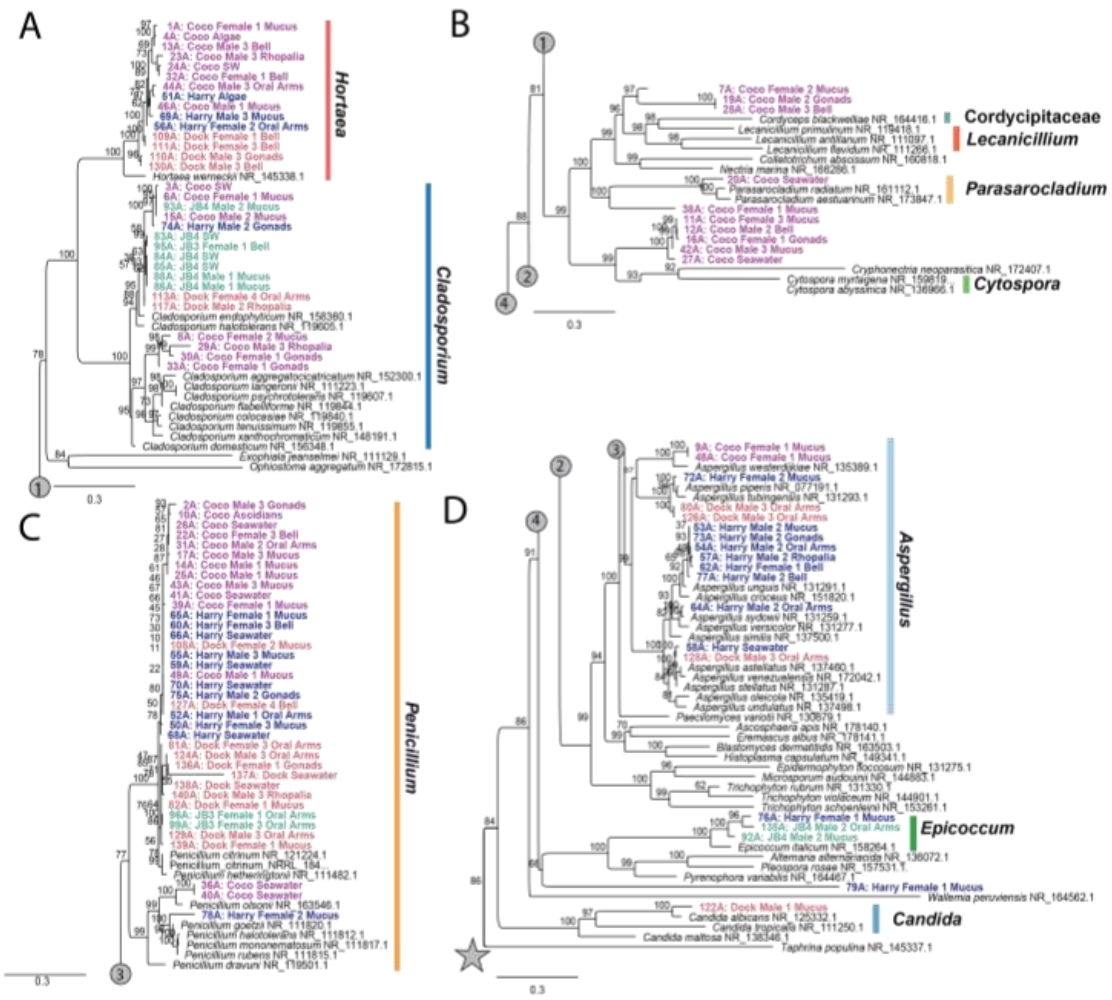


Figure 4-4: Ascomycota sequences aligned against NCBI Genbank fungal species. Fungal species labeled with sample ID, location, sex, and tissue. Labels color-coded by location. Connections between trees A-D shown by gray circles with numbers. Gray star shows connection to class Basidomycota (figure 4-5).

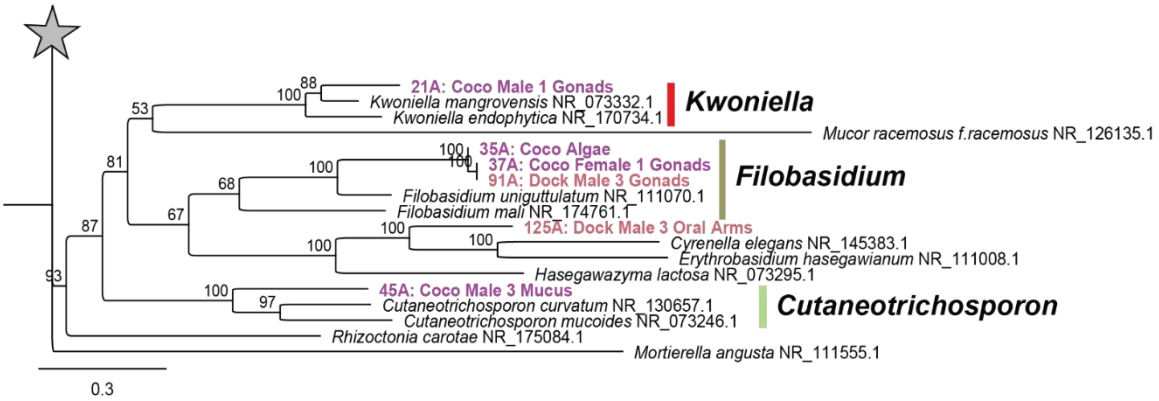


Figure 4-5: Basidomycota sequences aligned against NCBI Genbank fungal species. Fungal species labeled with sample ID, location, sex, and tissue. Labels color-coded by location. Gray star shows connection to class Ascomycota (figure 4-4).

Discussion

These results uncover for the first time endomycotal presence in *C. xamachana* medusa. All fungi isolated were identified as Ascomycota or Basidomycota (Figure 4-3), the two fungal taxa hosting the majority of currently known marine fungus (Amend et al. 2019). All genera identified through BLAST have been found in marine environments before (Li and Wang 2009; Wright et al. 2003; Ben-Dor Cohen et al. 2021; Richards et al. 2011; Jones et al. 2015). However, the fungal diversity within *C. xamachana* varies considerably from other described studies utilizing similar culturing techniques (Li and Wang 2009; Yue et al. 2015). While 12 sampled sponges from Li and Wang's 2009 study yielded over 200 isolates, culturable isolates from a pelagic jellyfish numbered as few as 5 (Yue et al. 2015). Our samples produced a range of isolates per individual depending on the location (Carnegie= 1-2; Dock= 1-9; Coco Plum= 2-11; Harry Harris= 2-7), but still fewer isolates than other sponge and coral studies.

Both free-living and lab-reared jellies had fungi present within their tissue, though the abundances and species diversity range significantly (Figure 4-2). The lab-reared jellies (Carnegie) only had 11 isolates for all 6 jellyfish, while Dock had 23 isolates, Coco Plum had 45 isolates, and Harry Harris had 26 isolates (Figure 4-2). Carnegie's isolates were disproportionally represented by *Cladosporium* species as well as missing major taxa cultured from the wild jellies such as *Aspergillus* (Figure 4-2). The lab-reared jellies were an F2 generation of *C. xamachana* originating from parents collected from the Florida Keys, USA, the same location our wild jellies were collected. Despite this, the Carnegie fungal isolates are significantly different in composition than all three wild locations (Figure 4-2). This suggests that while fungi exists in *C. xamachana*, its presence is not solely determined by host species. It also shows fungi are unlikely to persist over generations in a controlled lab setting. This could be due to a number of factors. The simplest explanation is that the original medusa collected for breeding in that lab had an extremely low fungi species count, but this is unlikely considering the diversity and abundance of culturable isolates we collected from wild jellyfish. Another possibility is fungi is able to exist within *C. xamachana* tissue but is not passed down through generations. *C. xamachana* are broadcast spawners but they are not known to pass down intracellular symbionts to offspring. Unlike several coral species which pass their photosymbiont into developing embryos (Baird et al. 2021), *C. xamachana* symbionts are absent in their planula (Medina et al. 2021). Thus, there already is a mechanism in this host preventing intracellular organisms from passing to offspring. Further research would need to be done to investigate this line of thought, starting with identifying where fungus is located within the jellyfish. We proved fungal species differ by tissue compartment, but did not separate the tissue into its layers (epidermis, mesoglea, and endodermis)(Medina et al. 2021) or explore if fungus can be found within cells. For determining inheritance, fungus from the gonads of a fertile female would need to be sequenced for species identification. Sequencing would then need to take place on the embryos, planula, and developing

polyps to determine if fungi can be passed from parent to offspring. Another likely possibility for the differences we saw between lab-reared and wild-caught jellyfish is in a controlled lab environment, the conditions are not ideal for multiple species of fungi to proliferate. Potentially the temperature, nutrient availability, or other abiotic factors in the seawater may influence the successful proliferation of various fungi species. Again, this would need to be explored further.

Fungal communities from the three wild locations also differed, though it is unclear what factors contributed to these differences between locations. Harry Harris was the hottest location with the highest salinity and jellyfish were collected from a man-made lagoon, giving those animals the closest physical association to potential land contamination (Table 4-1). The Dock location also had close association to land and human activity, though it was located behind a research station with limited pollution from the station inhabitants. While both places are characterized by close human interaction, the fungal communities are very different (Figure 4-2). Interestingly, the mucus and seawater samples had fungi genera that also appeared in multiple tissue compartments from the same locations, such as how Harry Harris' seawater sample was the only one out of all seawater samples to have *Aspergillus* grow, and *Aspergillus* was found in all Harry Harris jellyfish samples as well (Figure 4-2). This may suggest that if certain species are in high enough abundances in the environment to be collected from a water column sample, then they are more likely to enter and persist in *C. xamachana* tissue. Further sampling at these wild locations would be needed to fully characterize their yearly fungal species diversity.

The difference between tissue compartment and fungus genera did not achieve significance, but patterns can be seen in the isolate abundances. Consistently, gonads and rhopalia had low occurrence of fungal growth (Table 4-1; Figure 4-2). The lack of fungal growth from rhopalia may be explained by the small size of the organs. Rhopalia are the sense organs of *C. xamachana*, and are comprised of a tissue pocket with pigment and fluid inside to allow the animal to sense direction and light in the water. While adult *C. xamachana* have on average 16

rhopalia (Medina et al. 2021), their surface area is small and would have less physical space to accommodate fungal spores than other tissue. Additionally, rhopalia do not have a direct interface with the outside environment as they exist under the ectoderm of the bell. These physical characteristics may limit the abundance and diversity of fungi that can exist in the rhopalia pocket. However, rhopalia are critical for the prolonged survival of *C. xamachana*, and they will regenerate lost rhopalia if damaged (Cary 1916), so there may be antifungal mechanisms protecting the rhopalia from damage. There are numerous possibilities why rhopalia would have a low abundance of fungal growth, which needs to be further explored. Similarly gonads are of intense importance to an organism, and fungal pathogens can severely inhibit the production of gametes. In the bobtail squid *Euprymna scolopes*, the bacterial microbiome of the female reproductive gland stops infection with fungal pathogens. If the microbiome of the female reproductive gland is disturbed, fungus grows on the organ and the squid experience reduced hatch rates (Kerwin et al. 2019). *C. xamachana* may have similar microbiomes or other mechanisms to prevent fungal fouling of the gonads, as it is unlikely *C. xamachana* gonads do not have much culturable fungi by chance. The gonads of *C. xamachana* are located in the center of the gastrovascular canals (Medina et al. 2021), and the removal of gonads will often remove gastrovascular filaments as well (as seen through our sample collection). As this jellyfish ingests anything floating by in the water column (Medina et al. 2021), it can be assumed its gonads are frequently exposed to fungal spores. The low abundance of fungal isolates from collected gonads implies there may be an antifungal prevention in place to ensure healthy gonadal development. For instance, lab-reared jellyfish had no fungal isolates from gonadal tissue (Figure 4-2). As the lab-reared jellyfish were kept in a contained environment with regularly cleaned enclosures and limited outside contamination, it is likely they encountered fewer fungi. The lack of culturable isolates from the Carnegie samples confirm this theory. It is possible with fewer exposure events to fungi, the gonads were able to be kept mostly fungus-free through an antifouling mechanism.

Disruption tests of gonad microbiomes would need to be performed to explore potential anti-fungal mechanisms.

Coco Plum samples had a disproportionately high occurrence of isolates in gonadal tissue compared to the other locations, and contained an isolate from the genus *Kwoniella*, which was not seen in any other sample collected (Figure 4-2). It is unclear why this location had a higher occurrence of gonad fungi and further investigation would be needed on the nature of fungal spores in *C. xamachana* gonads. However, the Coco Plum location was a shallow lagoon at the end of a suburban street, and the entire lagoon was surrounded by mangrove trees. While a few mangrove trees were present several meters away from the Dock location, Coco Plum was the only site to have close association with mangrove trees. This is notable as *Kwoniella* spp. associate with the roots and sediment surrounding mangrove trees (Statzell-Tallman et al. 2008). It is notable that *Kwoniella* is the only mangrove-associated taxon we found in *C. xamachana.* Several taxa, including species in the genus *Acremonium,* are found on degrading mangrove leaves (Devadatha et al. 2021), but were noticeably absent in our samples. As *C. xamachana* polyps settle on the underside of degrading mangrove leaves (Medina et al. 2021), the animals would have close physical association with the fungi near the roots and leaves of mangrove trees. It is possible *Kwoniella* was transient in the jellyfish and does not persist in the host, but it is currently unclear along with why other mangrove-associated fungi were not cultured from our samples. Other sampling techniques or culture media may need to be used.

Several isolates were unique to jelly tissue or environmental samples (see Results), and they were not specific to location or tissue compartment (Supplemental Table 4-1). Many of these isolates did not cluster closely with described fungal species selected from the NCBI nucleotide database (Figures 4-4, 4-5). For these species, additional phylogenetic trees were run using the methods described in this study but using all published isolates in the NCBI Fungus ITS bioproject of that genus. The final selection of NCBI sequences included in the tree in Figure 4-3

are representatives of each genera for easier viewability of the tree. Sequences that were not closely associated with any isolate sequence (bootstrap < 50%) were excluded. For example, samples 29A and 30A/33A were identified as *Cladosporium*, but did not form a clade with any *Cladosporium* sequence published in the NCBI bioproject, so a reduced selection was included in Figure 3-4A. This is likely a product of the underrepresentation of marine fungus in online genetic databases. Additionally, only the ITS gene was used in this study, so multigene alignments were not performed that could have gotten down to the species level of identification.

The appearance of environmental and jellyfish-specific isolates in our samples suggest the potential of selection for fungi in the jellyfish. The *Cladosporium* and *Kwoniella* isolates described above for instance were not isolated from any environmental sample yet were found in host tissue. It is likely that fungal spores from these genera were present in the seawater, sediment, or neighboring marine organisms at one point in the jellyfish life cycle, and the fungi persisted within the jellyfish tissue. Likewise the appearance of environmental-specific isolates, such as the *Epicoccum* isolate from 76A (Harry Harris female mucus) which did not appear in any jellyfish tissue sample from the same location, shows that existence of fungi in the surrounding environment does not solely determine incorporation into host tissue. This could be driven by an antifungal microbiome, active selection by the host, or some competition within the endomycotal community.

This study records for the first time a comprehensive, geographically diverse, collection of fungal isolates from a benthic jellyfish. *C. xamachana* has a diverse internal fungal community, and fungi can persist in its tissue even in a laboratory setting. As research on marine fungi continues, sampling studies on marine organisms are necessary to explore the full fungal diversity of the oceans. Further research on *C. xamachana* fungal diversity using multiple agars or metabarcoding of fungal presence is needed to flush out the symbiotic community of this model organism.

References

Amend, Anthony, Gaetan Burgaud, Michael Cunliffe, Virginia P. Edgcomb, Cassandra L. Ettinger, M. H. Gutiérrez, Joseph Heitman, et al. “Fungi in the Marine Environment: Open Questions and Unsolved Problems.” mBio 10, no. 2 (March 5, 2019): 10.1128/mbio.01189-18. https://doi.org/10.1128/mbio.01189-18.

Baird, Andrew H., Irina M. Yakovleva, Saki Harii, Frederic Sinniger, and Michio Hidaka. “Environmental Constraints on the Mode of Symbiont Transmission in Corals.” Journal of Experimental Marine Biology and Ecology 538 (May 1, 2021): 151499. https://doi.org/10.1016/j.jembe.2020.151499.

Ben-Dor Cohen, Eyal, Micha Ilan, and Oded Yarden. “The Culturable Mycobiome of Mesophotic Agelas Oroides: Constituents and Changes Following Sponge Transplantation to Shallow Water.” Journal of Fungi 7, no. 7 (July 16, 2021): 567. https://doi.org/10.3390/jof7070567.

Cary, L. R. “The Influence of the Marginal Sense Organs on Metabolic Activity in Cassiopea Xamachana Bigelow.” Proceedings of the National Academy of Sciences of the United States of America 2, no. 12 (December 1916): 709. https://doi.org/10.1073/pnas.2.12.709.

De Domenico, Stefania, Gianluca De Rinaldis, Marta Mammone, Mar Bosch-Belmar, Stefano Piraino, and Antonella Leone. “The Zooxanthellate Jellyfish Holobiont Cassiopea Andromeda, a

Source of Soluble Bioactive Compounds." Marine Drugs 21, no. 5 (May 2023): 272. https://doi.org/10.3390/md21050272.

Devadatha, B., E. B. G. Jones, K. L. Pang, M. A. Abdel-Wahab, K. D. Hyde, J. Sakayaroj, A. H. Bahkali, et al. "Occurrence and Geographical Distribution of Mangrove Fungi." Fungal Diversity 106, no. 1 (January 1, 2021): 137–227. https://doi.org/10.1007/s13225-020-00468-0.

Durieux, David M., Gabrielle D. Scrogham, Christian Fender, David B. Lewis, Stephen M. Deban, and Brad J. Gemmell. "Benthic Jellyfish Act as Suction Pumps to Facilitate Release of Interstitial Porewater." Scientific Reports 13, no. 1 (March 7, 2023): 3770. https://doi.org/10.1038/s41598-023-30101-4.

Fields, S. C., L. Mireles-Lo, and B. C. Gerwick. "Hydroxycornexistin: A New Phytotoxin from Paecilomyces Variotii." Journal of Natural Products 59, no. 7 (January 1, 1996): 698–700. https://doi.org/10.1021/np960205e.

Haulena, Martin, Christy McKnight, and Frances M. D. Gulland. "IAAAM 2003." VIN.Com, May 10, 2003. https://www.vin.com/doc/?id=6693429.

Jantzen, Carin, Christian Wild, Mohammed Rasheed, Mohammed El-Zibdah, and Claudio Richter. "Enhanced Pore-Water Nutrient Fluxes by the Upside-down Jellyfish Cassiopea Sp. in a Red Sea Coral Reef." Marine Ecology Progress Series 411 (July 29, 2010): 117–25. https://doi.org/10.3354/meps08623.

Kavanagh, Kevin, ed. Fungi: Biology and Applications. Electronic resource. 2nd ed. Hoboken, NJ: John Wiley & Sons, 2011. http://SK8ES4MC2L.search.serialssolutions.com/?sid=sersol&SS_jc=TC0000536969&title=Fungi%20%3A%20biology%20and%20applications.

———, ed. Fungi: Biology and Applications. Electronic resource. 2nd ed. Hoboken, NJ: John Wiley & Sons, 2011. http://SK8ES4MC2L.search.serialssolutions.com/?sid=sersol&SS_jc=TC0000536969&title=Fungi%20%3A%20biology%20and%20applications.

Kerwin, Allison H., Samantha M. Gromek, Andrea M. Suria, Robert M. Samples, Dister J. Deoss, Kerry O'Donnell, Salvatore Frasca, et al. "Shielding the Next Generation: Symbiotic Bacteria from a Reproductive Organ Protect Bobtail Squid Eggs from Fungal Fouling." mBio 10, no. 5 (October 29, 2019): 10.1128/mbio.02376-19. https://doi.org/10.1128/mbio.02376-19.

Kohlmeyer, Jan, and Erika Kohlmeyer. Marine Mycology: The Higher Fungi. Elsevier, 2013.

Lawley, Jonathan W., Cheryl Lewis Ames, Bastian Bentlage, Angel Yanagihara, Roger Goodwill, Ehsan Kayal, Kikiana Hurwitz, and Allen G. Collins. "Box Jellyfish Alatina Alata Has a Circumtropical Distribution." The Biological Bulletin 231, no. 2 (October 2016): 152–69. https://doi.org/10.1086/690095.

Leone, Antonella, Raffaella Marina Lecci, Miriana Durante, Federica Meli, and Stefano Piraino. "The Bright Side of Gelatinous Blooms: Nutraceutical Value and Antioxidant Properties of Three Mediterranean Jellyfish (Scyphozoa)." Marine Drugs 13, no. 8 (August 2015): 4654–81. https://doi.org/10.3390/md13084654.

Li, Quanzi, and Guangyi Wang. “Diversity of Fungal Isolates from Three Hawaiian Marine Sponges.” Microbiological Research 164, no. 2 (January 1, 2009): 233–41. https://doi.org/10.1016/j.micres.2007.07.002.

Liu, Juan, Famei Li, Eun La Kim, Jian Lin Li, Jongki Hong, Kyung Sook Bae, Hae Young Chung, Hyung Sik Kim, and Jee H. Jung. “Antibacterial Polyketides from the Jellyfish-Derived Fungus Paecilomyces Variotii.” Journal of Natural Products 74, no. 8 (August 26, 2011): 1826–29. https://doi.org/10.1021/np200350b.

“Marine Fungi: Their Ecology and Molecular Diversity | Annual Reviews.” Accessed March 25, 2024. https://www.annualreviews.org/content/journals/10.1146/annurev-marine-120710-100802.

Medina, Mónica, Victoria Sharp, Aki Ohdera, Anthony Bellantuono, Justin Dalrymple, Edgar Gamero-Mora, Bailey Steinworth, et al. “The Upside-Down Jellyfish Cassiopea Xamachana as an Emerging Model System to Study Cnidarian–Algal Symbiosis.” In Handbook of Marine Model Organisms in Experimental Biology. CRC Press, 2021.

Oppong-Danquah, Ernest, Martina Miranda, Martina Blümel, and Deniz Tasdemir. “Bioactivity Profiling and Untargeted Metabolomics of Microbiota Associated with Mesopelagic Jellyfish Periphylla Periphylla.” Marine Drugs 21, no. 2 (February 17, 2023): 129. https://doi.org/10.3390/md21020129.

Paz, Z., M. Komon-Zelazowska, I. S. Druzhinina, M. M. Aveskamp, A. Shnaiderman, Y. Aluma, S. Carmeli, M. Ilan, and O. Yarden. “Diversity and Potential Antifungal Properties of Fungi

Associated with a Mediterranean Sponge." Fungal Diversity 42, no. 1 (May 1, 2010): 17–26. https://doi.org/10.1007/s13225-010-0020-x.

Raghukumar, Chandralata, and J. Ravindran. "Fungi and Their Role in Corals and Coral Reef Ecosystems." In Biology of Marine Fungi, edited by Chandralata Raghukumar, 89–113. Berlin, Heidelberg: Springer, 2012. https://doi.org/10.1007/978-3-642-23342-5_5.

Richards, Thomas A., Meredith D. M. Jones, Guy Leonard, and David Bass. "Marine Fungi: Their Ecology and Molecular Diversity." Annual Review of Marine Science 4, no. Volume 4, 2012 (January 15, 2012): 495–522. https://doi.org/10.1146/annurev-marine-120710-100802.

"Roadmap to Understanding Diversity and Function of Coral Reef-Associated Fungi | FEMS Microbiology Reviews | Oxford Academic." Accessed September 24, 2024. https://academic.oup.com/femsre/article/46/6/fuac028/6615459?login=false.

Soler-Hurtado, M. Mar, José Vladimir Sandoval-Sierra, Annie Machordom, and Javier Diéguez-Uribeondo. "Aspergillus Sydowii and Other Potential Fungal Pathogens in Gorgonian Octocorals of the Ecuadorian Pacific." PLOS ONE 11, no. 11 (November 30, 2016): e0165992. https://doi.org/10.1371/journal.pone.0165992.

Statzell-Tallman, Adele, Carmela Belloch, and Jack W. Fell. "Kwoniella Mangroviensis Gen. Nov., Sp. Nov. (Tremellales, Basidiomycota), a Teleomorphic Yeast from Mangrove Habitats in the Florida Everglades and Bahamas." FEMS Yeast Research 8, no. 1 (February 1, 2008): 103–13. https://doi.org/10.1111/j.1567-1364.2007.00314.x.

Thatoi, Hrudayanath, Bikash C. Behera, and Rashmi R. Mishra. “Ecological Role and Biotechnological Potential of Mangrove Fungi: A Review.” Mycology 4, no. 1 (March 1, 2013): 54–71. https://doi.org/10.1080/21501203.2013.785448.

Trischman, Jacqueline A., Dianne M. Tapiolas, Paul R. Jensen, Ryan Dwight, William Fenical, Tawnya C. McKee, Chris M. Ireland, Thomas J. Stout, and Jon Clardy. “Salinamides A and B: Anti-Inflammatory Depsipeptides from a Marine Streptomycete.” Journal of the American Chemical Society 116, no. 2 (January 1, 1994): 757–58. https://doi.org/10.1021/ja00081a042.

Wright, Anthony D., Claudia Osterhage, and Gabriele M. König. “Epicoccamide, a Novel Secondary Metabolite from a Jellyfish-Derived Culture of Epicoccum Purpurascens.” Organic & Biomolecular Chemistry 1, no. 3 (January 30, 2003): 507–10. https://doi.org/10.1039/B208588G.

Yarden, Oded. “Fungal Association with Sessile Marine Invertebrates.” Frontiers in Microbiology 5 (May 15, 2014). https://doi.org/10.3389/fmicb.2014.00228.

Yue, Yang, Huahua Yu, Rongfeng Li, Ronge Xing, Song Liu, and Pengcheng Li. “Exploring the Antibacterial and Antifungal Potential of Jellyfish-Associated Marine Fungi by Cultivation-Dependent Approaches.” PLOS ONE 10, no. 12 (December 4, 2015): e0144394. https://doi.org/10.1371/journal.pone.0144394.

Chapter 5

Conclusions and Future Directions

Cassiopea xamachana is a dynamic and robust model organism for a wide array of scientific inquiries. While studied for over a century, *C. xamachana* has rapidly popularized as a model organism in the last few decades (Medina et al. 2021). Researchers working on this organism frequently encounter the same questions on how to start working with *C. xamachana* and how to induce developmental symbiosis. While review papers have summarized the history of the model and its anatomy (see Medina, Sharp, et al. 2021), there has yet to be a comprehensive account of *C. xamachana* husbandry and its associated symbionts. The husbandry guide we created will work to establish the necessary baselines for sound data collection and healthy animals. Non-traditional model organisms experience fewer research regulations for proper handling, which results in few if none standardized descriptions of the organism and its life cycle (Ankeny and Lionelli 2020). Invertebrates in particular have few care standards set globally, and research conducted on animals with varying care parameters cannot be accurately compared. Environmental differences can affect development of organisms drastically (National Research Council 2011), as seen in the discussions in our husbandry guide. *C. xamachana* polyps raised exclusively in dark conditions can experience higher mortality (Chapter 1), but previous studies on aposymbiotic polyps have kept the animals in dark conditions (Fitt and Trench 1983). This impedes reproducibility in science. Consistent standards in animal care ensure research labs can accurately compare their data to other studies (National Research Council 2011). Also, the lack of standardization of animal husbandry can substantially block prospective researchers from incorporating a new model organism into their labs, which hampers scientific advancement. The work described in our husbandry guide summarizes years of tweaking care parameters to discover the best conditions for a healthy, reproductively active community of *C. xamachana* in the hopes of opening access to this unique system.

There are still unanswered questions in regards to *C. xamachana* ontogeny and care. For instance, natural fertilization of embryos has yet to be observed. While it is known keeping male

and female medusa in the same body of water will lead to fertilized embryos, the actual location of fertilization is unknown (Medina et al. 2021). In cases where researchers may want to cross-breed specific individuals, this uncertainty would make the process difficult. Also, it is theorized that light controls the timing of spawning as medusa kept in a 12:12 day/night cycle consistently produce fertilized embryos daily. However, the actual inducer of spawning has never been proven (Medina et al. 2021). Changing day/night cycles, including moonlight cycles in husbandry care, and changing other environmental parameters would systematically test what induces spawning once the location of fertilization has been determined. Also, changes in temperature can increase asexual reproduction in *C. xamachana* (Chapter 1), and it would be interesting to explore the role of temperature in spawning of these jellyfish.

Several other care parameters also need to be tested. For instance, light and circadian rhythms have been studied in adults but not polyps, where behavior is harder to measure (Nath et al. 2017). The responsiveness to stimuli like prodding or prey availability may be a metric for polyp responses and a way to measure their circadian rhythms. Work on this would supplement our observations from Chapter 1, where polyps kept in perpetual darkness experienced higher mortality compared to those kept in day/night conditions. It is possible exposure to perpetual darkness decreases metabolism function, which would influence how polyps grow and function. I have seen polyps kept in darkness are white in color while polyps kept on a day/night cycle develop a slight pink coloration. This shows a phenotypic change in polyps kept in darkness or light and suggests metabolic and genetic changes in the polyps as well. Transcriptomes or in situ hybridization could also be used to observe the activity of circadian rhythm-associated genes in polyps to supplement behavioral assays.

In Chapter 1, we tested nutritional supplements provided to *Artemia* nauplii to see if the additional nutrients would have a positive effect on polyp asexual reproduction. However, we only tested it on polyps, and the nutrient supplement blend was originally designed to feed to

adult moon jellies (Chapter 1). It is likely that the nutrient supplements would have a similarly positive effect on *C. xamachana* ephyra and medusa growth. This could be easily tested and would make a great project for undergraduate volunteers to work on.

In Chapter 2, we explored compatibility between a scyphozoan host and dinoflagellate symbionts. Photosymbiosis with Symbiodiniaceae is essential for the completion of *C. xamachana'*s life cycle and the establishment process of this symbiosis is both a question of *C. xamachana* husbandry and mechanisms of marine symbiosis. Previous research in the lab and the wild has shown polyps are able to acquire microalgal symbionts of various species (Mellas et al. 2014) yet predominantly associate with one species in the wild (Thornhill et al. 2006). The nature of this symbiont winnowing process remains unknown, but the results described in the third chapter of this thesis reveal potential selectivity. The lack of specificity in symbiont uptake as a polyp appears to confer some reproductive advantage to *C. xamachana*. Polyps associating with various symbionts from different Symbiodiniaceae were able to produce ephyra at different rates. Ephyra develop into sexually mature adults, and the ability to produce offspring quickly that can pass genetics along to a new generation is important for the survival of a species. The occurrence of wild medusa with only *S. microadriaticum* as its endosymbiont raises questions on the progression of symbiosis as ephyra turn into adults. It is possible that ephyra will begin to expel symbionts of different species as they mature, and preferentially uptake *S. microadriaticum*. Subsequent experiments would need to be performed. Ephyra colonized with a particular species could be placed in containers with an open exchange with the neighboring seawater containing symbionts of different species. Sequencing of the LSU gene for dinoflagellate identification would be performed along a timeline to map how symbiont composition within ephyra change when exposed to sources of other dinoflagellate species. Another interesting investigation would be on the origin of *S. microadriaticum* pools in a natural environment. Ephyra symbiotic with heterologous species could be left in dinoflagellate-free water, but with an adult *C. xamachana*

symbiotic with one or more species. Sequencing of symbiont composition of the ephyra could then be performed to explore if symbiont composition changes based on the availability of local symbiont pools. Other symbiotic cnidarians, such as *Exaiptasia* anemones associating with *Breviolum* symbionts, could be used as well to see if *C. xamachana* ephyra preferentially associate with symbionts expelled from their own host species. Bleached *Exaiptasia* has already been shown capable of establishing symbiosis with symbionts expelled from healthy *Exaiptasia* polyps. Symbiotic cnidarians naturally expel symbionts during their life, and this demonstrates that expelled dinoflagellates retain the ability to form symbiosis with a host (Sheahan et al. 2023). It would be a very interesting investigation to see if *C. xamachana* preferentially uptake expelled symbionts from other *C. xamachana* or if they can uptake symbionts expelled from foreign species. If they overwhelmingly uptake symbionts expelled from *C. xamachana*, this would suggest Symbiodiniaceae cell composition is somehow affected by forming symbiosis with a particular host.

As established in Chapter 1, small degree changes in temperature can affect strobilation rate, metabolism, and life span of *C. xamachana*. In our Chapter 2 data, we kept all polyps between 26-27°C. However, an experiment on *C. xamachana* symbiotic with different Symbiodiniaceae species with varying heat tolerance showed that *C. xamachana* is less likely to bleach when symbiotic with its homologous symbiont. Several heterologous symbionts in this experiment displayed higher heat tolerance in culture, suggesting a host-symbiont specificity that trumps adaptation against bleaching (Newkirk et al. 2020). It would be interesting to explore if the time to strobilation is affected by temperature of the culture conditions, or if this is also dependent on the symbiont specificity. Investigations into this would inform both future experimental parameters (e.g. what temperatures to keep polyps at when inducing strobilation) and the influence of environmental parameters on *C. xamachana* ontogeny. A major unanswered question in marine symbiosis research is what drives the establishment of symbiosis and the

specificity between host and symbiont (Davy et al. 2012). Further investigations into the extent this symbiosis is controlled by host, symbiont, or the environment would enlighten much of the process behind this phenotypic plasticity.

The full nature of the *C. xamachana* holobiont has yet to be characterized however, our precursor sampling of fungi within medusa tissue reveals a new community previously unknown. While other marine organisms have been known to associate with fungi (Yarden 2014), it has never been characterized from the internal environment of *C. xamachana*. This study established the presence of an endomycotal community, but it only tested one type of agar and antibiotic. It is likely other fungal species were present in our samples that either did not grow on the agar provided or were outcompeted by the other fungi growing. Subsequent experiments using other agar types and growth conditions would reveal hidden fungal diversity within this jelly. Importantly, we did not establish the role of fungus in *C. xamachana.* It is currently unclear if the fungi are commensals or symbionts. There are several ways to test if there are fungal symbionts within *C. xamachana* medusa. Lab-reared medusa with a low abundance of fungal presence could be used for this, or anti-fungal compounds could be used on medusa to remove the majority of fungi. The endomycotal community would confirmed either by barcoding sequencing or culturing. Once fungi are either reduced or removed, the health of individuals can be observed to see how the reduction of fungi in the jellyfish affects their growth and behavior. Also, several fungal isolates were recovered from multiple medusa across locations and may represent potential symbionts. These isolates, like several from *Aspergillus*, could be grown on petri dishes and manually inoculated in the medusa. Concurrently, marine fungal isolates could be grown that were not found in our samples, such as a genus of fungus found on mangrove roots, and inoculated to medusa. The comparison between *C. xamachana* response to a potentially symbiotic fungus and a heterologous one would explore the role of these fungi in the animal, and the jellyfish response to fungi exposure. The general response to fungi exposure is another interesting

question to explore. *C. xamachana* has never been reported to have symbiotic fungi or any kind, mutualistic or parasitic. Additionally, they do not have any reported diseases. Their fellow cnidarian coral however experience several fungal pathogens (Soler-Hurtado et al. 2016), and this presents an opportunity for cross-species comparisons on immune response to fungal pathogens. Octocorals can have the fungus *Aspergillus sydowii* as a commensal or a pathogen (Soler-Hurtado et al. 2016), and we found this same isolate in our samples. If fungi that can induce a disease response in coral do not induce the same response in *C. xamachana*, genotyping of RNA expression from these two organisms after exposure to inoculated *A. sydowii* culture may reveal mechanisms of disease tolerance.

As we saw different fungal composition in the various tissue compartments we sampled, the inoculations could occur in multiple places. Possibly the most interesting tissue compartment to inoculate is the gonads, which had overall low occurrence of fungal isolates and may have anti-fungal properties to prevent gonadal fouling. Gonads could be inoculated with a grown fungal culture and the medusa could be observed for developmental changes. It is possible to reach *C. xamachana* gonads through the subgenital pores with a pipette in a fairly non-invasive way (personal experience collecting gonad tissue), and this would provide limited stress to the animal that may affect their immune response. Gonad morphological changes can be observed, the production of gametes, and the production of fertilized embryos. Our work only explored fungi presence in medusa, and the presence of fungi in the other life stages of *C. xamachana* still needs to be studied. Once it is known if planula have internal fungi, the next question to be explored is if the fungi was passed down from the mother or the environment. If fungi are passed from mother to offspring, there would be evidence for the first time of vertically transmitted fungi in a marine organism. Fungal presence could be observed across the entire life cycle by keeping planula, polyps, ephyra, and then medusa in sterile seawater. If the fungal community remains consistent across all life stages, this would indicate fungi or their spores can be inherited. An

additional experiment could be done where fungi of known species are introduced to the water at set time points in ontogeny to see if *C. xamachana* will uptake artificially introduced fungi. There are many avenues to be explored in the *C. xamachana* endomycotal community, and our work has opened many future research directions.

In conclusion, our studies establish symbiont species diversity in *C. xamachana* and introduce techniques for optimal future experiments. By exploring symbiotic associations with *C. xamachana*, we create foundations for subsequent research on developmental symbiosis. These studies contribute to the broader context of marine symbiosis by deepening our understanding of marine symbiotic relationships in healthy animals.

References

Ankeny, Rachel A., and Sabina Leonelli. "Model Organisms." *Elements in the Philosophy of Biology*, November 2020. doi:10.1017/9781108593014.

Council, National Research, Division on Earth and Life Studies, Institute for Laboratory Animal Research, and Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. National Academies Press, 2010.

Davy, Simon K., Denis Allemand, and Virginia M. Weis. "Cell Biology of Cnidarian-Dinoflagellate Symbiosis." *Microbiology and Molecular Biology Reviews* 76, no. 2 (June 2012): 229–61. doi:10.1128/MMBR.05014-11.

Fitt, W. K., and R. K. Trench. "Endocytosis of the Symbiotic Dinoflagellate Symbiodinium Microadriaticum Freudenthal by Endodermal Cells of the Scyphistomae of Cassiopeia Xamachana and Resistance of the Algae to Host Digestion." *Journal of Cell Science* 64 (1983): 195–212.

Kavousi, Javid, Vianney Denis, Victoria Sharp, James Davis Reimer, Takashi Nakamura, and John Everett Parkinson. "Unique Combinations of Coral Host and Algal Symbiont Genotypes Reflect Intraspecific Variation in Heat Stress Responses among Colonies of the Reef-Building Coral, Montipora Digitata." *Marine Biology* 167, no. 2 (January 9, 2020): 23. doi:10.1007/s00227-019-3632-z.

Medina, Mónica, Victoria Sharp, Aki Ohdera, Anthony Bellantuono, Justin Dalrymple, Edgar Gamero-Mora, Bailey Steinworth, et al. “The Upside-Down Jellyfish Cassiopea Xamachana as an Emerging Model System to Study Cnidarian–Algal Symbiosis.” In *Handbook of Marine Model Organisms in Experimental Biology*. CRC Press, 2021.

Mellas, Rachel E., Shelby E. McIlroy, William K. Fitt, and Mary Alice Coffroth. “Variation in Symbiont Uptake in the Early Ontogeny of the Upside-down Jellyfish, Cassiopea Spp.” *Journal of Experimental Marine Biology and Ecology* 459 (2014): 38–44. doi:10.1016/j.jembe.2014.04.026.

Nath, Ravi D., Claire N. Bedbrook, Michael J. Abrams, Ty Basinger, Justin S. Bois, David A. Prober, Paul W. Sternberg, Viviana Gradinaru, and Lea Goentoro. “The Jellyfish Cassiopea Exhibits a Sleep-like State.” *Current Biology* 27, no. 19 (2017): 2984–90. doi:10.1016/j.cub.2017.08.014.

Newkirk, Casandra R., Thomas K. Frazer, Mark Q. Martindale, and Christine E. Schnitzler. “Adaptation to Bleaching: Are Thermotolerant Symbiodiniaceae Strains More Successful Than Other Strains Under Elevated Temperatures in a Model Symbiotic Cnidarian?” *Frontiers in Microbiology* 11 (2020): 822. doi:10.3389/fmicb.2020.00822.

Sheahan, Kathleen, Daniel Zarate, Bridget Chalifour, and Jingchun Li. “Intraspecific Transfer of Algal Symbionts Can Occur in Photosymbiotic Exaiptasia Sea Anemones.” *Symbiosis* 89, no. 1 (January 1, 2023): 139–48. doi:10.1007/s13199-022-00891-8.

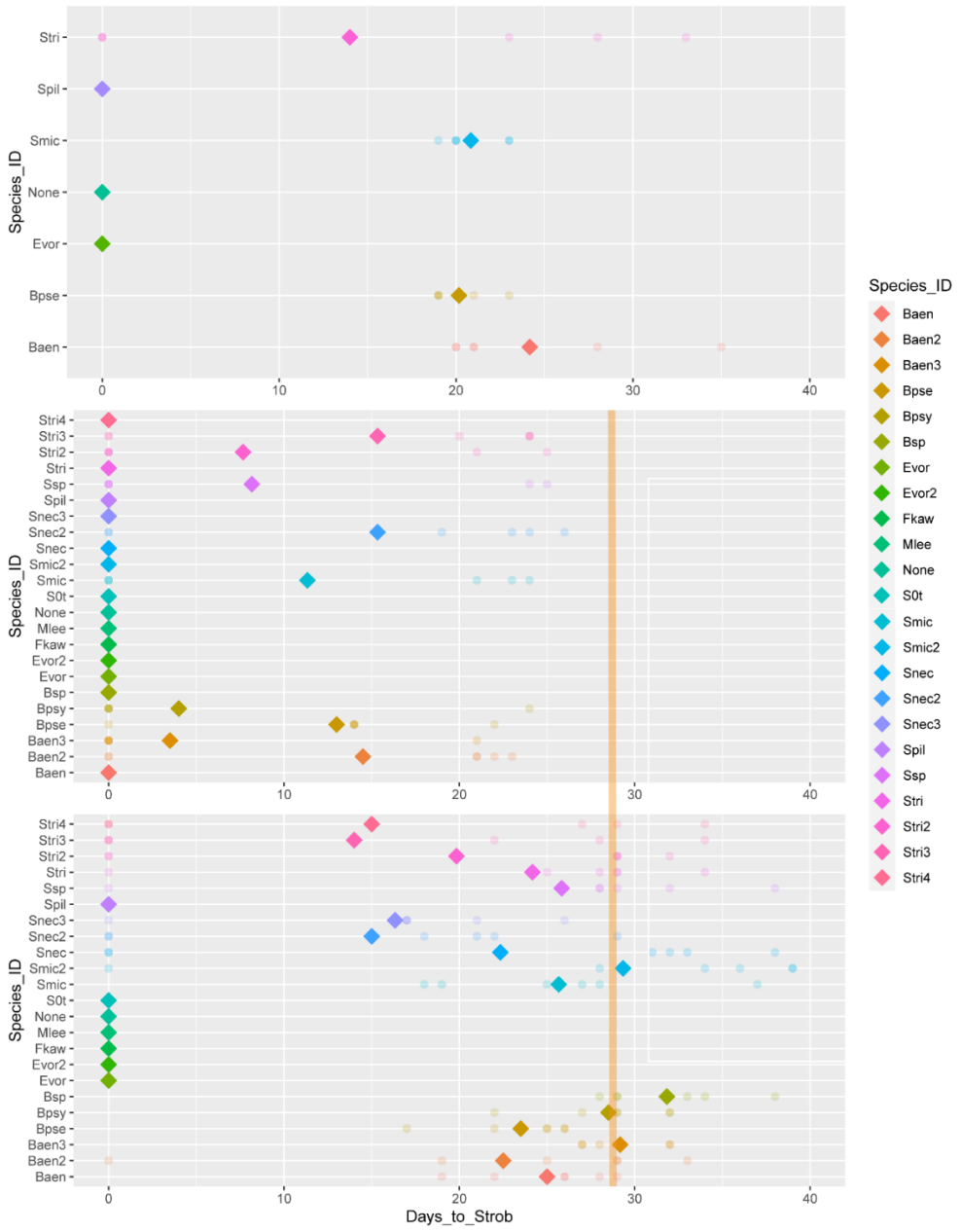
Soler-Hurtado, M. Mar, José Vladimir Sandoval-Sierra, Annie Machordom, and Javier Diéguez-Uribeondo. “Aspergillus Sydowii and Other Potential Fungal Pathogens in Gorgonian Octocorals of the Ecuadorian Pacific.” *PLOS ONE* 11, no. 11 (November 30, 2016): e0165992. doi:10.1371/journal.pone.0165992.

Thornhill, Daniel J., Michael W. Daniel, Todd C. LaJeunesse, Gregory W. Schmidt, and William K. Fitt. “Natural Infections of Aposymbiotic Cassiopea Xamachana Scyphistomae from Environmental Pools of Symbiodinium.” *Journal of Experimental Marine Biology and Ecology* 338, no. 1 (2006): 50–56.

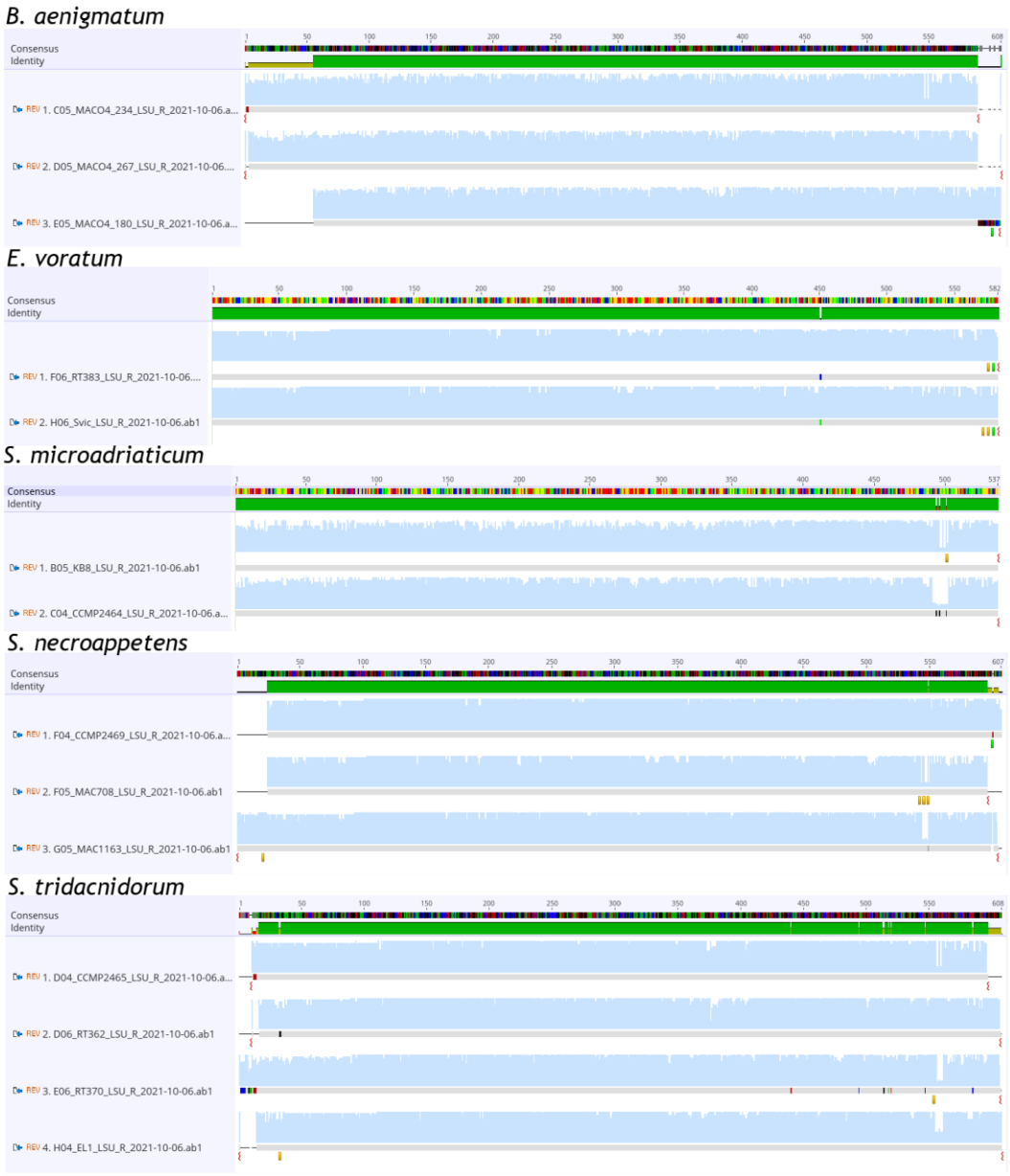
Yarden, Oded. “Fungal Association with Sessile Marine Invertebrates.” *Frontiers in Microbiology* 5 (May 15, 2014). doi:10.3389/fmicb.2014.00228.

Appendix A

Supplemental Material for Chapter 3



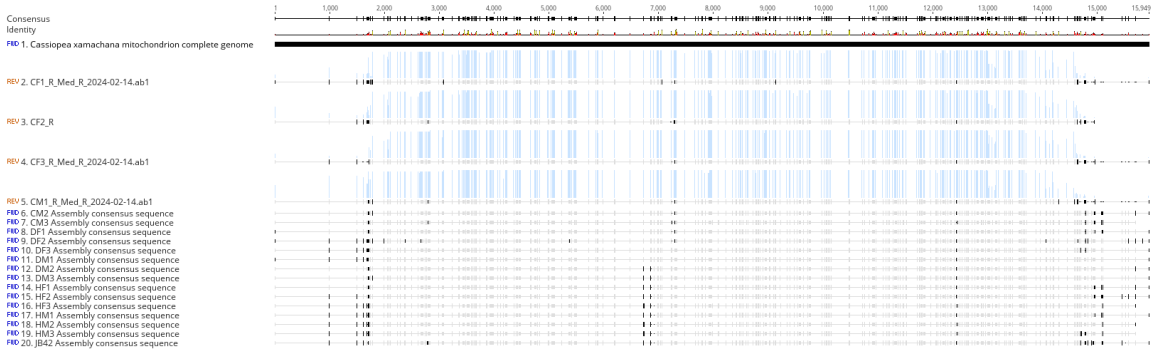
Supplemental Figure 3-1: Days to strobilation of each strain (y-axis) divided by trial 1 on top, trial 2 in the middle, and trial 3 on the bottom. Diamond points are the average number of days to strobilate for that strain within the trial. Square points represent individual polyps. The orange line indicates where trial 2 stopped, to illustrate how certain strains strobilated when given more time in trial 3.



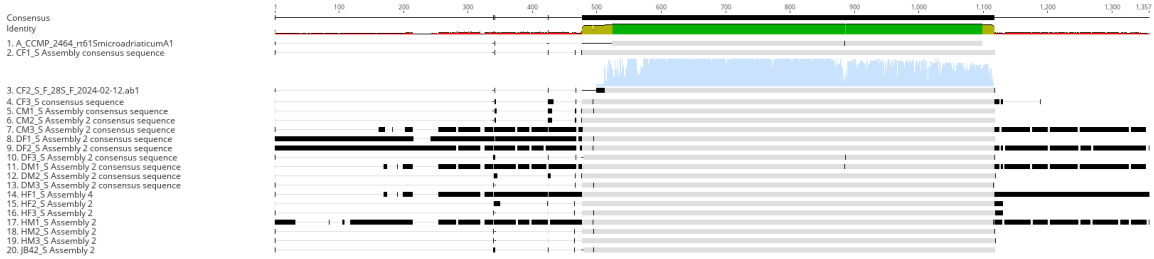
Supplemental Figure 3-2: LSU sequence alignments of the 5 species that had multiple strains. Alignments were done in Geneious.

Appendix B

Supplemental Material for Chapter 4



Supplemental Figure 4-1. Alignment of eukaryotic 16S region of all sampled jellyfish. Only one representative was taken from the lab-reared jellyfish for sequencing. Aligned against the closest match from the BLAST, NCBI Genbank sequence NC_016466.



Supplemental Figure 4-2. Alignment of the SSU gene of the Symbiodiniaceae symbionts of all sampled jellyfish. Only one representative was taken from Carnegie samples. Aligned against the closest match from the BLAST, the rt61 *Symbiodinium microadriaticum* sequence from LaJeunesse et al. 2018.

Supplemental Table 4-1: Sample metadata. Rows highlighted in yellow belong to Basidomycota, all others are Ascomycetes. The column “Unique” indicates if the sample was unique to either jellyfish tissue (JELLY) or environmental samples (ENV).

File	Sample	Location	Sex	Tissue	Species	Genus	Family	Class	Phylum	Unique
1	Coco Female 1 Mucus 1 (5/23)	Coco_Plum	Female	Mucus	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
2	Coco Male 3 Gonad 2	Coco_Plum	Male	Gonads	Penicillium sp. strain PP9	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	JELLY
3	Coco Seawater 2_3A	Coco_Plum	NA	Seawater	Cladosporium endophyticum	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	ENV
4	Coco Algae 1	Coco_Plum	NA	Algae	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
6	Coco Female 1 Mucus 4	Coco_Plum	Female	Mucus	Cladosporium endophyticum	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	ENV
7	Coco Female 2 Mucus 1	Coco_Plum	Female	Mucus	Fungal sp. isolate F_0038	Unknown	Unknown		Unknown	ENV
8	Coco Female 2 Mucus 2	Coco_Plum	Female	Mucus	Cladosporium tenuissimum	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	ENV
9	Coco Female 1 Mucus 2	Coco_Plum	Female	Mucus	Aspergillus westerdijkiae	Aspergillus	Trichocomaceae		Ascomycota	ENV
10	Coco Ascidians	Coco_Plum	NA	Ascidians	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
11	Coco Female 3 Mucus	Coco_Plum	Female	Mucus	Cytospora rhizophorae	Cytospora	Valsaceae		Ascomycota	
12	Coco Male 2 Bell	Coco_Plum	Male	Bell	Cytospora rhizophorae	Cytospora	Valsaceae		Ascomycota	
13	Coco Male 3 Bell	Coco_Plum	Male	Bell	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
14	Coco Male 1 Mucus 4	Coco_Plum	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
15	Coco Male 2 Mucus 2	Coco_Plum	Male	Mucus	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
16	Coco Female 1 Gonads 2	Coco_Plum	Female	Gonads	Cytospora rhizophorae	Cytospora	Valsaceae		Ascomycota	
17	Coco Male 3 Mucus 3	Coco_Plum	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
19	Coco Male 2 Gonads	Coco_Plum	Male	Gonads	Lecanicillium sp. isolate W3	Lecanicillium	Cordycipitaceae		Ascomycota	JELLY
20	Coco Seawater 1 (6/2)	Coco_Plum	NA	Seawater	Parasarocladium radiatum	Parasarocladium	Sarocladiaceae		Ascomycota	ENV
21	Coco Male 1 Gonads Culture A	Coco_Plum	Male	Gonads	Kwoniella mangrovensis	Kwoniella	Tremellales incertae sedis		Basidiomycota	JELLY
22	Coco Female 3 Bell	Coco_Plum	Female	Bell	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
23	Coco Male 3 Rhopalia 3	Coco_Plum	Male	Rhopalia	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
24	Coco Seawater 3?	Coco_Plum	NA	Seawater	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
25	Coco Male 1 Mucus 1	Coco_Plum	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
26	Coco Seawater 2_1 A	Coco_Plum	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
27	Coco Seawater 2_2 A	Coco_Plum	NA	Seawater	Cytospora rhizophorae	Cytospora	Valsaceae		Ascomycota	
28	Coco Male 3 Bell 2	Coco_Plum	Male	Bell	Lecanicillium sp. isolate W3	Cordycipitaceae	Cordycipitaceae		Ascomycota	JELLY
29	Coco Male 3 Rhopalia 2	Coco_Plum	Male	Rhopalia	Cladosporium sp. strain Kvan-sym	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	JELLY
30	Coco F1 Gonads A_2	Coco_Plum	Female	Gonads	Cladosporium xanthochromaticum	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	JELLY
31	Coco Male 2 Oral Arms A_2	Coco_Plum	Male	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
32	Coco Female 1 Bell	Coco_Plum	Female	Bell	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
33	Coco F1 Gonads A_1	Coco_Plum	Female	Gonads	Cladosporium xanthochromaticum	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	JELLY
35	Coco Algae 2_2	Coco_Plum	NA	Algae	Filobasidium uniguttulatum	Filobasidium	Filobasidiaceae		Basidiomycota	
36	Coco Seawater 2.2_3	Coco_Plum	NA	Seawater	Penicillium olsonii	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	ENV
37	Coco F1 Gonads 1_3	Coco_Plum	Female	Gonads	Filobasidium uniguttulatum	Filobasidium	Filobasidiaceae		Basidiomycota	
38	Coco F1 Mucus 3 _1	Coco_Plum	Female	Mucus	Uncultured fungus clone 4248_32	Unknown	Unknown		Unknown	ENV
39	Coco F1 Mucus 3_4	Coco_Plum	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
40	Coco Seawater 2.2_1	Coco_Plum	NA	Seawater	Penicillium olsonii	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	ENV
41	Coco Seawater 2.2_2	Coco_Plum	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
42	Coco Male 3 Mucus 2.1	Coco_Plum	Male	Mucus	Cytospora sp. HKA4	Cytospora	Valsaceae		Ascomycota	ENV
43	Coco Male 3 Mucus 2.2	Coco_Plum	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
44	Coco Male 3 Oral Arms	Coco_Plum	Male	Oral_Arms	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
45	Coco Male 3 Mucus 1	Coco_Plum	Male	Mucus	Cutaneotrichosporon curvatum	Cutaneotrichosporon	Trichosporonaceae		Basidiomycota	ENV
46	Coco Male 1 Mucus 3	Coco_Plum	Male	Mucus	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
48	Coco Female 1 Mucus 1 (6/2)	Coco_Plum	Female	Mucus	Aspergillus westerdijkiae	Aspergillus	Trichocomaceae		Ascomycota	ENV
49	Coco Male 1 Mucus 2	Coco_Plum	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
50	Harry Female 3 Mucus	Harry_Harris	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
51	Harry Algae	Harry_Harris	NA	Algae	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
52	Harry Male 1 Oral Arms	Harry_Harris	Male	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
53	Harry Male 2 Mucus 2	Harry_Harris	Male	Mucus	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
54	Harry Male 2 Oral Arms 2	Harry_Harris	Male	Oral_Arms	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
55	Harry Male 3 Mucus 2	Harry_Harris	Male	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
56	Harry Female 2 Oral Arms 1	Harry_Harris	Female	Oral_Arms	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
57	Harry Male 2 Rhopalia	Harry_Harris	Male	Rhopalia	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
58	Harry Seawater 1_2 or 3?	Harry_Harris	NA	Seawater	Aspergillus stellatus	Aspergillus	Trichocomaceae		Ascomycota	ENV
59	Harry Seawater 2_3	Harry_Harris	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
60	Harry Female 3 Bell	Harry_Harris	Female	Bell	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
62	Harry Female 1 Bell	Harry_Harris	Female	Bell	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
64	Harry Male 2 Oral Arms 1	Harry_Harris	Male	Oral_Arms	Aspergillus sydowii	Aspergillus	Trichocomaceae		Ascomycota	JELLY

65	Harry Female 1 Mucus 2 6/2/23	Harry_Harris	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
66	Harry Seawater 1	Harry_Harris	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
68	Harry Seawater 2_1	Harry_Harris	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
69	Harry Male 3 Mucus 1	Harry_Harris	Male	Mucus	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
70	Harry Seawater 2_2	Harry_Harris	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
72	Harry Female 2 Mucus 1	Harry_Harris	Female	Mucus	Aspergillus sp. isolate AUMS56	Aspergillus	Trichocomaceae		Ascomycota	ENV
73	Harry Male 2 Gonads 2	Harry_Harris	Male	Gonads	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
74	Harry Male 2 Gonads 3	Harry_Harris	Male	Gonads	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
75	Harry Male 2 Gonads 1	Harry_Harris	Male	Gonads	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
76	Harry Female 1 Mucus 1	Harry_Harris	Female	Mucus	Epicoccum nigrum	Epicoccum	Didymellaceae		Ascomycota	ENV
77	Harry Male 2 Bell	Harry_Harris	Male	Bell	Aspergillus unguis	Aspergillus	Trichocomaceae		Ascomycota	
78	Harry Female 2 Mucus 2	Harry_Harris	Female	Mucus	Penicillium rubens strain DTO269C2	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	ENV
79	Harry Female 1 Mucus 2 5/23/23	Harry_Harris	Female	Mucus	Fuscolachnum sp. voucher SBRH927	Fuscolachnum	Hyaloscyphaceae		Ascomycota	ENV
80	Dock Male 3 Oral Arms 2_5	Dock	Male	Oral_Arms	Aspergillus luchuensis	Aspergillus	Trichocomaceae		Ascomycota	JELLY
81	Dock Female 3 Oral Arms 1	Dock	Female	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
82	Dock Female 1 Mucus 1	Dock	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
83	JB4 seawater 1	Carnegie	NA	Seawater	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
84	JB4 seawater 2	Carnegie	NA	Seawater	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
85	JB4 seawater 3	Carnegie	NA	Seawater	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
86	JB4 1 Mucus 1	Carnegie	Male	Mucus	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
88	JB4 1 Mucus 3	Carnegie	Male	Mucus	Cladosporium parahalotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	ENV
91	Dock M3 Gonads 2	Dock	Male	Gonads	Filobasidium uniguttulatum	Filobasidium	Filobasidiaceae		Basidiomycota	
92	JB4 2 Mucus B	Carnegie	Male	Mucus	Epicoccum sp. strain csbf018	Epicoccum	Didymellaceae		Ascomycota	ENV
93	JB4 2 Mucus A	Carnegie	Male	Mucus	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
95	JB3 1 Bell	Carnegie	Female	Bell	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
96	JB3 1 Oral Arms	Carnegie	Female	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
99	JB3 3 Oral Arms 2	Carnegie	Female	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
108	Dock Female 2 Mucus 2	Dock	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
109	Dock Female 1 Bell	Dock	Female	Bell	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
110	Dock Male 3 Gonads 1	Dock	Male	Gonads	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Unknown	
111	Dock Female 3 Bell	Dock	Female	Bell	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
113	Dock Female 4 Oral Arms	Dock	Female	Oral_Arms	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
117	Dock Male 2 Rhopalia	Dock	Male	Rhopalia	Cladosporium halotolerans	Cladosporium	Cladosporiaceae	Dothideomycetes	Ascomycota	
122	Dock Male 1 Mucus 1	Dock	Male	Mucus	Candida albicans	Candida	Saccharomycetales incertae sedis		Ascomycota	ENV
124	Dock Male 3 Oral Arms 2_3	Dock	Male	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
125	Dock Male 3 Oral Arms 2_4	Dock	Male	Oral_Arms	Uncultured fungus clone S44T_69	Unknown	Unknown		Unknown	JELLY
126	Dock Male 3 Oral Arms 2_1	Dock	Male	Oral_Arms	Aspergillus costaricaensis	Aspergillus	Trichocomaceae		Ascomycota	JELLY
127	Dock Female 4 Bell	Dock	Female	Bell	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
128	Dock Male 3 Oral Arms 2_2	Dock	Male	Oral_Arms	Aspergillus stellifer	Aspergillus	Trichocomaceae		Ascomycota	JELLY
129	Dock Male 3 Oral Arms	Dock	Male	Oral_Arms	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
130	Dock Male 3 Bell	Dock	Male	Bell	Hortaea werneckii	Hortaea	Teratosphaeriaceae		Ascomycota	
135	JB4 Oral Arms 2_1	Carnegie	Male	Oral_Arms	Epicoccum italicum	Epicoccum	Didymellaceae		Ascomycota	JELLY
136	Dock Female 1 Gonads	Dock	Female	Gonads	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
137	Dock Seawater 2	Dock	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
138	Dock Seawater 1	Dock	NA	Seawater	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
139	Dock Female 1 Mucus 2	Dock	Female	Mucus	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	
140	Dock Male 3 Rhopalia	Dock	Male	Rhopalia	Penicillium citrinum	Penicillium	Trichocomaceae	Eurotiomycetes	Ascomycota	

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Publications

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Mammone, M. and Sharp, V., Hewitt, M., Medina, M. The influence of photosymbiosis in Cassiopea xamachana regenerative success. Symbiosis 90, 1 (2023). DOI: 10.1007/s13199-023-00920-0

Medina, M., Sharp, V., Ohdera, A., Bellantuono, A., Dalrymple, J., Gamero-Mora, E., Steinworth, B., Hofmann, D.K., Martindale, M.Q., Morandini, A.C., Degennaro, M., and Fitt, W.K., "The Upside-Down Jellyfish Cassiopea xamachana as an Emerging Model System to Study Cnidarian–Algal Symbiosis", in Handbook of Marine Model Organisms in Experimental Biology ed. Agnès Boutet and Bernd Schierwater (Boca Raton: CRC Press, 14 Dec 2021), Routledge Handbooks Online.