

4th International Cassiopea Workshop May 20-23, 2021

The Fourth International *Cassiopea* Workshop will be held online across four sessions, from 10:00 AM to 3:00 PM Eastern Standard Time (USA).

This year, the workshop will be held virtually, but similar to previous workshops, our goal is to grow our community, generate collaborations, and encourage sharing of knowledge and ideas. In order to offer an experience beyond Zoom presentations, we are planning virtual 'mini-workshops' centered around tutorials on topics such as anatomical identification of *Cassiopea*, spawning/ husbandry, embryo injections, computational fluid dynamics, etc. We will also organize a virtual space for attendees to converse with others, participate in trivia, and make new colleagues. This meeting is for those working on *Cassiopea* and those interested in working in *Cassiopea*! Past attendees have diverse research backgrounds and questions, but we all come away with much to gain.

Social hours will be held over Gather Town (gather.town). Gather.town is a virtual networking service which allows attendees to move their avatar from room to room, in and out of conversations, and interact with colleagues in a virtual space. You do not need an account to participate, and a demo is available on the website if you would like to explore before the workshop. Links to access the workshop gather.town space will be released to attendees during the week of the workshop.

Thursday, May 20

<i>EST (USA)</i>	<i>Title</i>	<i>Presenter</i>
10:00	Welcoming Remarks	Mónica Medina <i>(Pennsylvania State University)</i>
10:15	History of <i>Cassiopea</i> (Dedication to Hugo Freudenthal)	Dietrich Hoffman William Fitt <i>University of Georgia</i>
10:30	<i>Cassiopea sp.</i> Life Cycle	Dietrich Hoffman William Fitt <i>University of Georgia</i>
10:45	Tutorial: Spawning and Husbandry of <i>Cassiopea</i>	Aki Ohdera <i>Caltech</i> Victoria Sharp <i>Pennsylvania State University</i>
11:30	<i>BREAK</i>	
12:00	Tutorial: JGI Genome Browser	Sajeet Haridas <i>Joint Genome Institute</i>
12:45	<i>BREAK</i>	
13:00	Tutorial: Embryo Micro-injections	<i>DeGennaro Lab</i> <i>Florida International University</i>
14:00	<i>SOCIAL</i> (gather.town)	

Friday, May 21

<i>EST (USA)</i>	<i>Title</i>	<i>Presenter</i>
10:00	Ramifications of viewing symbiosis as a loop of context dependent states	Tamar Goulet <i>U. of Mississippi</i>
10:15	Conserved symbiotic mechanisms in invertebrate-algal symbioses	Angus Thies <i>Scripps Inst. of Oceanography</i>
10:30	Amoebocytes facilitate efficient nutrient transfer in <i>Cassiopea</i>	Niclas Lyndby <i>Swiss Federal Inst. for Technology in Lausanne</i>
10:45	Quo vadis, Symbiodiniaceae miRNA evolution?	Viridiana Avila-Magaña <i>U. of Colorado Boulder</i>
11:00	BREAK	
11:15	The relationship between diverse Symbiodiniaceae species, strobilation, and resulting host phenotypes in <i>Cassiopea xamachana</i>	Victoria Sharp <i>Pennsylvania. St. U.</i>
11:30	A deep dive into <i>Cassiopea</i> sleep: complementary behavioral, RNAseq, and pharmacology experiments implicate homeostasis as a key function of sleep	Michael Abrams <i>UC Berkeley</i>
11:45	Behavioral differentiation among <i>Mastigias papua</i> subpopulations in an isolated marine lake (Lightning Talk)	Karly Higgins-Poling <i>UC Merced</i>
11:53	TBA (Lightning Talk)	
12:00	Feeding Behavior of <i>Cassiopea xamachana</i>	Kendra Pfeil <i>Pennsylvania. St. U.</i>
12:15	<i>Cassiopea</i> care and culture at the Maritime Aquarium	Rachel Stein <i>Maritime Aquarium</i>
12:30	BREAK	
13:15	Plenary Talk: The fluid dynamics of <i>Cassiopea</i> sp.	Laura Miller <i>U. of Arizona</i>
13:30	BREAK	
14:00	Hydrodynamic studies of <i>Cassiopea</i> feeding and exchange currents	Arvind Santhanakrishnan <i>Oklahoma St. U.</i>
14:15	Hopscotching Jellyfish: combining different duty cycles can lead to enhanced swimming performance	Nicholas Battista <i>College of New Jersey</i>
14:30	Exploring the Benthic Fluid Dynamics of <i>Cassiopea</i> with 3D Computational Models	Alexander Hoover <i>U. of Akron</i>
14:45	Introducing Planktos: an agent-based modeling framework for small organisms in fluid flow	Christopher Strickland <i>U. of Tennessee, Knoxville</i>
15:00	SOCIAL (gather.town)	

Saturday, May 22

<i>EST (USA)</i>	<i>Title</i>	<i>Presenter</i>
10:00	Using <i>Cassiopea cf. xamachana</i> as a model to study the effects of temperature and pH on symbiotic interactions	Gloria Lorena Velazquez Mejia <i>Universidad del Mar</i>
10:15	In vivo evaluation of bioenergetic parameters in heat-stressed <i>Cassiopea</i>	Edmée Royen <i>U. of Liège</i>
10:30	Influence of Salinity, Temperature, and Light Intensity on the Rate of Strobilation of <i>Cassiopea xamachana</i>	Marcela Prado-Zapata, Raian Counsman, Caroline Link <i>New College of Florida</i>
10:45	Mitochondrial dysfunction and reductions in symbiont colonization occur in tandem during thermal stress in <i>Cassiopea xamachana</i>	Bradford Dimos <i>U. of Texas at Arlington</i>
11:00	BREAK	
11:15	Symbiont Genotype Influences Host Response to Temperature in a Model Cnidarian-Dinoflagellate Mutualism	Jennica Moffat <i>California St. U., Northridge</i>
11:30	Untangling genetic tentacles: Characterizing population diversity in a model jellyfish	Megan Maloney <i>Auburn U.</i>
11:45	Symbioses in 3D: diversity and dynamics in pelagic photosymbioses	Michael Dawson <i>UC Merced</i>
12:00	A chemist in the ocean - exploration of the microbial community and chemistry of <i>Cassiopea</i>	Sandra Loesgen <i>U. of Florida</i>
12:15	The bacterial community of <i>Cassiopea xamachana</i>	Natalia Carabantes <i>Universidad Nacional Autónoma de México</i>
12:30	BREAK	
13:00	Plenary Talk: Adaptation to the environment by endosymbiosis - a model systems' approach	Annika Guse <i>Heidelberg U.</i>
13:30	BREAK	
14:00	High photosynthetic plasticity may reinforce invasiveness of upside-down zooxanthellate jellyfish in Mediterranean coastal waters	Marta Mammone <i>Università del Salento</i>
14:15	Upside-down jellyfish (<i>Cassiopea</i>) as a model organism for biomonitoring environmental variability	Madeline McKenzie <i>James Cook U.</i>
14:30	<i>Cassiopea</i> as a biomonitoring tool	Shelley Templeman <i>TropWATER, James Cook U.</i>
14:45	Upside-down down under: identification, distribution, and population dynamics of the invasive <i>Cassiopea</i> jellyfish in Lake Macquarie, Australia	Claire Rowe <i>U. of Sydney</i>
15:00	SOCIAL (gather.town)	

Sunday, May 23

<i>EST (USA)</i>	<i>Title</i>	<i>Presenter</i>
10:00	Tutorial: A quick tutorial on numerically simulating mathematical models relevant to Cassiopea	Laura Miller University of Arizona Christina Hamelt Bucknell University
11:00	<i>BREAK</i>	
11:30	Tutorial: Symbiodinium Culturing	Todd LaJeunesse <i>Pennsylvania State University</i> William Fitt <i>University of Georgia</i>
12:00	<i>BREAK</i>	
12:30	Tutorial: <i>Cassiopea</i> Morphology	Andre Morandini <i>University of São Paulo</i> Edgar Gamero <i>University of São Paulo</i>
13:30	Winner of best talk	Mónica Medina <i>Pennsylvania State University</i>
13:45	Closing Remarks	Mónica Medina <i>Pennsylvania State University</i>
14:00	<i>SOCIAL</i> (gather.town)	

Abstracts

Plenary

Adaptation to the environment by endosymbiosis - a model systems' approach

Annika Guse / Heidelberg University

Symbiotic interactions between organisms occur in all domains of life. A prime example is the symbiosis between corals and eukaryotic, photosynthetic dinoflagellates. Each generation, initially symbiont-free coral larvae take up dinoflagellates from the environment and a new, stable symbiotic interaction is established. Symbionts provide essential nutrients such as sugars, amino acids and lipids to their host powering the productivity of reefs ecosystems. This 'photo-symbiosis' is evolutionary ancient and considered a key adaptation to shallow, sunlit tropical oceans where food is scarce, and likely the main driver for coral diversification as well as onset of reef formation in the Triassic. Despite of its importance, key aspects about coral symbiosis establishment, maintenance, its evolution and ecosystem functions are still largely unknown. Here I will present our advances in developing *Aiptasia*, a marine sea anemone, as a tractable model to dissect fundamental aspects of symbiosis establishment at the mechanistic level. I will summarize our currently available resources and experimental toolkit for *Aiptasia*. Based on our findings combining *Aiptasia* work at the bench and comparative work with corals collected in the field, I will give an overview over our current understanding of the mechanisms underlying symbiont uptake via phagocytosis, how the symbionts escape the hosts' defensive strategies to persist intracellularly and conversely, how hosts prevent invasion by non-symbiotic organisms, how symbionts integrate into host cell metabolism and transfer nutrients such as sterols to the host. We provide fundamental insight into how two very distinct cells, an animal host and a dinoflagellate symbiont cell, coordinate their cellular functions to adapt to nutrient-poor environments and drive the productivity and biodiversity of the ecosystem.

Presentations

1. Ramifications of viewing symbiosis as a loop of context dependent states

Tamar Goulet / University of Mississippi

In 1878, de Bary defined symbiosis as the "living together of differently named organisms." de Bary's definition of symbiosis described a phenomenon, not its ramifications. Subsequently, attributes were assigned to symbioses based on benefits and costs, from mutualism (both partners benefit), commensalism (one partner benefits while the other is neither harmed nor gains a benefit), parasitism (one partner benefits while the other is harmed), to the recently added amensalism (one partner is harmed while the other is neither harmed nor gains a benefit). Although the symbiotic states may appear as discrete boxes, or a sliding continuum, neither portrayal captures the complexities of symbioses, since the former assumes that a symbiosis is constrained into one category while the latter implies that a mutualism needs to transition into a commensalism or amensalism before it may morph into a parasitism, and vice versa. I posit that symbiotic states may oscillate from one to another based on the context. Changing environmental conditions, which often occur in conjunction with climate change, may be an impetus for such symbiotic shifts. Conversely, if the symbiotic partner(s) rely on a certain symbiotic state, environmental changes may lead to symbiosis breakdown and even partner death. Viewing symbiosis via a transitional loop lens opens the door to symbiosis comprehension not confined or constrained by a narrow prism.

2. Conserved symbiotic mechanisms in invertebrate-algal symbioses

Angus Thies / *Scripps Institution of Oceanography*

Despite having evolved independently across several phyla, symbiotic associations between invertebrate hosts and algal symbionts are characterized by a conserved set of morphological arrangements and physiological conditions. The best studied of these is the carbon concentrating mechanism (CCM) present in corals (cnidarians) and giant clams (mollusks). Here, we report evidence that the same CCM is present in symbiotic medusa-stage *Cassiopea*.

3. Amoebocytes facilitate efficient nutrient transfer in *Cassiopea*

Niclas Lyndby / Swiss Federal Institute for Technology in Lausanne

The upside-down jellyfish *Cassiopea* engages in symbiosis with photosynthetic microalgae that facilitate uptake and recycling of inorganic nutrients. By contrast to most other symbiotic cnidarians, algal endosymbionts in *Cassiopea* are not restricted to the gastroderm but are found in amoebocyte cells within the mesoglea. While symbiont-bearing amoebocytes are highly abundant, their role in nutrient uptake and cycling in *Cassiopea* remains unknown. By combining isotopic labelling experiments with correlated scanning electron microscopy, and Nano-scale secondary ion mass spectrometry (NanoSIMS) imaging, we quantified the anabolic assimilation of inorganic carbon and nitrogen at the subcellular level in juvenile *Cassiopea* medusae bell tissue. Amoebocytes were clustered near the sub-umbrella epidermis and facilitated efficient assimilation of inorganic nutrients. Photosynthetically fixed carbon was efficiently translocated between endosymbionts, amoebocytes and host epidermis at rates similar to or exceeding those observed in corals. Amoebocytes thus play a vital role for the assimilation and translocation of nutrients in *Cassiopea*, providing an interesting new model for studies of metabolic interactions in photosymbiotic marine organisms.

4. Quo vadis, Symbiodiniaceae miRNA evolution?

Viridiana Avila-Magaña / University of Colorado Boulder

5. The relationship between diverse Symbiodiniaceae species, strobilation, and resulting host phenotypes in *Cassiopea xamachana*

Victoria Sharp / Pennsylvania State University

6. A deep dive into *Cassiopea* sleep: complementary behavioral, RNAseq, and pharmacology experiments implicate homeostasis as a key function of sleep

Michael Abrams / University of California Berkeley

Cassiopea, like other cnidarians, does not have a centralized nervous system (CNS), and yet the animal can be behaviorally asleep. *Cassiopea* has radially spaced marginal ganglia (called rhopalia) that control muscle contractions and responses to stimuli. By tracking their pulsing activity over days and nights we have determined that each ganglion has a temporal propensity for pulse initiation, displaying long-term ganglia usage hierarchies that exchange day to day – quintessential homeostatic regulation. Excitingly, the temporally dominant ganglion dictates the behavioral state, transitioning the whole animal between wake and sleep – evidence of local control of sleep in a decentralized nervous system. We have also found that sleep deprivation (SD) can have a variable affect, but RNA sequencing and analysis of SD animals suggests that expression changes correlate with level of sleep disturbance. Finally, using pharmacological perturbations of cholinergic and GABAergic systems, we are beginning to reveal the mechanisms of ganglia regulation. *Cassiopea*, therefore, is well positioned to become a new model system to study the role of sleep in an animal without a CNS, to make progress towards deriving the most basic functions of sleep.

7. Behavioral differentiation among *Mastigias papua* subpopulations in an isolated marine lake

Karly Higgins-Poling / University of California Merced

Cassiopea xamachana is rapidly becoming a model for (jellyfish) symbioses, illuminating a variety of behaviors, symbiotic interactions and associated life histories. We are interested in exploring the utility and range of applicability of this model for other jellyfishes. Rapidly evolving populations of genetically, morphologically and behaviorally diverse *Mastigias papua* inhabit marine lakes in Palau. Like *C. xamachana*, these medusae normally contain endosymbiotic, photosynthetic zooxanthellae contributing to their common name: golden jellyfish. These animals have distinct horizontal migratory patterns adapted to each lake, but their interaction with the zooxanthellae is unknown. Unexpectedly, in 2016, ENSO decimated a mass occurrence of millions of *M. papua* in Jellyfish Lake, Palau. Upon population recovery we noticed a number of white medusae, apparently lacking zooxanthellae. This natural phenomenon provided the opportunity to assess the impacts of missing symbionts in medusae with a direct comparison between the white and golden phenotype. We swam transects and collected data on swimming direction, showing white medusae did not have significant migratory patterns in contrast to the largely documented horizontal migration of the golden medusae. This observational study is the first to suggest symbionts mediated behavioral differentiation within a single lake population in Palau.

8. Feeding Behavior of *Cassiopea xamachana*

Kendra Pfeil / Pennsylvania State University

The purpose of this experiment is to determine the effects of strobilation among *Cassiopea xamachana* polyps when fed during different variables. The variables consist of feeding polyps one, three, and seven days a week, during the day and during the night for 30 minutes. This experiment is important as it can lead to a feeding strategy that is beneficial for quicker strobilation periods in research laboratories.

9. *Cassiopea* care and culture at the Maritime Aquarium

Rachel Stein / The Maritime Aquarium

The Maritime Aquarium (TMA) at Norwalk, CT is considered a moderately-sized aquarium, with over 250,000 gallons of water and over 6,000 species of fish. Despite its moderate size, TMA has a very robust jelly program, with 9 different species in culture, 13 jelly display and holding tanks, and 3 culture racks ranging in size from 80 to 1,600 gallons. *Cassiopea* are an important display animal at TMA, and have been kept and cultured at the facility since since 2011. This talk will discuss the techniques used to keep, culture, and display *Cassiopea* at TMA.

10. Hydrodynamic studies of *Cassiopea* feeding and exchange currents

Arvind Santhanakrishnan / Oklahoma State University

Through periodic bell pulsations, epibenthic *Cassiopea* jellyfish drive jets into the water column for suspension feeding, excretion and exchange of nutrients and gases. Hydrodynamic interactions of medusan jets with ambient low-speed flow plays a critical role in prey capture and benthic nutrient fluxes. In addition, pore water release by bell pulsations can assist in nutrient cycling and benthic-pelagic coupling. However, the roles of the oral arm structure, bell diameter and pulsing frequency on currents generated by bell pulsations remain unclear. We use non-invasive imaging techniques to experimentally examine the following questions in medusae in laboratory aquaria: 1) how does interaction of *Cassiopea*-induced currents with a background flow impact suspension feeding?, 2) how does medusa size and pulsing frequency affect pore water release?, and 3) how does the oral arm structure assist in prey capture? We conducted particle image velocimetry (PIV) measurements of *Cassiopea* individuals of varying bell diameters in a recirculating flow tank under continuous background flow speeds from 0.5 cm/s to 2 cm/s. Interaction of the background flow with the branches of the oral arms results in

shedding small-scale vortices, resulting in a larger spatial region where suspended particles can be brought closer to medusa feeding structures. Low background flow of 0.5 cm/s decreased escape efficiency and increased feeding efficiency. Increasing background flow to 2 cm/s increased escape efficiency and decreased feeding efficiency. Planar laser-induced fluorescence (PLIF) and PIV measurements on individuals of varying bell diameters were used to examine pore water release. Near-field PLIF measurements showed that pore water was drawn through starting vortices generated near the bell margin. Smaller individuals pulsed frequently and relied on interaction of starting vortices from multiple pulsing cycles to mix the released pore water with ambient water. In contrast, larger medusae pulsed less frequently but generated wider jets that aided in pore water mixing. Three-dimensional particle tracking velocimetry (PTV) measurements showed that the oral arms are not necessary for maintaining a continuous upward jet above the medusa. However, the breakdown of the coherent starting vortex by the oral arms aids in slowing flow near the medusa and facilitates suspension feeding.

11. Hopscotching Jellyfish: combining different duty cycles can lead to enhanced swimming performance

Nicholas Battista / The College of New Jersey

Much effort has been placed into understanding jellyfish maneuverability and swimming performance by the computational fluid dynamics community. However, there is still much to be learned about the jet propulsive locomotive gait displayed by prolate jellyfish. Traditionally, computational models have assumed uniform duty cycle kinematics from cycle to cycle. However, we determined that possible enhancements in swimming performance were possible by shuffling different duty cycles together across multiple Reynolds numbers and contraction frequencies. Increases in speed and reductions in cost of transport were observed as high as 80% and 50%, respectively. Generally, the net effects were greater for cases involving lower contraction frequencies. Overall, robust duty cycle combinations were determined that led to enhanced or impeded performance.

12. Exploring the Benthic Fluid Dynamics of Cassiopea with 3D Computational Models

Alexander Hoover / The University of Akron

Cassiopea spp., or upside-down jellyfish, represent a prime example of a benthic medusae. Their behavior is characterized by forming carpets with other upside-down jellyfish that collectively act as nutrient pump in the shallow water biomes that they inhabit. In this talk we present a 3D computational model that describes the biomechanics of upside-down jellyfish and characterizes the flow patterns generated by their motion. With this model we examine approaches for modeling a *Cassiopea*'s oral arms and their influence on the resulting fluid dynamics.

13. Introducing Planktos: an agent-based modeling framework for small organisms in fluid flow

Christopher Strickland / University of Tennessee – Knoxville

In this talk, I will introduce a computational framework written in Python for modeling the collective motion of small organisms in 2D or 3D fluid flow. Features include the automated import of VTK data sets specifying time-varying fluid velocity fields, import of static mesh structures (vertices or stl format) which act as solid barriers to agents, an Ito stochastic differential equation solver along with the ability to specify more ad-hoc agent behavior, and plotting of results. Work is ongoing with plans to include a method for calculating the FTLE field which can operate on generalized, deterministic movement.

14. Using *Cassiopea cf. xamachana* as a model to study the effects of temperature and pH on symbiotic interactions

Gloria Lorena Velazquez Mejia / Universidad del Mar

The increasing of the temperature and acidification in the ocean as a result of anthropogenic activities generate negative effects on the function and structure of marine ecosystems, among the most affected are corals reef which are highly diverse and valuable ecosystems. Despite the importance, studying them in the laboratory presents difficulties, such as their slow growth, limits on acclimatization variables, and protected status. The upside-down jellyfish *Cassiopea xamachana* has emerged as a study model of corals, as it is easily cultured in laboratory, its life cycle is controlled, and the *Cassiopea* genus is highly dependent on its symbiosis with microalgae of the *Symbiodinium* genus as are the corals.

In this study, we evaluate how different combinations of temperature and pH affect the density of *Symbiodinium microadriaticum* (reinfection) in bleached polyps of *C. cf. xamachana*. Polyps were subject to high temperatures, menthol and saccharides to initiate algae expulsion and obtain bleached polyps. Bleaching was confirmed by optic and epifluorescence microscopy. Bleached polyps were subjected to different treatments (infected with fresh cells of *S. microadriaticum* isolated from an adult medusa), and controls (without *S. microadriaticum*); both with replicates and different combinations of temperature (29, 32 and 34 °C) and pH (8.20, 7.50 and 7.20) for 16 days. Polyps were collected at 2, 4, 8 and 16 days post treatments; reinfection was confirmed and imaged by optic and epifluorescence microscopy. Images were edited with ImageJ program to determine the *S. microadriaticum* density. The results for the bleaching induction showed that high temperatures were not effective to obtain bleached polyps; however it induced the strobilation. Menthol dose used was lethal for the polyps; while saccharides treatment was the most effective method to induce bleaching. In addition, the reinfection observed as density was higher in a neutral pH (8.20) and temperature (29 °C), these density decreases in an acid pH (7.20) and higher temperature (34 °C).

15. In vivo evaluation of bioenergetic parameters in heat-stressed *Cassiopea*

Edmee Royen / University of Liège

The symbiotic partnership between cnidarians and dinoflagellates from the family Symbiodiniaceae constitutes the basis of remarkably diverse ecosystems. This tight partnership between the cnidarian host and the intracellular photosymbionts rests upon metabolic exchanges, and the holobiont displays a complex energetic metabolism, involving respiration from both partners and photosynthesis from the symbionts. Despite the major importance of these two critical processes, their interplay and regulations remain poorly understood. To address this topic, our first approach consisted in probing photosynthetic parameters in *Cassiopea*, an established model organism for photosynthetic jellyfish, subjected to a hyperthermic stress. Our preliminary results, obtained by concurrently carrying out chlorophyll a fluorescence and P700 absorbance measurements, suggest that the electron transport rate (ETR) through photosystem (PS) II is substantially less impacted by hyperthermic stress than ETR through PSI. This could suggest the occurrence of cyclic electron flow around PSI. Besides, the acceptor side limitation of PSI was also increased at elevated temperatures, potentially suggesting an inhibition of the Calvin-Benson cycle. All in all, these first results illustrate the benefits of using biophysical and spectroscopic techniques in the context of the mutualistic partnership between cnidarians and dinoflagellates, as well as its disruption. They will be performed thoroughly, along with investigation of the expression of essential respiratory and photosynthetic proteins.

16. Influence of Salinity, Temperature, and Light Intensity on the Rate of Strobilation of *Cassiopea xamachana*

Marcela Prado-Zapata, Raian Counsman, Caroline Link / New College of Florida

Cassiopea xamachana, commonly referred to as upside-down jellyfish, serve as extremely valuable model organisms when studying the mechanisms of establishment, maintenance, and stress tolerance

of marine symbioses (Ohdera et. al, 2018). In particular, *Cassiopea* is notable for their symbiotic relationship with photosynthetic dinoflagellates within the family Symbiodiniaceae and are most commonly used as a more easily cultured and managed alternative to stony corals (Rädecker et. al, 2017). The asexual reproduction of *Cassiopea* in the scyphistoma stage, known as strobilation, is particularly interesting because its initiation requires the suppression of host immune response, allowing for the colonization of Symbiodiniaceae (Hoffman et. al, 1996). Previous studies have found that Symbiodiniaceae growth and survival is influenced by environmental factors such as salinity, temperature, and light intensity, with different species varying in their tolerance to these external influences (Díaz-Almeyda et. al, 2017; Rogers and Davis, 2006). Notably, the rate of strobilation is dependent on the relative health of the Symbiodiniaceae endosymbionts (Hoffman et. al, 1996). While this symbiotic pair has been well studied within separate adult medusa stage *Cassiopea* (Hopf et. al, 2013) and isolated Symbiodiniaceae cultures (Klueter et. al, 2017), there is a lack of insight pertaining to the effects these external factors may have on the fitness of the early-stage symbiosis. This study aims to contribute to this gap by tracking the strobilation rate of *Cassiopea xamachana* scyphistoma infected with *Symbiodinium microadriaticum* (CassKB8) exposed to varying levels of salinity, temperature, and light exposure. No distinction in strobilation rate was observed between scyphistomae grown at varying light and salinity levels, however, increased temperature stimulated a faster strobilation process compared to control treatments. Furthering our understanding of external influences on the early stages of this model symbiosis will allow for future applications relating to selective colonization of endosymbionts in cnidarians. This in turn could be utilized in the study of environmental effects on symbiotic systems, and the understudied symbiont in these systems, Symbiodiniaceae.

17. Mitochondrial dysfunction and reductions in symbiont colonization occur in tandem during thermal stress in *Cassiopea xamachana*

Bradford Dimos / University of Texas at Arlington

Thermally induced breakdown in the symbiosis formed between symbiotic cnidarians and dinoflagellate algae in the family Symbiodiniaceae threatens the future of reefs worldwide. This process is thought to be initiated by photosynthetic dysfunction of the algal symbiont, however new research also implicates host mitochondria as potentially playing a role in the process of bleaching. However, the effect thermal stress has on host mitochondrial function and how this relates to symbiosis breakdown in Cnidarians, has not been investigated. By using the model organism *Cassiopea xamachana* we test the hypothesis that exposure to increased temperatures leads to damage to the host mitochondria and that these changes accompany bleaching. We show that exposure to temperature stress increases the demand placed on mitochondria leading to mitochondrial damage in the form of reduced membrane potential. These physiological changes co-occur with reductions in symbiont colonization, opening up new lines of investigation into the interaction between mitochondrial function and the breakdown of symbiosis.

18. Symbiont Genotype Influences Host Response to Temperature in a Model Cnidarian-Dinoflagellate Mutualism

Jennica Moffat / California State University, Northridge

Species in tight-knit mutualisms have disproportionately strong influences on each other as the fitness of each species involved is dependent on the performance of its partner, and can therefore have dynamic eco-evolutionary relationships, especially in the context of a rapidly changing environment. Many obligate mutualisms are composed of relatively small, fast-growing symbionts with a greater potential to evolve on ecologically-relevant time scales than their relatively large, slower-growing hosts. There is therefore great interest in the opportunity for selection on symbionts and the potential for evolutionary rescue of holobionts threatened by climate change. To explore this possibility, I used the

upside-down sea jelly *Cassiopea xamachana* and its microalgal endosymbiont, *Symbiodinium microadriaticum*, as a model cnidarian-dinoflagellate system to test for variation in traits among genotypes of the endosymbiont in response to thermal stress and if this variation affected host fitness in response to thermal stress. To investigate if there is sufficient variation in symbiont populations to respond to strong selection pressure, I measured the growth rate and physiological responses of five *S. microadriaticum* genotypes to three temperatures (26°C, 30°C, and 32°C) in culture and found that symbiont genotypes varied in their response to temperature for all measurements, suggesting sufficient opportunity for selection. I then introduced these genotypes to aposymbiotic *Cassiopea* polyps to determine if symbiont trait differences affected host fitness response to temperature. I found that the effect of temperature on developmental timing and asexual reproduction was dependent on the genotype of the symbiont that the polyp was hosting. This significant interaction between symbiont genotype and temperature demonstrates the influence of symbiont identity on holobiont fitness and its response to a changing environment, and a potential avenue for evolutionary rescue of the holobiont by the symbiont communities. This eco-evolutionary interaction may be a critical component of understanding species resilience in an increasingly stressful environment.

19. Untangling genetic tentacles: Characterizing population diversity in a model jellyfish

Megan Maloney / Auburn University

The upside-down jellyfish (*Cassiopea xamachana*) is an emerging model organism used to understand symbiotic relationships between cnidarian hosts and dinoflagellate algae. As sea surface temperatures increase, the geographical ranges of *C. xamachana* is expanding rapidly. However, our understanding of population structure, genetic variation, and the extent of clonality amongst *C. xamachana* populations remains limited. Because *C. xamachana* from the Florida Keys are often chosen for symbiosis research, it is imperative to identify potential host genetic factors that contribute to their phenotypes. Further, the morphological variation of *C. xamachana* is conspicuous but what drives this remains unknown. Therefore, we will attempt to find connections between morphological variation and genetics across this environmental range. The objective of this project is to characterize the populations structure of *C. xamachana* and understand capacity of this organisms to adapt to a changing climate. Adult medusa have been collected from Florida Bay and Atlantic sites across the Florida Keys. These results will provide the foundation for studies on the role of phenotypic plasticity in *C. xamachana* and their ability to thrive in a changing climate. Genetic data will enhance our understanding of variation across a relevant geographical range and help identify factors involved in their resilience to a changing climate.

20. Symbioses in 3D: diversity and dynamics in pelagic photosymbioses

Michael Dawson /University of California, Merced

The open ocean presents numerous physiological and biological challenges to which animals exhibit various adaptations. These adaptations have given pelagic fauna such as the jellyfishes powerful ecosystem roles as predators and prey, capable of altering ecosystem structures. For some of these animals, symbiosis also is a crucial adaptation to occupying this niche, with various biological implications including nutrition, communication, and development. However, much is still unknown about the role of symbiosis in the evolution, ecology, and biology of pelagic animals. As part of a project studying four major lineages of pelagic animals (pyrosomes, medusozoans, ctenophores, acoels) and their symbionts (proteobacteria, zooxanthellae, flagellates, green algae), we will sequence a comprehensive set of genomes for computational testing and hypotheses generation, testable across our various laboratory models (*Cassiopea*, *Symsagittifera*, etc.). We are considering also what happens when a pelagic symbiosis returns to the benthos, i.e. the case of *Cassiopea*. Our goal is to accelerate understanding of the eco-evolutionary assembly and disassembly of host-specific symbioses from a

diverse pool of pelagic microbes, specificity and plasticity to changing environments, and how symbioses influence life history and shape ecosystem dynamics (and vice versa).

21. A chemist in the ocean - exploration of the microbial community and chemistry of *Cassiopea*

Sandra Loesgen / University of Florida

Microbial chemistry plays key roles in marine environments. Inter/intra species signaling is of importance and chemical cues are involved in mating, defense, settlement, development of marine life, including *Cassiopea*. We are interested in the microbiome associated with *Cassiopea*, its chemical components and its function in the Cnidarian life cycle.

22. The bacterial community of *Cassiopea xamachana*

Natalia Carabantes / Universidad Nacional Autónoma de México

Cassiopea xamachana has been considered a model organism for studies in biological and ecological aspects of the symbiosis in coral reef systems. One important aspect of the study of this organism is the knowledge of its microbial community. The microbial community associated with cnidarian hosts has a major role in the protection and recycling of nutrients, contributing to their health. We present the first characterization of the bacterial community associated with the surface mucus layer of *Cassiopea xamachana* collected from Nichupté lagoon, México. We evaluated the effect of the loss of symbionts (experimental bleaching) on the bacterial composition of this layer and compared their diversity in summer and winter, through the analysis of 16S rRNA gene high-throughput sequences. The density of symbionts, was experimentally diminished using a mix of sugars. In summer, bacterial composition in symbiotic medusas was more diverse than in winter. The loss of symbionts reduced the bacterial diversity of medusas sampled in summer, but the bacterial diversity in winter was higher in bleached medusas. Bacterial community analysis suggested that season and symbiotic condition were both important in defining the bacterial composition of medusas. However, the LDA analysis showed that although genera were different between seasons, the dominant genera did not change with the season. The bacterial community of symbiotic medusas was dominated by the genus *Endozoicomonas*, while the genus *Vibrio* dominated under the bleached condition, consistent with other studies in corals. Our results suggest that symbionts have a major influence in structuring the bacterial community associated with the surface mucus layer of *C. xamachana*.

23. High photosynthetic plasticity may reinforce invasiveness of upside-down zooxanthellate jellyfish in Mediterranean coastal waters

Marta Mammone / Università del Salento

Ecological profiling of non-native species is essential to predict their dispersal and invasiveness potential across different areas of the world. *Cassiopea* is a monophyletic taxonomic group of scyphozoan mixotrophic jellyfish including *C. andromeda*, a recent colonizer of sheltered, shallow-water habitats of the Mediterranean Sea, such as harbors and other light-limited, eutrophic coastal habitats. To assess the ecophysiological plasticity of *Cassiopea* jellyfish and their potential to spread across the Mare Nostrum by secondary introductions, we investigated rapid photosynthetic responses of jellyfish to irradiance transitions - from reduced to increased irradiance conditions (as paradigm of transition from harbors to coastal, meso/oligotrophic habitats). Laboratory incubation experiments were carried out to compare oxygen fluxes and photobiological variables in *Cassiopea* sp. immature specimens pre-acclimated to low irradiance (PAR=200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and specimens rapidly exposed to higher irradiance levels (PAR=500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Comparable photosynthetic potential and high photosynthetic rates were measured at both irradiance values, as also shown by the rapid light curves. No significant differences were observed in terms of symbiont abundance between control and treated specimens. However, jellyfish kept at the low irradiance showed a higher content in chlorophyll a and c

($0.76 \pm 0.51SD$ vs $0.46 \pm 0.13SD$ mg g⁻¹ AFDW) and a higher C_i (amount of chlorophyll per cell) compared to jellyfish exposed to higher irradiance levels. The ratio between gross photosynthesis and respiration (P:R) was >1, indicating a significant input from the autotrophic metabolism. *Cassiopea* sp. specimens showed high photosynthetic performances, at both low and high irradiance, demonstrating high potential to adapt to sudden changes in light exposure. Such photosynthetic plasticity, combined with *Cassiopea* eurythermal tolerance and mixotrophic behavior, jointly suggest the upside-down jellyfish as a potentially successful invader in the scenario of a warming Mediterranean Sea.

24. Upside-down jellyfish (*Cassiopea*) as a model organism for biomonitoring environmental variability

Madeline McKenzie / James Cook University

Coastal marine systems are exposed to a diversity of contaminants, and for many, their influence on marine ecosystems is unknown. Historically, biomonitors have been used as effective tools to monitor the occurrence and level of contaminants in ecosystems. Sedentary organisms like molluscs are commonly used, however their utility to detect short-term pulse exposure events is low. Recent studies have highlighted the potential of the upside-down jellyfish, *Cassiopea*, as a biomonitor. This jellyfish possesses many of the key criteria of an ideal biomonitor, such as: 1) they accumulate contaminants in tissues, 2) are a sedentary species, 3) are tolerant to changes in water quality, 4) are tolerant to handling, and 5) are easily identifiable. *Cassiopea* have also been shown to accumulate contaminants over a period of days to weeks, and can excrete them within a similar time interval, highlighting their potential to detect short-term pulse events. Using laboratory and field experiments, *Cassiopea* will be exposed to contaminants singly, in combination, and in the presence of an environmental factor to assess their responses. This research will determine the effectiveness of *Cassiopea* as a biomonitor to contaminants found in coastal marine systems. Ultimately this will aid in the development of early detection and quantification tools to help assess risks and help to fill knowledge gaps on the effects of contaminants on marine organisms.

25. *Cassiopea* as a biomonitoring tool

Shelley Templeman / TropWATER, James Cook University

Cassiopea possess a range of characteristics that offer potential for in situ biomonitoring of xenobiotics in coastal marine systems. We will review some of these characteristics along with recent research that demonstrate their potential. Furthermore, we will discuss opportunities for further research in this area.

26. Upside-down down under: identification, distribution, and population dynamics of the invasive *Cassiopea* jellyfish in Lake Macquarie, Australia

Claire Rowe / University of Sydney

Cassiopea, the upside-down jellyfishes, have been reported as invasive on a global scale. In Australia, records have typically been confined to the tropics, but since 2013 there have been reports further south in the temperate eastern seaboard in three widely separated estuarine lakes. This study focuses on one of these populations in Lake Macquarie with the objective of providing baseline information to inform management decisions on the presence and impact of *Cassiopea* within the lake. My research has shown that *Cassiopea* are present in Lake Macquarie from February until August, peaking in May and disappearing in June-July. In order to test if this apparent disappearance could be attributed to reaching the lower thermal limit of the jellyfish, an aquarium-based study measured bell pulsation rate, changes in bell size, and routine metabolic rate in response to a regime equivalent to the natural decrease in temperatures in Lake Macquarie with change of season and onset of Winter. This was compared to predicted climate change temperatures; stable temperatures held at 20°C as found

immediately prior to Winter decrease; and increasing temperatures equivalent to those found in summer. Results show that with decreasing temperatures these jellyfish have decreased performance, including reduced bell size, pulsation and resting metabolic rate. This suggests that the disappearance of *Cassiopea* from Lake Macquarie in colder months is due to senescence in response to reduced winter temperatures, and that with increasing sea surface temperatures because of climate change, the jellyfish will increase their performance in Lake Macquarie possibly with a presence of the medusae throughout the year with potential for blooms in higher numbers.