Research

My research group, the Integrative Marine Genomics and Symbiosis (IMaGeS) laboratory, focuses on the study of the symbiotic interactions between microbial organisms and cnidarian hosts, in particular reef-building corals, from different perspectives, including cell biology, eco-physiology and molecular ecology to answer broad questions on symbiosis evolution. Reef building corals harbor diverse microbial communities integral to their metabolism and physiology that are important in nutrient cycling, pathogenicity, pathogen defense and environmental resilience. The interdependence of the coral host and its diverse microbiome has manifested the recognition of the coral holobiont, or the sum of all microbes and the coral host.
While an important component of our research is conducted on reef-building coral hosts, we have also developed a cnidarian model system as a proxy for studying corals in the laboratory, the sea anemone *Exaiptasia pallida*. Coral are very slow-growing organisms that in average extend their calcareous skeleton a few centimetres per year, and consequently poses a challenge to setup well replicated experiments that can be conducted in a reasonable timeframe to study microbiome manipulations within the host and to evaluate its response under different environmental settings. To overcome this limitation we are using successfully the sea anemone as a surrogate model to study corals. The important features considered in this selection were attributed to the fact that these anemones engage symbiotically with similar microorganisms as corals do and therefore make them good proxies to study coral biology and ecology. Furthermore, these anemones are fast growers and easy to culture in captivity in laboratory conditions. A great advantage of this system is that clonal populations of anemones can be raised from a dozen of individuals to populations of hundreds in a matter of a month in the lab. All these features make the anemones the appropriate and convenient laboratory mouse to study corals.
The following are five of the major research themes that we are currently pursuing in my laboratory:

1) Ecological and functional role of Symbiodiniaceae diversity within the context of the response to ocean warming (Funded by NSF-BIO OCE)
There are no doubts that coral reefs are among the most biologically diverse and economically important marine ecosystems on the planet, providing ecological services that are vital to human societies and industries through fisheries, coastal protection, pharmaceutical compounds, and tourism. It has been estimated that coral reefs provide goods and services worth about $375 billion each year. The high biodiversity and productivity of these ecosystems is, however, perplexing considering that coral reef organisms grow in nutrient-low waters within sub-tropical and tropical latitudes. Corals have thriven in these environments due to highly efficient nutrient cycling provided by their mutualistic relationship with photosynthetic dinoflagellates of the family Symbiodiniaceae, which live inside the hosting coral cells. In this coral-symbiont interaction, the corals provide their algal symbionts with shelter and nutrients utilized partially for photosynthesis, while the symbionts provide the coral with up to 95% of the fixed carbon they produce. However, coral reef ecosystems are currently undergoing deterioration because of a variety of natural and anthropogenic processes occurring both at a local (e.g. overfishing, nutrient loading and diseases) and global (e.g. global warming and ocean acidification) scale. Most of the coral mortality, and thus coral reef decline, has been attributed to coral bleaching, which is the massive loss of the intracellular Symbiodiniaceae population caused by abnormal increase of seawater temperature - a phenomenon linked to global warming, and exacerbated by high light intensities. This breakdown between coral and dinoflagellate symbiosis is detrimental as the reproductive capacity, growth, and disease resistance and/or survivorship of affected corals is extremely reduced.
So far the mechanisms linking patterns of bleaching and recovery is not well understood. We have learned that the Symbiodiniaceae family comprises many genetically diverse types? or species with different phenologies that are associated with a multitude of hosts, including Foraminifera, Mollusca, Porifera and Cnidaria. Therefore one important question that we are exploring in my laboratory focus on determine the influences of composition of the mutualistic Symbiodiniaceae species on coral bleaching likelihood and recovery using high resolution of next generation DNA sequencing approaches. The overall hypothesis is that corals and their symbionts/associates are building-block communities that can be rearranged in response to environmental change and that some combinations will foster resistance and/or rapid recovery while others will reflect slow recovery that might be followed by mortality. Over the last five years we have gathered a unique and extensive pre-thermal stress baseline of genotypic identity of coral hosts and Symbiodiniaceae species in Caribbean coral reefs, which has offered an unprecedented opportunity to analyze changes associated with natural recurrent bleaching events that have occurred over the last few years. Repeated sampling of the same coral colonies has allowed us to compare holobiont compositions before, during, and after bleaching (recovery).

2) Stability and resilience of coral microbiome in the face of ocean warming and bleaching disturbance (Funded by NASA and NSF-BIO OCE)
While Symbiodiniaceae has been the most well studied symbiont in coral symbioses, it is well known that the coral microbiome comprises species from bacteria, archaea, fungi, protista and viruses, which the sum of all give rise to what we refer as ‘the coral holobiont’. These associated microbial assemblages has been shown to be integral to the coral metabolism and physiology contributing in nutrient cycling, pathogen defense and environmental resilience.

Moreover, recent efforts from my group and colleagues from other research institutes using highly sensitive next-generation sequencing have discovered that the coral microbiome is far more diverse than previously thought. However, under current and future predicted environmental scenarios it remains practically unknown the effect the recurrent thermal disturbances will have on coral-associated microbial community structure and dynamics. It is clear that the understanding of the responses of the coral-associated microbial symbionts to thermal stress is of paramount importance in the face of intensifying threats on the world’s coral reefs.

In my lab we are particularly interested to understand the resistance and recovery capacity of coral-associated bacterial communities in response to anomalous ocean warming events (i.e. ecological resilience). For this we study the changes of the composition, structure and functionality the bacterial communities under simulated and natural experiments of thermal disturbances. Significant questions within this context include: 1) How resilient are coral-associated bacterial communities to bleaching episodes? 2) Are these microbiomes resistant to new species invasion during the recovery period? 3) Do spatially different microbiome assemblages respond similarly following recurrent bleaching events? 4) Is the trajectory response different as function of the community baseline state? And 5) How is the response different from single bleaching events from annually consecutive events?
Within the context of acclimatization and adaptive response of corals to thermal stress, we are also studying the influence of the microbial community associated with corals in the acclimatization process (thermal tolerance) to elevated seawater temperatures linked to global warming. We are focusing to address the following questions: 1) How do changes in microbial community composition and structure from acclimatized corals compare with corals that are thermally injured and undergo coral bleaching? 2) Is there a functional metabolic shift of the microbial community during the process of thermal tolerance of the coral holobiont? 3) How transient are these structural/functional shifts of the microbial community in thermally acclimatized corals?

Some recent results from natural experiments before hyper-thermal anomalies have shown that the associated bacterial assemblages in corals differed from reef locations. However, regardless of these site-specific baseline differences all bacterial microbiomes become similar in structure immediately after the thermal anomaly and even more they respond with the same trajectory during the recovery process. Additionally we have discovered that these coral-associated microbial assemblages show to be resilient but this might be altered by the composition of Symbiodiniaceae that become established during the recovery after thermal/bleaching event. These interesting results suggest the existence of non-random transitory community states that might facilitate the recovery of stable baseline states. It also suggest that rather than temperature the major disturbance might be related to a change of nutritional status and/or coral metabolic shift due to the loss of algal symbionts (bleaching). This is on-going research that has also been extended in the lab using the *Exaiptasia pallida* anemone model system.
With the goals of connecting and applying my work on microbial/coral symbiosis to a broader program of ecosystem restoration, I started a project in partnership with the NASA Extreme Exploration Mission Operation (NEEMO) since 2015—a project that has been extended to 2021 because of its success. In this project, we provide a marine-science platform during the training of NASA Astronauts every summer by conducting marine science research with us in the 60-feet deep underwater Marine Laboratory Medina Aquarius in the Florida Keys. NASA astronauts benefit from these activities since this project provides a real extreme condition scenario for training, as an analog of planetary missions. As a result of this scientific work, we have built experimental coral farms where different types of coral/microbe combinations are being assessed through time under different reef environments. The application of microbial ecology to provide solutions to large ecological problems is an activity that motivates and excites students.

3) Molecular mechanisms of symbiosis regulation using the *Exaiptasia pallida* host model (Funded by NSF IOS and FIU)
From the project #1 described above, we have learned that while corals host a diverse community of intracellular algal symbionts, Symbiodiniaceae, the main bulk of symbiont is dominated by single strain/species. Moreover, we have documented that shifts of the dominance of these symbionts take place after environmental disturbance and even correlate with increased physiological advantages under subsequent appearance of environmental disturbances (See Project #1 above). These findings have fuelled our interest to understand the cellular and molecular mechanisms that underlie the successful engagement of symbiosis by different Symbiodiniacea species? a phenomenon still largely unknown. I also think figuring out the underlying molecular and cell biology of the symbiotic partners, and of the mechanisms by which they recognize each other and maintain a stable relationship will facilitate addressing the ecological and conservation issues affecting reef corals.
Unlike corals, *Exaiptasia pallida* anemones represents again an ideal system to investigate the molecular basis of symbiosis regulation by comparing anemones harboring different Symbiodiniaceae species with anemones lacking the algal symbionts. Currently in my laboratory after a rigorous process of symbiont depletion and presentation/acquisition of new strains we have created different assemblages of host/Symbiodiniaceae symbiosis with distinct physiological characteristics. This has provided us with the comparative power to determine differences and similarities of molecular regulation in the host as a function of symbiont species. We use proteomic and transcriptomic approaches to determine differences in the gene and protein profiles of these anemones. These approaches have allowed my research group to address previously intractable questions concerning the cnidarian-microalgae symbiosis, including both genetic analysis and cell biology. Significant questions about the nature of these associations include: 1) Are there newly evolved genes specific to the symbiosis, and/or what genes are co-opted from pre-existing programs? 2) How the expression of core symbiosis genes is affected by the species of Symbiodiniaceae being harbored by the host? 3) Are there Symbiodiniaceae species-specific induced host genes?

4) Functional genomics analysis and genome editing of Symbiodiniaceae species to study symbiont/host associations and thermal tolerance (Recently funded by NSF-IOS-EDGE)
From projects #1 and 3, we have been learning and identifying several Symbiodiniaceae species with physiologies that facilitate the cnidarian host (corals and anemones) to better tolerate environmental stressors and therefore display more resistance to bleaching (the dysbiosis of the host/Symbiodiniaceae partnership). From these findings, we have started one major genomic and several transcriptomic studies to understand the genomic architecture and adaptation of one of the most thermal tolerant symbiotic microalgal species, *Durusdinium trenchi*. We just completed the sequencing and assembly of the genome for this species and are in the genome comparative stage of analysis for this project. The quality of the genomic data is high with 28,531 annotated genes and a NP50 of 1,470 bp (Table 1). Along with this, my lab has started several transcriptomic projects on *D. trenchii* under different thermal experimental setup with the goals to discover genes and pathways responsible to the thermal resistance phenotype of these microbial symbionts. The transcriptomic information is being also informative to improve gene models for the genome project.

5) **Unveiling the molecular basis of the immune defense system of cnidarians** (Funded by NSF-IOS-CAREER)
Coral reefs are not only threatened by abiotic factors linked to global warming but also disease-causing infectious agents, which are of great concern since they have also emerged over recent decades as a significant threat to coral reef ecosystems. In order to understand and influence the persistence of coral reefs under current challenges, another important aspect of research in my laboratory is to measure how corals (Cnidarians) develop mechanisms of resistance to pathogenic stressors.

The fact that invertebrates do not possess the adaptive immune system as those well described in vertebrate has filled the notion over many years that in general invertebrates do not display any capacity of increasing immune competence during their life span. And perhaps in organism with short-life span of few days to few weeks such in the cases of many small invertebrates there is a lack of adaptive immune systems. However that is not the case for corals, as they are long-lived organisms that can live for hundreds of years and hence it is not difficult to expect that they encounter during their lifespans same common pathogens in many occasions. Yet it remains unknown whether these evolutionary early-diverged animals possess some type of immunological reaction that strengthens the defense response upon repeated infections, such as that described as immunological memory in more evolutionary derived organisms, such as humans and many other vertebrates.

Interesting recent evidence has shown that some of coral species can no longer be infected by the same strain pathogen once proposed to be disease-causing infectious agents. An example of this is the case for the Caribbean coral *Acropora palmata* and whitepox disease. These observations have generated a number of hypotheses to explain the acquired resistance by corals to pathogenic agents, including the hologenome theory of evolution, which postulates that corals can acquire beneficial partners that prevent pathogens from colonizing or entering host tissue. However, experimental support for this hypothesis is lacking. Other explanatory hypotheses postulated in the literature include the loss of virulence in recent years by known coral pathogens, the natural selection for disease-resistant coral genotypes, and the suggestion that coral diseases are a collection...
In my laboratory we are testing an alternative hypothesis to explain the increase of resistance to known disease-causing microbial pathogens: The sub-lethal exposure of corals to a pathogen induces a defense response that is memorized and expressed in an accelerated manner upon subsequent exposure; a process that might resemble a primitive form of immune priming. This hypothesis relies on the fact that while immunological memory was conventionally considered a characteristic of the vertebrate adaptive immune system, there is well-accepted evidence that immune responses of invertebrates, such as insect and crustaceans, are strengthened upon repeated infections.

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