Funding generously provided by:

GORDON AND BETTY
MOORE
FOUNDATION

11th Annual Yosemite Symbiosis Workshop 2023

Friday May 5th

Landsnaes House 2667 English Lane, Wawona CA
Arrival & Welcome Party 7:00pm – 10:00pm

Saturday May 6th

Wawona Community Center (see attached map)

Lunch 11:30pm – 12:25pm

- **12:25** Welcome to the 2023 Yosemite Symbiosis Workshop (Carolin Frank, Joel Sachs)

Session I Microbe-microbe interactions

- **12:30 Emily Aguirre** The role of *Roseibium* in the Symbiodiniaceae phycosphere
- **12:45 Satya Spandana Boddu** Variation in *C. elegans* gut microbiome is a result of host heterogeneity
- **1:00 Pedro Perez** Crossing communication barriers: Autoinducer production between symbiotic Vibrio species from *Sepiola atlantica* and *Sepiola affi*
- **1:15 Brian Pipes** Your genes or mine? Regulation of Competence and Natural transformation in the *Vibrio fischeri – Euprymna* Mutualism

30 minute break for coffee and snacks

Session II Global change and anthropogenic effects on symbiosis

- **2:00 Julia A. Boyle** Soil microbiomes under climate change: effects on plant performance and symbiosis
- **2:15 Rowan McLachlan** Impacts of nutrient enrichment & overfishing on the physiological health & microbial community dynamics of *Pocillopora* corals
- **2:30 Candace L. Williams** Examining microbial drivers of wildlife fitness in the growing anthropogenic landscape
- **2:45 Claire E. Williams** Characterizing rapid shifts in the *Anolis* gut microbiome after introduction to a novel environment

30 minute break for coffee and snacks

Session III Partner specificity and coadaptation

- **3:30 Chris Carlson** The Evolution of Partner Specificity in Mutualism (VIDEO TALK)
- **3:45 Alison Gould** Distinct strain-level symbiont communities between individuals and populations of a bioluminescent fish host
- **4:00 Jessica Maccaro** Vulture bee metagenomes reveal the role of the microbiome in carrion digestion
- **4:15 Nickole Villabona** Conserved, yet disruption-prone, gut microbiomes in neotropical bumblebees
- **4:30 Anna Simonsen** Horizontally transmitted symbionts often form highly complex specialisation patterns with their hosts.

30 minute break for snacks, beer and wine

- **5:15-615 KEYNOTE: Monica Medina** Dissecting a Marine Holobiont: How far I have come in 20 years?

Dinner 6:30-800
Poster Session 8:00-9:30pm

Sunday May 7

Wawona Community Center (see attached map)

Breakfast 8:30-9:15am

Session IV Microbial communities, dispersal and services

- **9:15 Jacob S. Francis** Yeast does confess it pays itself to distract: agent based models suggest pollinator learning of microbial cues and floral reward modification mediates microbial dispersal
- **9:30 Tinkara Bizjak** Presence and activity of nitrogen-fixing bacteria inside Scots pine needles in the Swedish boreal forest
- **9:45 Iolanda Ramalho da Silva** Multiscale patterns of bacterial community composition associated with lodgepole pine: a dominant conifer species in the Sierra
- **10:00 Jessie Wang** Testing the outcomes of host-microbiome interactions across 1000 environments
- **10:15 Magdalena L. Warren** Bacteria in the honeybee crop are decoupled from those in the mouth

30 minute break for coffee and snacks

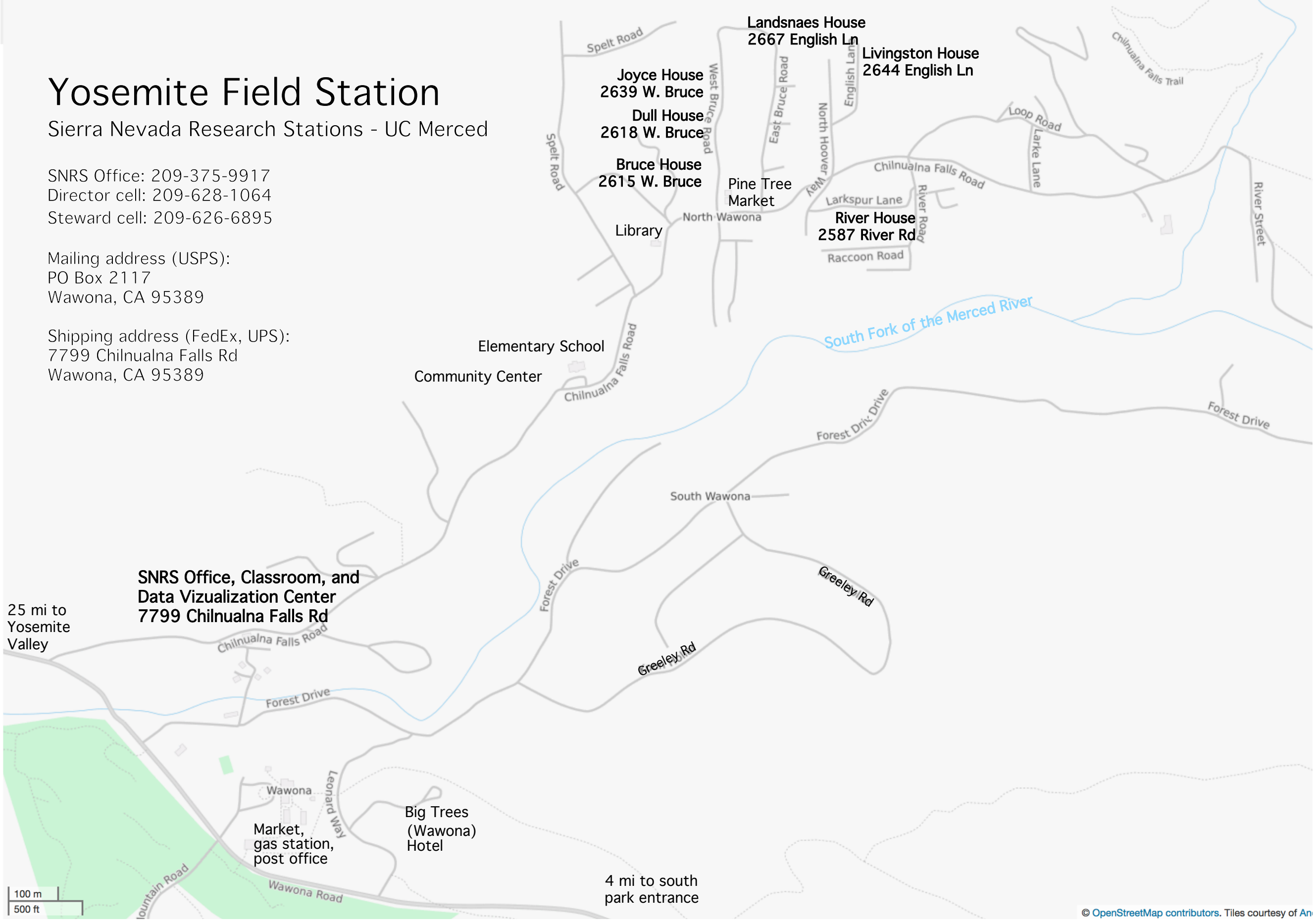
Session V Origins and evolution of symbioses

- **11:00 Sarah Frail** Emergence of a nitrogen-fixing organelle in eukaryotes
- **11:15 Tobin Hammer** Why do hosts malfunction without microbes? Missing benefits versus evolutionary addiction
- **11:30 Pu Wang** Co-existence of uni-multicellularity in the adaptation of collective action in *Kluyveromyces lactis*
- **11:45 Michele Nishiguchi** INSITE- The Institute for symbiotic interactions, training, and education
- **12:00 Carolin Frank, Joel Sachs** Closing remarks

Lunch 12:00-1:00

<u>End of conference</u>

11th Annual Yosemite Symbiosis Workshop 2023



Yosemite Field Station

Sierra Nevada Research Stations - UC Merced

SNRS Office: 209-375-9917
Director cell: 209-628-1064
Steward cell: 209-626-6895

Mailing address (USPS):
PO Box 2117
Wawona, CA 95389

Shipping address (FedEx, UPS):
7799 Chilnualna Falls Rd
Wawona, CA 95389

Talks

1. Emily Aguirre emilyagu@usc.edu University of Southern California

The role of Roseibium in the Symbiodiniaceae phycosphere

Emily G Aguirre, Marissa J Fine, J Cameron Thrash, Carly D Kenkel

It is unclear whether bacterial players influence the persistence of the cnidarian-algal symbiosis and the mechanisms involved in cnidarian-bacterial and algal (Symbiodniniaceae)-bacterial signaling remain uncharacterized. Possible roles for bacteria within the cnidarian holobiont include providing a steady supply of micronutrients, antimicrobials, and/or essential vitamins. Bacterial products are clearly exchanged, but what are the mechanisms that link these services to the success of the cnidarian-algal symbiosis? This multi-faceted question can be explored by focusing on the exchange of compounds that are essential for the algae, which they cannot themselves produce. *Symbiodinium linucheae*, the algal symbiont of Aiptasia anemones (*Exaiptasia diaphana*), possesses the cobalamin (B12)-dependent methionine synthase gene, which makes it auxotrophic for B12, an essential, prokaryote-produced vitamin. We isolated a putative B12-producer, *Roseibium*, a core member of the Symbiodiniaceae microbiome, and sequenced its genome to verify its metabolic potential for B12 synthesis. Axenic *S. linucheae* (strain SSA01) and *Roseibium* cocultures were grown in B12-limited media for 12 weeks and RNA was extracted for differential gene expression analysis with controls (axenic SSA01 or axenic *Roseibium*). Overall, growth was robustly enhanced for both organisms in coculture, suggesting bacteria like *Roseibium*, play a role in enhancing growth of the algal symbiont.

2. Tinkara Bizjak, tinkara.bizjak@slu.se Swedish University of Agricultural Sciences

Presence and activity of nitrogen-fixing bacteria inside Scots pine needles in the Swedish boreal forest

Tinkara Bizjak, Anita Sellstedt, Regina Gratz & Annika Nordin

In recent years, endophytic nitrogen-fixing bacteria have been detected using sequencing and isolated using culturing methods from diverse native conifer trees, growing predominately in North American boreal forests. Furthermore, their nitrogen-fixing activity has been measured using acetylene-reduction assay. It has been suggested this could be especially beneficial for trees growing in nitrogen-limited environments. To assess whether nitrogen-fixing bacteria are also present in Scots pine trees native to the nitrogen-limited Swedish boreal forest, we isolated nitrogen-fixing bacteria on nitrogen-free media and confirmed their nitrogen-fixation ability by in- vitro measurements of acetylene reduction. The isolated bacteria belonged to *Bacillus, Variovorax, Microbacterium, Sphingomonas, Novosphingobium* and *Priestia* genera. Additionally, we compared the presence and activity of nitrogen-fixing bacteria between control plots and nitrogen fertilized plots in a long-term inorganic nitrogen addition experimental forest. The hypothesis was that the activity of nitrogen-fixing bacteria would decrease in fertilized plots as was previously observed for nitrogen-fixing bacteria associated with mosses growing at the same field site. We used an immunodetection method to detect a subunit of the nitrogenase enzyme in needle protein extract to quantify the presence of nitrogen-fixing bacteria and we used acetylene-reduction assay to measure their activity inside Scots pine needles. In contrast to our hypothesis of decreased nitrogen fixation in the fertilized forest plots, the presence and the rate of nitrogen fixation was similar between the two treatments.

3. Satya Spandana Boddu, satya.spandana.boddu@emory.edu Emory University

Variation in C. elegans gut microbiome is a result of host heterogeneity

Satya Spandana Boddu, Michael K Martini, Ilya Nemenman, Nic Vega

Microbiome composition varies across hosts and within hosts over time. The rules of assembly of microbiomes and the microbial interactions that define the structure and variation within those microbiomes are not yet understood. We use a combination of experiments and a simple stochastic model as a bottom-up approach to understand heterogeneity in colonization dynamics and identify sources of variation within a minimal microbiome. Seven bacterial species that are part of the native microbiome of C. elegans are used to colonize wildtype worms and gut contents are measured across timepoints to quantify colonization process. We find that demographic noise is not enough to explain the observed variation. Our results indicate hidden heterogeneity among hosts is an important source of microbiome community variation. These results will be used to modify the existing model and better understand structure of microbiomes.

4. Julia Boyle julia.boyle@mail.utoronto.ca University of Toronto
Soil microbiomes under climate change: effects on plant performance and symbiosis
Julia A. Boyle, Bridget Murphy, Fangming Teng, Ingo Ensminger, John R. Stinchcombe, Megan. E. Frederickson

The impacts of climate change on soil microbial communities are important for predicting plant fitness and plant evolutionary responses to climate change, given the ubiquitous nature of symbioses between plants and microbes. While past studies have demonstrated climate can shift soil microbial communities, the actual eco-evolutionary impact of altered soil microbiomes on plants was not addressed. Climate conditions can affect microbes differently, creating new microbial competitive interactions and potentially disrupting or improving the likelihood of different symbiotic interactions and subsequent plant performance. Legumes have a close mutualism with rhizobia bacteria, making them especially good organisms to assess how symbiosis and plant performance may change. To address eco-evolutionary impacts of climate change, we first examined how a history of heat and drought affect bacterial and fungal soil community composition and fungal functional guilds, then we applied the soil to legumes in a growth chamber to test the effect of the altered microbiome on plant performance and symbiosis. Testing how climate-treated soil affects the plant, without the plant experiencing the climate itself, will isolate the effect of climate-treated microbiomes. We collected soil samples from warming array plots at the Koffler Scientific Reserve, Ontario, Canada, that have a multiyear history of being heated, droughted, heated and droughted, or left natural. Sequencing demonstrated that fungal community composition was significantly different between each climate treatment, with drought significantly decreasing observed fungal species richness while increasing parasite abundance. Additionally, bacterial community composition was significantly different due to drought, and rhizobial diversity increased in heated and droughted treatments. After applying the soils to *Medicago lupulina* in a growth chamber, we found that soils with a history of heat significantly reduced rhizobial symbiosis, and that soils with a history of drought significantly increased legume mortality. However, we found no effect on plant biomass. Data analysis is ongoing, but preliminary results suggest that symbiosis may be disrupted and plant survival reduced when paired with microbiomes that have experienced climate change. As the frequency and severity of extreme weather events increases, understanding the eco-evolutionary dynamics between plants and microbes under climate change will be crucial to predicting plant response and mitigating negative effects on their performance.

5. Christopher Carlson, christopheriancarlson@gmail.com University of Toronto (video presentation)
The Evolution of Partner Specificity in Mutualism
Chris Carlson, Erol Akcay, Bryce Morsky

Mutualistic species vary in their level of partner specificity, which has important evolutionary, ecological, and management implications. Yet, the evolutionary and ecological mechanisms which underpin partner specificity are not fully understood. Most work on specialization focuses on the trade-off between generalism and specialism, where specialists receive more benefits from preferred partners at the expense of benefits from non-preferred partners, while generalists receive similar benefits from all partners. Because all mutualisms involve some degree of both cooperation and conflict between partners, we highlight that specialization to a mutualistic partner can be cooperative, increasing benefit to a focal species and a partner, or antagonistic, increasing resource extraction by a focal species from a partner.

We devise an evolutionary game theoretic model to assess the evolutionary dynamics of cooperative specialization, antagonistic specialization, and generalism. Our model shows that cooperative specialization leads to bistability: stable equilibria with a specialist host and its preferred partner excluding all others. We also show that under cooperative specialization with spatial effects, generalists can thrive at the boundaries between differing specialist patches. Under antagonistic specialization, generalism is evolutionarily stable.

We extend this game theoretic model to examine how variation in environment quality shapes the evolution of the genotypic match between mutualistic partners, and variation in mutualist quality. We find that variation in habitat quality across space promotes coexistence of specialist phenotypes, enhancing variation in mutualist quality. Ecological trade-offs between matching mutualistic partners and the local habitat had divergent implications for the evolution of partner specificity. We provide predictions for how a cooperation-antagonism continuum, and variation in habitat quality may determine the patterns of partner specificity that develop within mutualistic relationships.

6. Sarah Frail, sfrail@stanford.edu Stanford University
Emergence of a nitrogen-fixing organelle in eukaryotes
Sarah Frail, Jon Doenier, Solene Moulin, Ellen Yeh

Mitochondria and chloroplast organelles arose from ancient events (~2Gya, ~1Gya respectively) in which one bacterium was engulfed by another organism and assimilated into an organelle. The functions conferred by these endosymbiotic bacteria opened evolutionary doors for the host organisms, enabling considerable genomic expansion and diversification. Such organellogenesis events were critical to shaping biological diversity on Earth; yet because they occurred so long ago, the molecular and genetic adaptations required to give rise to an endosymbiotic organelle are obscured by billions of years of evolution. While endosymbiotic organelles have only evolved few times, bacterial symbioses are widespread in eukaryotes, especially in aquatic environments. One exceptional example is the epithemioid family of diatoms, a globally widespread algae that harbor a nitrogen-fixing cyanobacterial endosymbiont, allowing the diatom host to survive under nitrogen-limited conditions. Furthermore, the endosymbiont exhibits hallmarks of early transition to an organelle, having lost considerable portions of its genome including critical metabolic pathways and photosynthesis. Strikingly, this endosymbiotic relationship is only about 12 Mya. We have sequenced the genome of the host diatom, and aim to leverage this genome to define the status of the epithemioid endosymbiont in the transition from endosymbiont to bona-fide organelle. In doing so, we will apply it as a model and a window back in time, through which essential, defining, and rate-limiting steps of endosymbiotic organellogenesis can be explored.

7. Jake Francis jacob.franci@gmail.com UC Davis
Yeast does confess it pays itself to distract: agent based models suggest pollinator learning of microbial cues and floral reward modification mediates microbial dispersal
Jacob S. Francis, Daniel J. Kliebenstein, Rachel L. Vannette

Nectar-inhabiting microbes rely on animals for dispersal and show intraspecific variation in dispersal limitation. Many of these microbes are not passive stowaways on pollinators and flowers. Rather, some produce pollinator-perceptible volatile compounds or chemically modify nectar rewards in ways that change pollinator preference. While it is intuitively clear that these signaling and reward strategies should shape microbe dispersal, the consequences of microbial effects on nectar attractiveness and palatability for microbial abundance and persistence in nectar metacommunities is unclear. Here we use an agent-based simulation model that incorporates realistic pollinator learning and memory parameters to test how signaling and reward strategy impacts microbial dispersal. Inspired by empirically tested microbial traits, we modeled focal microbes that 1) either did or did not produce volatile cues, and 2) modified nectar palatability for pollinators. We then tested for differences in the rate of dispersal, landscape level incidence, and total population size of these focal microbes when competing against a neutral microbial competitor (non-signaling, no reward change). Preliminary simulations suggest that microbes which decrease pollinator preference and signal microbial presence colonize new flowers more quickly and have higher landscape level incidence than competitors. Alternatively, microbes that increase pollinator preference and signal microbial presence do not spread more quickly, but instead reach higher densities in the flowers where they occur. Currently we are testing how the success of these strategies might depend on floral longevity and interact with pollinator memory duration in further simulations as a first step in predicting how microbial metabolism impacts dispersal. The models generate testable hypotheses about the relationship between microbial signaling/reward strategy and landscape level patterns of floral microbiomes. This is highlights the importance of animal cognition in shaping dispersal limited epi-floral communities, and raising the question of whether similar dynamics might be important for animal dispersed microbes in other systems.

8. Alison Gould, agould@calacademy.org California Academy of Sciences

Distinct strain-level symbiont communities between individuals and populations of a bioluminescent fish host

A Gould, S Donohoo, E Román, and E Neff

The bioluminescent symbiosis involving the sea urchin cardinalfish, *Siphamia tubifer*, and *Photobacterium mandapamensis*, a luminous member of the Vibrionaceae, is highly conserved and specific over both space and time. Despite its high degree of specificity, patterns of genetic diversity have been observed for the symbionts from hosts sampled over relatively small spatial scales (<100km). We characterized and compared sub-species, strain-level symbiont diversity within individual fish hosts and between populations of hosts sampled from both the Philippines and Japan using a PCR fingerprinting approach. We then used whole genome sequencing of the unique symbiont genotypes to investigate the underlying genetic diversity of the symbiont community and characterize the symbiont pangenome. We determined that an individual S. tubifer light organ hosts approximately 6 symbiont genotypes, although the number of strains present ranged from 1-13. Surprisingly, there was little overlap in strains between hosts from the same location. However, a phylogenetic analysis of the unique symbionts indicated a Japan- and Philippine- specific clade, suggesting some genetic differentiation in the symbionts between these two locations. We were also able to identify symbiont genes that were variable between strains, including luxF, a gene in the lux operon, which is responsible for light production, and could have important consequences for the symbiosis. This study highlights the importance of examining strain-level diversity in microbial symbioses and is providing new insight into the underlying genetic architecture of symbiont communities within a host.

9. Tobin Hammer, hammert@uci.edu UC Irvine

Why do hosts malfunction without microbes? Missing benefits versus evolutionary addiction

Microbiome studies increasingly use experiments comparing microbe-free and microbe-colonized hosts. Microbe-free hosts often exhibit defects in behavior, development, metabolism, immunity, or other traits. Most studies focus on the underlying mechanisms, but in mainstream microbiome discourse, why these effects occur in the first place is rarely asked or critically evaluated. The standard interpretation is “missing benefits”: hosts exhibit defects because they lack their beneficial symbiotic partners. Benefits imply that a novel or useful service is being provided, and that the origin of the symbiosis was adaptive for hosts. This hypothesis clearly holds in many cases, but missing benefits cannot explain the numerous examples of defects in basic host processes—such as misregulated gene expression—that arise in the absence of symbionts. In my talk I will outline evolutionary addiction, an alternate pathway whereby the association between microbes and the host trait in question was not originally adaptive for hosts, and dependencies evolved secondarily. Although empirical evidence for evolutionary addiction (EA) has existed for decades, it is not widely known in the microbiome field. My talk will synthesize the main arguments from an opinion article I am writing that is focused on EA. I argue that, far from being a minor oddity, evolutionary addiction likely underlies the majority of traits that malfunction in microbe-free hosts. I will also discuss ways to differentiate these processes, and potential implications of EA for host evolutionary responses to microbiome disturbance.

10. Jessica Maccaro, jmacc003@ucr.edu UC Riverside
Vulture bee metagenomes reveal the role of the microbiome in carrion digestion
Jessica Maccaro, Laura Figueroa, Erin Krichilsky, Quinn McFrederick

Diet and gut microbiomes are intricately linked on both short and long timescales. Changes in diet can alter the microbiome, while microbes in turn allow hosts to access novel diets. Bees are wasps that switched to a vegetarian lifestyle, and the vast majority of bees feed on pollen and nectar. Some stingless bee species, however, also collect carrion, and a few have fully reverted to a necrophagous lifestyle, relying on carrion for protein and forgoing flower visitation altogether. These “vulture” bees belong to the corbiculate apid clade, which is known for its ancient association with a small group of core microbiome phylotypes. Here, we investigate the vulture bee microbiome, along with closely related facultatively necrophagous and obligately pollinivorous species, to understand how these diets interact with microbiome function. Via shotgun metagenomic sequencing and functional analyses, we find that vulture bee microbiomes appear to play a role in amino acid and fatty acid digestion, protein processing, and more.

11. Rowan McLachlan, rowan.mclachlan@oregonstate.edu Oregon State University
Impacts of nutrient enrichment & overfishing on the physiological health & microbial community dynamics of Pocillopora corals
McLachlan RH, Speare KE, Epstein HE, Silva DP, Vompe A, Thurber A, Burkepile DE, Adam TC, Vega Thurber RL

Coral reef ecosystems, and the services that they provide, are being degraded at an alarming rate due to various global (e.g., ocean warming and acidification) and local stressors (e.g., coral disease outbreaks, nutrient pollution, and overfishing). Nutrient pollution and overfishing are among the most important local stressors affecting coral health and can decrease reef resilience in several ways. The reduction of herbivorous fish stocks due to overfishing can lead to prolonged recovery times of corals after disturbances and cause an increase in fleshy seaweed overgrowth on reefs. Nutrient pollution also stimulates seaweed growth and increases competition with corals. Recent research indicates that the coral microbiome (host-associated microbial community) is strongly associated with reef resilience to nutrient pollution and overfishing. However, to fully understand the relationship between microbial dynamics and ecological resilience, comprehensive integration of the physiological health characteristics of the coral holobiont – which includes the cnidarian host, the algal endosymbionts –, and microbial community dynamics should be conducted simultaneously. Here we describe the results of a long-term (3-year) field experiment to better understand how chronic low-level nutrient enrichment and overfishing of herbivorous fishes influences the combination of physiological and microbial health of *Pocillopora* coral colonies in Moorea, French Polynesia. We measured a variety of physiological health metrics, including tissue biomass, total lipid content, and chlorophyll a concentration. Microbiome health (a.k.a., dysbiosis) was assessed by monitoring changes in the bacterial community composition, diversity, function, and the relative abundance of pathogenic microbes. Preliminary results are surprising and indicate that chronic low-level nutrient enrichment may have had a positive influence on coral holobiont health through increases in tissue biomass. Additional preliminary results will be presented at the meeting. Overall, the goal of this research is to understand how coral reefs will respond to abiotic (nutrient pollution) and biotic (reduced herbivory) threats through a holobiont-innovative approach by integrating physiological traits and microbial communities' analysis. The integration of these data may help identify the potential interacting roles of these associated microbial communities in the nutrition and health of the coral animal and its protist symbionts and reveal a more holistic and mechanistic understanding of how marine holobionts are resistant or resilient to environmental changes.

12. Michele Nishiguchi, nish@ucmerced.edu, UC Merced
INSITE- The Institute for symbiotic interactions, training, and education

Environmental policies are integrating climate change scenarios and projected impacts to their species and ecosystem conservation strategies at the international, national, and local levels. However, the general approach to species conservation and protection has ignored the fact that all animals and plants are essentially symbiotic systems that depend on stable interactions with microbes for essential biological and ecological services. Since each partner is likely to suffer differential impacts of climate change, these associations are likely more susceptible to rapid environmental change leading to instability and eventual dysbiosis of the interaction. Moreover, climate change is known to substantially alter microbial communities in the environment, which is likely to cause shifts away from beneficial interactions towards detrimental ones, and eventually lead to their extinction. To better predict the trajectory of biodiversity under climate change, we need to assess how hosts and their microbes will respond to the rapidly shifting climate. Our vision is to discern key indicators of climate change through a microbial lens and develop useful methods that predict the potential for biodiversity loss- thereby offering insight as to what changes are necessary to alleviate such devastation. To fill knowledge gaps and accelerate the integration of symbiosis into climate-based science, education, and outreach, we propose a Biology Integration Institute (BII): INSITE, the INstitute for Symbiotic Interactions, Training, and Education in the Face of a Changing Climate.

13. Pedro Perez, pperez40@ucmerced.edu UC Merced
Crossing communication barriers: Autoinducer production between symbiotic Vibrio species from Sepiola atlantica and Sepiola affi
P. Perez, R. Lami, and M.K. Nishiguchi

Two species of bacteria, *Vibrio fischeri* and *Vibrio logei*, can occur within the light organs of squids in the genus *Sepiola* (Cephalopoda: Sepiolidae). The ability to produce light by these bacteria is necessary for the squid to camouflage due to a phenomena termed counterillumination. Light production depends on the accumulation of small chemicals called autoinducers, which trigger this response at high concentrations. Interestingly, it is still unknown whether both species can communicate with one another to coordinate bioluminescence during symbiosis. We therefore measured the production of the Acyl-homoserine lactones C6 and oxo-C10, and the Autoinducer-2 (AI-2) in symbiont isolates from *Sepiola atlantica* and *S. affinis* using the biosensors Pseudomonas putida F117, Escherichia coli MT102 and *Vibrio harveyi* MM32. Our findings yielded a different pattern among and within the isolates from the two locations. These differences were also observed using High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS) analysis, which can detect each autoinducer molecule and their concentration. Validation of these AHLs was through the Global Natural Products Social Molecular Networking (GNPS). Our analysis gives a first approximation about how these two species of bacteria could communication within the light organs of two allopatric Sepiola species, and how they govern the patterns of counterillumination behavior in this beneficial symbiosis.

14. Brian Pipes, bpipes@ucmerced.edu UC Merced
Your genes or mine? Regulation of Competence and Natural transformation in the *Vibrio fischeri* – *Euprymna* Mutualism
Pipes, B and M.K.Nishiguchi

Symbiotic *Vibrio fischeri* are found in dense populations within a specialized light-emitting organ in *Euprymna scolopes*, undergoing a daily cycle of expulsion and re-growth of the remaining population. The selective pressure of this cycling can drive horizontal genetic exchange, which plays a role in bacterial adaptation and contributes to widespread heterogeneity in genome sequences and plasticity found in many bacterial species. Additionally, chitin induced natural competence and transformation has been instrumental in gene swapping, particularly in several Vibrio species. Therefore, we examined whether different inducing signals and cues (restricted nutrient availability, high cell density) regulated the induction of *V. fischeri* natural transformation during the nocturnal cycle within the light-organ. Chitin exposure did not induce natural transformation in *V. fischeri*, unless the regulatory components of the competence machinery are genetically manipulated for constitutive DNA uptake. Additionally, we show that natural transformation rates in *V. fischeri* grown in culture are highly variable and strain-dependent, even with the supplementation of chitin oligosaccharides and the presence of quorum-signaling inducers. This suggests that there are different response regulators depending on strain type and host location, as well as induction of transformation due to abiotic stressors (UV, pH). Ongoing research in other environmental and genetic factors controlling the regulation of *V. fischeri* natural competence will provide further insight in microbial adaptation to environmental cues for symbiosis.

15. Iolanda Ramalho da Silva, iramalhodasilva@ucmerced.edu UC Merced
Multiscale patterns of bacterial community composition associated with lodgepole pine: a dominant conifer species in the Sierra
Iolanda Ramalho da Silva, Andrea Salinas, and Carolin Frank

Forests are recognized as the richest and most diverse environments for biological groups and plant leaf surfaces are one of the largest microbial habitats on earth. However, the rhizosphere microbial communities are generally better studied than microbial communities associated with aboveground parts of plants (phyllosphere). This study aimed to determine the multiscale patterns of bacterial community composition associated with lodgepole pine (*Pinus contorta*). Three twigs of lodgepole pine plants growing in different geographic locations were collected in a nested design approach considering multiple spatial scales: (i) regions (two regions, c. 50 - 100 km apart), (ii) sites (three sites within each region, c. 1 - 5 km apart), (iii) subsites (distinct sections within plant populations, c. 10 m apart), and (iv) neighborhood scales (three trees within each subsite, c. 25 cm apart) in Yosemite and Sequoia National Parks, California, USA. We characterized the bacterial epiphyte communities using 16S rRNA high-throughput Illumina sequencing. Our results showed that the phylogenetic diversity did not change across space suggesting that the host plant may be the main driver of alpha diversity of bacteria. However, strong differences in bacterial community assembly especially at the regional scale indicate that the environment plays a key role in the beta-diversity of epiphyte bacteria on the lodgepole pine phyllosphere.

16. Anna Simonsen, asimonse@fiu.edu Florida International University

Consequences of symbiont genomic properties on patterns of specialisation to its plant hosts in a diverse legume-rhizobia mutualism

Horizontally transmitted symbionts often form highly complex specialisation patterns with their hosts. Bacteria are well known to have flexible genomes, with different strains conferring different gene content. However, it is still unclear how this flexibility in gene content impacts bacterial symbiont interactions with their hosts. In the legume-rhizobia mutualism, where legumes form symbiosis with horizontally transmitted nitrogen-fixing bacteria (rhizobia) in their root systems. Using this study system, my research has identified clear patterns of genome variation within a single species of nitrogen-fixing symbiont (*Bradyrhizobium diazoefficiens*, n=375 strains) isolated from a single endemic host species (*Acacia acuminata*), which correlates strongly with abiotic conditions of the symbionts' soil origin. Gene loss was widespread across the genome, with hotspots of low gene loss in close spatial proximity to core genes, suggesting that Bradyrhizobium has evolved to cluster essential-function genes in discrete regions to maintain viability during genomic decay. Symbiosis function of rhizobia isolates were tested by inoculating 21 Acacia species (from diverse ecoregions) in the glasshouse with a mixed culture containing strain subset (n=220) and quantifying strain occupancy on root nodules from sequenced root nodules. Incorporating a robust phylogenetic tree constructed from individual transcriptomes of all host species into the analysis, we found that structural aspects of symbiont genomes, such as their size, explained the breadth of host range.

17. Nickole Villabona, nvillabo@uci.edu UC Irvine

Conserved, yet disruption-prone, gut microbiomes in neotropical bumblebees

Nickole Villabona, Tobin Hammer, Nancy Moran, Alejandro Reyes

Bumblebees are important pollinators in natural ecosystems and agriculture, but many species are in decline. Temperate-zone bumblebees have host-specific and beneficial gut microbiomes, which may have a role in mediating the effects of environmental stressors. However, there is almost no information on gut microbiomes of tropical bumblebees. As temperate and tropical bumblebees encounter different floral resources and environmental conditions, their microbiomes could be distinct. Here, we characterized the gut microbiomes of four neotropical Bombus species and co-occurring solitary bees in the genus *Thygater*. We collected wild foraging bees from multiple sites in central Colombia and used 16S rRNA gene sequencing to characterize their gut microbiomes. DNA barcoding and morphology were used to identify bumblebee species. We found that neotropical bumblebees generally harbor microbiomes with similar diversity and composition to microbiomes of temperate-zone species. This result suggests that the microbiome was conserved during bumblebee dispersal from North America, despite major shifts in ecology and life history. As previously observed in temperate-zone species, some neotropical bumble bees have highly disrupted microbiomes, in which conserved gut bacterial symbionts are replaced by environmental microbes. In these individuals the gut microbial profile is more like that of solitary bees than of conspecifics. The gut parasites *Nosema* and *Crithidia* are also prevalent and are associated with microbiome disruption. Our findings provide insights into the biogeography of bee gut microbiomes and a foundation for studying bee-microbe-stressor interactions in the neotropics.

18. Candace Williams, cwilliams@sdzwa.org San Diego Zoo & Wildlife Alliance

Examining microbial drivers of wildlife fitness in the growing anthropogenic landscape

Due to numerous anthropogenic threats, the need for human management of threatened and endangered species exists, ranging from assist-rearing offspring to genetic reservoirs for species facing imminent extinction. For many threatened species, offspring reared in human-care destined for release are necessary to recover wild populations. Yet, given the associations between human-care and changes in both the microbiome and reduced animal fitness, we lack a mechanistic understanding of how human care impacts the microbiota of wildlife that may subsequently lead to the observed fitness loss. Here, we will demonstrate that by integrating multiple disciplines, we can begin to unravel the microbial and environmental drivers of host-fitness across a broad range of animal taxa, leading to greater conservation outcomes.

19. Claire Williams williams.claire.e@gmail.com University of Nevada Reno
Characterizing rapid shifts in the Anolis gut microbiome after introduction to a novel environment
Claire E. Williams, Maria Alcivar, Anabarbara Gonzalez, Kelly Lin Wuthrich, Leah Bakewell, Renata Pirani, Noa Ratia, Daniel Romero, Noah D. Gripshover, John David Curlis, Samir Gulati, Karla Alujevic, Guillermo Garcia Costoya, Akhila C. Gopal, W. Owen McMillan, Candace L. Williams, Christian L. Cox, Michael L. Logan

As rising temperatures threaten biodiversity worldwide, tropical ectotherms may be particularly vulnerable due to their narrow thermal tolerance ranges. Although most studies of ectotherm responses to rapid environmental change focus on tolerance traits of the host, resident gut microbes may also affect thermal physiology and can change rapidly in response to the environmental conditions experienced by the host. Thus, shifts in gut microbiome communities might play a major role in adaptation of hosts when their thermal environments change quickly. Regardless, real-time field studies on the responses of gut microbial communities to changing conditions are exceptionally rare. We transplanted slender anoles (*Anolis apletophallus*) from a mainland population to four islands in the Panama Canal—several of which were warmer than the source environment. We collected fecal samples from all individuals prior to transplantation, and repeatedly in the weeks afterwards to collect a time series of shifts in their gut microbiomes (characterized using 16S rRNA sequencing) as they acclimated to the warmer island environments. We also compared shifts in the microbiomes of lizards from these four new islands to those of four additional island populations that we transplanted in previous years to examine the extent to which microbiome structure converges across similar thermal environments. We found that the population level composition of the gut microbiome on newly transplanted lizards changes rapidly in response to transplantation and converges toward that of islands which had been transplanted in previous years. We also found that selection on gut microbiome composition played a role in this shift on at least one island, as lizards with specific gut microbiota compositions were less likely to survive. Ultimately, we plan to leverage our discoveries about shifts in microbiomes to understand how this process impacts host fitness in our rapidly changing world."

20. Jessie Wang, jae.wang@mail.utoronto.ca University of Toronto
Testing the outcomes of host-microbiome interactions across 1000 environments
Jessie Wang, Talha Kose, Tiago Lins, Oxana Pogoutse, David Sinton, Megan Frederickson

As human activities continue to change the environment at an unprecedented rate, organisms will face novel challenges in their surroundings. The excess deposition of nutrients that often result from human activities may affect the outcomes of species interactions. At low concentrations, these compounds may have positive effects on plant growth and performance. However, at higher concentrations, nutrient loading can impose stress and hamper plant performance. In this study, we tested the outcomes of duckweed-microbiome interactions across environmental gradients of nitrogen, phosphorus, and potassium. This allowed a fine-tuned resolution of the variation in plant responses to environmental stressors, as well as the identification of “optimum” nutrient levels for plant performance. In addition, we investigated the impact of nutrient stress on the composition and diversity of the duckweed microbiome.

21. Pu Wang, wangpu@grinnell.edu Grinnell University

Co-existence of uni-multicellularity in the adaptation of collective action in *Kluyveromyces lactis*

Pu Wang, Michael Travisano

Although multicellularity has had a significant impact on the world, most life still consists of unicellular organisms. The transition to multicellular life is a crucial event in evolution that has been the subject of extensive research. However, there has been limited investigation into the effects of this transition on contemporary unicellular life. In this study, we experimentally evolved *Kluyveromyces lactis* to develop collective action. We discovered that in all of our *K. lactis* lineages, unicellular organisms were able to persist alongside an evolved multicellular form, which we called the ""snowflake"" phenotype. We investigated the genetic basis of each phenotype and reconstructed the evolutionary trajectories of each population. Population genomic analysis demonstrated that the evolution of the multicellular snowflake phenotype altered the selection pressure on the remaining unicells, opening up alternative evolutionary pathways. Previously, the evolution of multicellularity was often viewed as a competitive outcome against unicellular organisms. However, our results showed that multicellularity can actually provide new niches for unicellular organisms and promote multi-unicellular symbiosis.

22. Magdalena Warren, mlwarren@stanford.edu Stanford

Bacteria in the honeybee crop are decoupled from those in the mouth

Magdalena L. Warren, Kaoru Tsuji, Leslie E. Decker, Jihoon Yang, Adina Howe, Manabu Kishi, Tadashi Fukami

Gut bacteria in honeybees have recently garnered much attention as a factor affecting bee health. However, unlike the hindgut, which has been the primary focus of this research, the crop, or the honey stomach, is often assumed to be dominated by environmentally acquired transient taxa that matter little to the bees. To evaluate this assumption, we compared crop- and mouth-derived bacterial 16S rRNA amplicon sequences in foraging adults of two species, *Apis mellifera* and *A. cerana japonica*, caught in summer and winter in the Minabe-Tanabe region of Japan. Analysis of 221 bees from 12 sites in the region indicated that the mouth samples had mostly non-overlapping bacterial compositions between summer and winter, with a sharp drop in alpha diversity and a large increase in beta diversity from summer to winter. In contrast, the crop remained similar in bacterial composition and alpha and beta diversity across the two seasons, lending support to the hypothesis of a conserved crop bacterial community that does not follow the seasonal changes seen in environmental bacteria. These findings build on previous studies that indicated positive effects of crop-inhabiting bacteria on honeybee health and suggest that further studies of crop bacterial communities are needed to better understand the relationship between honeybees and their gut bacteria.

Poster presentations

23. Heidi Abresch, heidi.abresch@umontana.edu University of Montana

Fixing nitrogen on the day shift: Altered gene expression in a cyanobacterial endosymbiont

Heidi Abresch, Scott R. Miller, Tisza Bell

In many environments, biologically available nitrogen (N) is scarce, and many organisms have formed symbiotic relationships with N-fixing bacteria to overcome this challenge. One notable example of this is diatoms in the family Rhopalodiaceae that host unicellular, N-fixing cyanobacterial endosymbionts called spheroid bodies (SBs). Although this relationship is very young (~35 million years old), it shares many key features with older endosymbiotic relationships, including coordinated cell division and large-scale genome reduction. The Rhopalodiaceae-SB system provides a remarkable opportunity to understand the early stages of endosymbiosis and address the crucial process of how endosymbionts become genetically and metabolically integrated with their hosts. While SBs have lost several metabolic pathways required for an independent lifestyle, they have retained all genes required for N-fixation. In closely related, free-living unicellular cyanobacteria, N-fixation is strictly regulated over day-night cycles along with many other central metabolism pathways. Unlike their free-living relatives, SBs fix N mostly during the day instead of at night. The reasons for this shift in activity remain unknown. Here, we obtained transcriptomic data from the *Rhopalodia gibba* SB from mid-light and mid-dark timepoints to understand how its gene expression has changed compared with the diurnal patterns observed in free-living cyanobacteria. We found that SB gene expression has changed dramatically compared to free-living relatives. In particular, catabolism and N-fixing genes are more highly expressed during the day. This shift to daytime catabolism could support the demands of N-fixation in coordination with host photosynthesis.

24. Christopher Bivins, cbivins@ucmerced.edu UC Merced

Fungal Endophytes in Cynipid Wasp Galls

Christopher Bivins, Carolin Frank

The Cynipid wasps are a unique group of gall-forming wasps that all rely on various oak species for reproduction. These wasps lay their eggs inside of oak tissues, and in response, the oak cells form a gall to house, nourish, and protect the developing wasp larvae. The Blue Oak (*Quercus douglasii*) hosts over 50 different species of Cynipid wasps. Most of them are restricted to laying their eggs in oak leaves. We also know that oak leaves are hosts to very poorly understood fungal endophytes. How oak leaf fungal community compositions vary in response to galling by Cynipid wasps has never been studied before with modern molecular techniques. In this project, we use DNA metabarcoding to explore how fungal endophyte communities differ between two species of Blue-Oak specific Cynpid galls, the leaves the galls occur on, and leaves that don't have any galls on them.

25. Daravuth Cheam, dcheam@ucmerced.edu UC Merced

Model behavior: Predicting how bacterial biofilms respond to predatory protozoans

Cheam, D., Yeakel, J., and M. K. Nishiguchi

Vibrio fischeri are bioluminescent symbiotic bacteria that form cellular aggregate communities known as biofilms both within and outside their hosts. These biofilms are resilient during their symbiotic state inside the light organs of sepiolids squids, but can be susceptible to grazing from protozoans during their free-living cycle outside the host. Long-term grazing effects may be difficult to examine both in the field and experimentally in the lab, but can be estimated using predictive mathematical models. We therefore generated several models to determine whether grazing upon V. fischeri biofilms affected their growth and evolution over multiple generations. The accuracy of these models was tested by comparing their output with results from in-lab experiments. New parameters such as mortality rates were added into an existing model to account for long-term population dynamics. Results indicated that changes to specific parameters have significant effects on certain variables. For example, increasing the growth rate of planktonic cells decreases the size of other variables such as carbon source and biofilm population size. Given our predictions of biofilm modality, models generated from this work provide insight to populations dynamics in V. fischeri biofilms and how they might be affected by protozoan predation, providing the foundation for determining how the environment selects for key attributes used in this beneficial symbiosis.

26. Rebecca Crust, rcrus002@ucr.edu UC Riverside

The quest for native beneficial rhizobia: Investigating California agricultural lands for competitive cowpea symbionts.

Rebecca M. Crust, David Fronk, Fatima Macedo, and Joel Sachs

Cowpea (*Vigna unguiculata*), also known as blackeye pea, is an ideal legume crop for California thanks to its tolerance for heat and drought. As a legume, it forms a symbiosis with nitrogen fixing soil bacteria known as rhizobia, which is utilized by farmers in lieu of chemical fertilizers. However, commercially available rhizobia inoculants generally fail to give plants benefits in a field setting. This is due to the so-called rhizobia competition problem, wherein introduced strains are outcompeted for root space and resources by better adapted native bacteria. Our objectives are to i) characterize the rhizobia communities in California agricultural regions, and ii) quantify their capacity to colonize and enhance the growth of cowpeas. Soil was sampled from 10 fields with a history of cowpea planting across a 1000 km transect in California. Soil rinsates were used to inoculate cowpeas, soybeans, and lima beans in a greenhouse. At the stage of reproductive maturity, plants were harvested and dissected for root tips (where rhizobia colonization is initiated) and root nodules (symbiosis structure). Rhizobia were selectively cultured from these tissues, and DNA was extracted for community sequencing of bacteria. Soy and lima nodulated poorly compared to cowpea, suggesting little cross compatibility. We are weighing dried plant tissue to calculate biomass and host growth response data. Also underway is collection of cultural practices by growers to compare the effects of management on soil microbiome and plant growth benefits. In the future we plan to create a database of rhizobia genomes and their relative locations and metadata. This research is valuable for both California farmers and researchers of rhizobia alike.

27. Jesse Espinoza, jespinoza5@sfsu.edu San Francisco State University

How Seasonality and the Intertidal Location of Anthopleura sola Impacts Symbiosis with *Breviolum muscatinei*

Espinoza, Jesse; Esquerra, Raymond; Chung, Isabella; Chan, Annette; Ling, Lorraine; Cohen, C. Sarah

Symbiotic algae can yield significant primary production while providing nutrients to improve fitness and survival in their Cnidarian hosts. The state of this symbiotic relationship is sensitive to the local ecosystem and anthropogenic environmental changes are likely to disrupt this balance. The anemone *Anthopleura sola* is undergoing northern geographic expansion along the Pacific Coast consistent with heat waves associated with climate change. Within the temperate rocky intertidal, A. sola has a broad tidal height distribution that is subject to different exposure regimes related to heat, desiccation, and light. The facultative relationship between temperate anemones and their symbionts in response to abiotic variation over short and long timescales is not well understood. We use close spatial and temporal sampling to quantify algal density and photosynthetic potential in *A. sola* and symbiont *Breviolum muscatinei* over eight months in a natural population at Amarai Point, Pacifica, CA, within the intertidal zone. During bi-monthly low tide series, two tentacles are collected from 35 individuals selected for repeat collection. Each selected anemone had its positioning within the intertidal quantified: (1) The intertidal zone was separated into three subzones equal in length: the upper, middle, and lower intertidal zones with nearly equal anemone representation in each; (2) absolute vertical height was quantified using a laser level to further differentiate intertidal positioning among individuals found within pools, on channel shelves, or on top of the channel itself (vertical positioning range of sampled population: -0.75m to 0.13m). On a spatial scale, we hypothesized individuals in the upper intertidal zone would have lower algal densities and chlorophyll concentrations from increased exposure times. On a temporal scale, we predicted summer would show lower average algal density due to increased nutrient availability from seasonal upwelling in the California Current System. Previous studies have found A. sola had reduced algal densities with increased heterotrophic feeding. Sampling, beginning in July 2022, spans the large atmospheric river storm series that began in late December 2022 and continues through March 2023. Data analyzed thus far indicate population wide algal bleaching mid-November. Seven months into the study, we have collected over 500 samples from the 35 anemone individuals. The population has not recovered to pre bleaching algal densities since this event, potentially due to continued storms. Regarding zonation comparisons, anemones within the middle intertidal zone have higher algal densities on average compared to the lower and upper intertidal zones (Kruskal-Wallis $p < 0.05$). However, the difference in chlorophyll concentration between the three zones is minimal, indicating that algal cells within individuals in the upper and lower zones have adjusted to reach similar primary productivity as the middle zone. Additionally, the absolute vertical height positioning of anemone individuals appears to have little impact on algal density and chlorophyll concentration.

28. Perla Gonzalez Moreno, pgonzalezmoreno@ucmerced.edu UC Merced
How to get rid of your neighbor: Type VI secretion system (T6SS) between conspecific V. fischeri
Perla J. Gonzalez Moreno, Sarif Morningstar and Michele K. Nishiguchi

Symbioses between sepiolid squids (Cephalopoda: Sepiolidae) and the bioluminescent bacterium *Vibrio fischeri* serve as a model to examine the dynamics of host colonization. Multiple strains of *V. fischeri* are present in seawater, yet only a few successfully colonize and dominate squid light organs. Since the light organ represents a haven for bacterial replication, multiple factors have led to competition for this niche. Therefore, we examined whether T6SS-facilitated intraspecific competition may regulate the diversity and spatial distribution of *V. fischeri* strains found within the light organ of *Euprymna.* The T6SS is a molecular syringe that delivers toxic effectors to targeted competitor cells and can be regulated by changes in pH encountered within the host. Candidate T6SS-encoding strains were coincubated with T6SS-deficient strains isolated from Indo-West Pacific host populations. Five T6SS-candidate strains exhibited a lethal phenotype against competitor strains under neutral pH conditions. We subsequently examined whether pH affects T6SS expression in our lethal strains during this competition. Lethal strains had distinguished T6SS expression levels when experimentally evolved to different pH conditions compared to their ancestor. Results imply that lethal strains can extirpate potential competitors during host colonization and adapt to express T6SSs under host-specific cues (pH). Thus, the T6SS may serve as a major driving force in regulating the diversity and spatial distribution of symbiont strains found within the light organ of various host populations.

29. Arik Joukhajian ajouk001@ucr.edu UC Riverside
Characterization of the Eastern Joshua Tree Arbuscular Mycorrhizal Community
Arik Joukhajian, Sydney Glassman

The 2020 Dome Fire in the California Mojave Desert burned through the Cima Dome, killing over a million Eastern Joshua trees (*Yucca jaegeriana*). The Cima Dome made up a climate refugia in the event of the predicted 90% shrinkage of reproductive Joshua tree range due to climate change, but a quarter of the forest in this region was badly burned with poor natural recovery outcomes. Therefore, replanting efforts have already begun to sustain the local population, and several aboveground methods of ensuring seedling survival after replanting are being tested by land managers. Arbuscular mycorrhizal fungi (AMF) are commonly found inhabiting the roots of desert plants, since they greatly increase water and mineral uptake which are critical in arid environments. AMF inoculation may be necessary to improve Eastern Joshua tree regeneration since the shrubs that act as nursery plants and possible sources of AMF inoculum were practically wiped out within the burn scar. However, the AMF community of the Eastern Joshua tree has never been characterized, and there is only one study characterizing the AMF community of its sister genus *Yucca brevifolia*. We therefore sought out to characterize the AMF community of healthy *Y. jaegeriana* to provide baseline information for future restoration efforts. We selected twenty healthy focal trees and collected soils and roots at the base of each tree during the summer, fall, winter, and spring to fully characterize AMF composition and abundance and understand how it changes seasonally. We used Illumina MiSeq sequencing of the 18S rRNA region using the WANDA-AML2 primer pair and visual confirmation through spore counting of desert soil of each season from Summer 2021 to Spring 2022. We identified 1706 amplicon sequence variants, representing 42 distinct virtual taxa of AMF. The most abundant taxon Glomus VTX00294, was detected in every season, with greater sequence abundance in fall and spring. In total, we found 5 AMF families including Glomeraceae, Claroideoglomeraceae, Archeaosporaceae, Paraglomeraceae, and Diversisporaceae. On average, we found 11.9 ± 0.39 virtual taxa in soil samples compared to 8.6 ± 0.35 virtual taxa in root samples. Overall richness did not significantly vary across seasons, but winter samples had the most overall taxa as well as 3 unique taxa not present in the other seasons. These findings establish a baseline for sourcing potential mycorrhizal inocula in Eastern Joshua tree seedling survival studies within the burn scar.

30. Leta Landucci, llanducci@ucdavis.edu UC Davis

Floral nectar hydrogen peroxide as a microbial filter

Leta Landucci, Rachel L. Vannette

Flowering plants offer nectar to attract pollinators. Meanwhile, plants protect their nectar resources by reducing unwanted microbial growth. The antimicrobial hypothesis argues that “repellent” compounds present in nectar may be a defensive adaptation against nectarivorous microbes. However, the antimicrobial hypothesis currently lacks wide applicability given that its assumptions are based on measurements from a limited number of plant species and has not been tested using microbes that are specialized to nectar and other harsh environments. Here we test the hypotheses that (1) flowering plants produce hydrogen peroxide at levels sufficient to combat nectarivorous microbial growth, (2) nectar-specialized microbes are more tolerant to elevated hydrogen peroxide nectar conditions compared to microbes from other environments, (3) plants can increase nectar H2O2 levels in the presence of microbes as antimicrobial defense and (4) bumble bees may also use hydrogen peroxide within a microbial filter to protect their honey pot stores. We used bioassays to examine microbial growth of strains isolated from floral nectar in a field-relevant range of H2O2 concentrations. Preliminary results suggest that ecologically relevant H2O2 concentrations reduce growth of microbes except for a specialized nectar yeast which only experienced reduced growth at extremely high H2O2 concentrations. Additionally, we tested if plants induced with methyl-jasmonate increase floral nectar H2O2. Preliminary results show that treatment with Me-JA significantly increases nectar H2O2 concentration. Future work will incorporate field-based studies to explore the role of hydrogen peroxide within nectar microbial filtration and to investigate how microbes themselves may modify H2O2 concentrations via detoxification mechanisms. Finally, to probe the presence of H2O2 in *Bombus impatiens* honey pot stores we used a colorimetric assay and found high levels of H_2O_2. Preliminary results suggest that H_2O_2 may be a widespread regulator of microbial growth in floral nectar and pollinator-associated environments and that microbes specialized to these environments show adaptations to grow despite high H_2O_2 concentrations.

31. Jeanmaire Molina jmolina2@pace.edu Pace University

A symbiotic trifecta: the world's largest parasitic flowers, their host plant, and the microbes that live within

Denia Diaz, Anastasiia Kirdiianova, Jeanmaire Molina

The plant family Rafflesiaceae, composed of *Rafflesia, Sapria, and Rhizanthes*, is known for producing the largest iconic flowers in the world, but are endangered, being restricted to the fast-disappearing tropical forests of Southeast Asia. They are solely parasitic to the plant genus *Tetrastigma* of the grape family. Since Rafflesiaceae species are parasites, and it is unknown how they infect their *Tetrastigma* hosts, it has been difficult to propagate and conserve them ex situ by traditional horticultural techniques. However, bacterial endophytes--bacteria that reside inside plant tissues--have shown to play a major role in promoting the growth of plants. We hypothesize that Rafflesiaceae species may also rely on certain bacteria to facilitate their growth inside the host plant. In this study, we characterized and analyzed the microbiome of *Tetrastigma* roots infected with Rafflesia speciosa and *Sapria himalayana* and compared this to that of uninfected *Tetrastigma* using metagenomic sequencing. We found evidence that Rafflesiaceae-infected hosts have a different microbiome compared to uninfected *Tetrastigma*. This could suggest that either these plant parasites transfer their bacterial endophytes into their host altering host microbial composition for their benefit, or that only *Tetrastigma* roots with a certain microbiome are vulnerable to Rafflesiaceae infection. These newly characterized bacterial endophytes could potentially serve as bioinoculants that may have applications in the *ex situ* propagation and conservation of Rafflesiaceae species.

32. Michelle Munguia Figueroa mmunguiafigueroa@ucsd.edu UC San Diego

Establishing a model system to study coevolution between an animal and its microbiome

Michelle Munguia-Figueroa, Justin Meyer

Animal bodies, particularly the gut, provide a favorable habitat for the colonization, survival, and remodeling of microbial communities. Microbial communities, in return, influence the physiology and fitness of their host. Moreover, they have been shown to foster adaptation and acclimatization to noxious environmental conditions, like toxins. Because of this, it has been hypothesized that hosts and their microbiomes may coevolve to enhance these benefits. However, studies on the effects of long-term associations and coevolution between hosts and their associated bacteria are still scarce and inconclusive. We are introducing a model system using the rotifer species *Brachiounus calyciflorus*, a microscopic freshwater zooplankton, and their associated bacteria, to establish a setting for the study of animal-microbe interactions over evolutionary time scales. Using rotifers for evolution studies provides several advantages due to their short generation time (around 48 hours), and ease of manipulation, maintenance and characterization of individuals and large populations. We have developed a series of protocols for the sterilization of rotifers to efficiently make germ-free strains, and methods for inoculation of rotifers. Likewise, we have developed high throughput methods to characterize rotifer life history traits. Using these techniques, we have observed that the microbiome affects rotifer's population growth by modulating their reproductive and survival rates under a standard microalgae diet. We will next inoculate germ-free rotifers with individual bacteria or microbial assemblages from different natural sources to test how increasing microbiome diversity can enhance metagenomic plasticity and aid in the health and/or adaptation of their host. We will also inoculate rotifers with microbiomes that have coevolved with rotifers for varying amounts of time to test the role coevolution plays in strengthening their interactions and benefits. For this poster we will report our progress and explore future studies.

33. Emily Neff, eneff@calacademy.org California Academy of Sciences
The genetic diversity of a bioluminescent fish-bacteria symbiosis over a broad geographic range
Emily Neff, Alison Gould

Hosts have a plethora of options when choosing their microbial symbionts via horizontal transfer, yet they continue to exhibit host-symbiont specificity. The tropical fish *Siphamia tubifer* and their luminous bacterial symbiont *Photobacterium mandapamensis* show high specificity within a small geographic range in Japan, however the extent of this specificity over the host's broad geographic distribution from the Red Sea to Micronesia remains unknown. To answer this question, I obtained *S. tubifer* specimens from one site in Japan and two sites in the Philippines, one of which was much deeper than the other, and used their light organs to perform whole genome sequencing (WGS). I extracted total DNA from 11-13 light organs from each site, resulting in WGS data for both the host fish and its bacterial symbiont. I then identified single nucleotide variants (SNVs) for the host and symbiont and examined patterns of genetic divergence of both. I also inferred the phylogeny of the symbiotic bacteria to determine whether there are divergent clades associated with different host populations or regions. Our preliminary results indicate that all light organ symbionts were identified as members of Clade II of *P. mandapamensis*, which confirms previous findings that the association is highly specific. However, there is no evidence of genetic divergence between the three sampling locations, which indicates that hosts in Japan and the Philippines have overlapping symbiont populations. We are now expanding our sampling to include *S. tubifer* collected from the Red Sea and Sri Lanka to establish whether this highly specific association is maintained throughout the host's broad Indo-Pacific range.

34. Amaury Payelleville, amauryp@stanford.edu Stanford University
Entomopathogenic nematodes: From the concept of a mono-symbiont to the existence of a microbial community
A. Payelleville and T. Fukami

Entomopathogenic nematodes (EPNs) are found in the soil and are pathogenic to a broad range of insects. These nematodes are from two genera, *Heterorhabditis* and *Steinernema*, and are associated with specific symbiotic bacteria, *Photorhabdus* and *Xenorhabdus*, respectively. However, Ogier et al. (2020) have recently shown that in vitro strains of *Steinernema* are also associated with other bacteria belonging to diverse genera and that some of those species might be important to complete the nematode's life cycle. In a project we recently started, we aim to understand this association with a diversity of bacterial symbionts in the environment and their effects on the life cycle of the nemato-bacterial complex. We are focusing on EPNs pathogenic to the filbertworm *Cydia latiferreana* and the filbert weevil Curculio occidentis, both of which feed on acorns of California oaks. We are identifying EPNs in the soil at Jasper Ridge Biological Preserve located in the Santa Cruz Mountains, and characterizing the symbionts associated with each EPN. We are also identifying all associated bacteria throughout the EPNs' life cycle using meta-barcoding. Based on these results, we will investigate the symbionts' role in EPN-insect interactions using experimental evolution and molecular microbiology. We also plan to investigate the effects of wildfire and cultural burns on insect-nematode-bacteria interactions and their abundance in California oak woodlands.

35. Ryan J. Quaal, rquaal1206@gmail.com, UC Riverside
Effect of Long-Term Fire Retardants on Fungal Communities
Ryan J. Quaal, Dylan J. Enright, Sydney I. Glassman

As fires begin to increase in both size and severity, the application of long term fire retardants (LTFR) are becoming increasingly commonplace for forest fire prevention and home defense. LTFRs have been shown to drastically alter soil chemistry for years after application.The effect this may have on soil mycorrhizal fungal communities is largely unknown and drastically understudied, however changes in soil chemistry have been shown to alter the lifestyles and decrease the size of these fungal communities. With the help of the UCNRS, we applied Phos-Chek, a commonly used LTFR by California firefighters, to Q. agrifolia trees in the Emerson Oaks Nature Reserve. We analyzed roots for both ectomycorrhizal and arbuscular mycorrhizal fungal colonization, finding a decrease of 29.03% in ectomycorrhizal colonization and a decrease of 44.19% in arbuscular mycorrhizal colonization. Additionally, we analyzed soil nutrient composition, finding that LTFRs significantly increased soil phosphorus levels by 215.51%. We also found that LTFRs slightly altered the amount of nitrogen based nutrients, but did not lead to a significant change. Lastly, we analyzed fungal biomass in the soil using qPCR techniques, finding no significant change in soil fungal biomass but a trend downwards. Fire prevention is a necessity in our forests, however LTFRs may alter the size and function of fungal communities, potentially affecting their interaction with local vegetation and having far reaching impacts on post-fire environments.

36. Emma Roman, eroman@calacademy.org California Academy of Sciences
Priority effects in luminous bacteria from the light organ of a coral reef fish
Emma D. Román, Tadashi Fukami, Alison L. Gould

Symbiotic microorganisms rarely exist in isolation. Even in binary symbioses, many form multi-strain communities in their hosts, and the composition and function of these communities can be highly variable. Although some of this variation can be explained by the hosts' characteristics, even when the host environment appears identical across individuals, symbiotic communities can still differ greatly. One potential reason for this seemingly random variation is that communities develop differently depending on colonization history. Based on the colonization history, priority effects, which is when the initial relative abundance of strains determines how strains interact with one another, could further explain how multi-strain communities form. However, experimental tests documenting priority effects in symbiotic communities remain rare. We are investigating if strain-level differences can influence symbiont community assembly, using the luminous bacterium *Photobacterium mandapamensis* isolated from the gut-associated light organ of the coral reef fish *Siphamia tubifer*. In their natural environment, each *S. tubifer* fish hosts multiple strains of *P. mandapamensis* in the light organ, but strain composition varies among individuals with almost no overlap among them. Furthermore, strains differ in luminosity and growth rate, suggesting that they could be functionally different as symbiotic partners. In the in vitro experiments we have conducted so far, where the initial abundance of two strains was manipulated, we found strong priority effects. Specifically, when the abundance of a strain was initially low, how well it grew depended on the initial abundance of the other strain. The strain grew well when the other strain was absent or initially low in abundance, but did not grow at all when the other strain was initially abundant. In contrast, if a strain had high initial abundance, they grew equally well regardless of initial abundance of the other strain. These results suggest priority effects may be responsible for some of the unexplained variation in the strain composition and function of P. mandapamensis among S. tubifer individuals. We plan to extend these experiments to study interactions among more than two strains and also conduct similar experiments in vivo using captive fish.

37. Andrea Salinas Aguilar, ab.salinas28@gmail.com, UC Merced
Plant Microbiome Structure Across the Species Range of *Mimulus Laciniatus*
Andrea Beatriz Salinas Aguilar, Carolin Frank, and Jackie E. Shay

Plant microbiomes are an essential; component of the plant host and are widely known to ameliorate stress response. In the California Sierra Nevada, the endemic cutleaf monkeyflower (*Mimulus laciniatus*) is experiencing and adapting to various levels of stress associated with climate change, however, it is unclear to what extent the *M. laciniatus* microbiome is engaged in responding to this stress. This study aimed to determine the monkeyflower microbiome along with their growing substrate (moss or soil) across a steep elevation gradient in the species range during the 2019 growing season. Monkeyflower plants were collected and divided into compartments subsamples (roots, shoots, soil, and moss substrate) to asses the bacterial communities of each compartment by 16S rRNA gene sequencing to answer the following: (i) Do the *M. laciniatus* endophyte communities differ in composition and diversity across the *M. laciniatus* species range, and does this composition vary by elevation? (ii) Does the *M. laciniatus* endophyte community differ between plant compartments (e.g. root and shoot)? (iii) Do soil and moss, from which M. laciniatus grows, serve as a source of *M. laciniatus* endophytes, and do soil and moss communities vary in structure across the *M. laciniatus* species range? The results showed that the bacterial community structure of both plant compartments and the substrate differed across the range of *M. laciniatus*, and that compartment strongly influenced bacterial communities. Elevation played a role in community variation, and differences in bacterial community structure could be due to local climate or metacommunity. This study highlights the importance of plant compartment and local climate or metacommunity in shaping bacterial communities along the elevation gradient of plant species.

38. Dino Lorenzo Sbardellati, dlsbardellati@ucdavis.edu, UC Davis
Examining how *Acinetobacter* bacteriophage adapt to the ecology of their hosts
Dino L. Sbardellati, Rachel L. Vannette

Bacteriophage (phage), the viruses which infect bacteria, are important biological entities and play key roles in bacterial population and community dynamics. While phage are known to specialize in infecting particular species and strains of bacteria, how phage adapt in response to the ecology of their host is less well understood. *Acinetobacter* is a taxonomically and ecologically diverse genus of bacteria. Here, we leverage *Acinetobacter* diversity to examine if, and how, phage adapt to the ecology of their bacterial hosts. We first download NCBI *Acinetobacter* assemblies from four broad ecological groups (human, animal, plant, environment) and bioinformatically mine these assemblies for integrated phage signatures using the program VirSorter2. After extracting putative phage sequences from assemblies, we examined the distribution of inferred phage across the range of our *Acinetobacter* assemblies. Next, we annotated putative phage and constructed gene and genome networks to compare phage gene content and gene sharing across the taxonomic and ecological diversity of our phage sequences. Overall, we identified 2,268 putative phage from 1,114 *Acinetobacter* assemblies. When combined with 64 known Acinetobacter phage genomes, our putative phage genomes encoded a total of 99,007 genes which clustered into 13,154 unique gene clusters (35% AAI). Our results show that some gene clusters were present in phage from each of the ecological groups of *Acinetobacter* considered, indicating a broad importance for *Acinetobacter* phage. In contrast, some clusters were only found in phage infecting Acinetobacter from particular ecological habitats, indicating key genes important for infecting particular Acinetobacter species or ecological groups.

39. Peter Zee, zee@olemiss.eduUniversity of Mississippi
The role of disturbance in the evolution of mutualism
Peter C. Zee, Nayan Chawla

Microbes often exist in spatially structured environments where interactions among individuals are mediated by extracellular chemical interactions. These complex community interactions range from antagonistic to mutualistic. A body of both empirical and theoretical work has demonstrated the importance of spatial structure for the evolutionary maintenance of facilitative interactions both within and among species. Here, we explore the effects of temporal variation in spatial structure on the emergence of mutualistic interactions from antagonistic ancestry. More specifically, we explore whether spatial structure may constrain the emergence of facilitative interactions in microbial communities in certain cases. We developed a spatially-explicit model of microbial biofilms where individual fitness is determined by extracellular compounds released by neighboring cells. In this eco-evolutionary model, we track both species densities and allele frequencies simultaneously. Confirming results from previous work, we find that spatial structure and local dispersal facilitate the maintenance of mutualistic interactions in communities. However, we also find that intermittent lapses in spatial structure have the ability to promote the emergence of facilitative interactions. We test a range of scenarios varying mutation rates, disturbance regimes, modes of dispersal, and sizes of interaction neighborhoods for their impacts on evolution of ecological interaction types in the community.

Attendees (no presentation)

40. Francine Arroyo farroyo@mail.fresnostate.edu Fresno State University
41. Fernanda Batistel fernandabatistel@ufl.edu University of Florida
42. Celia Bertrand cbertrand2@ucmerced.edu UC Merced
43. Sarah Cohen sarahcoh@sfsu.edu San Francisco State University
44. Carolin Frank cfrank3@ucmerced.edu UC Merced
45. Nora Ismail nisma004@ucr.edu UC Riverside
46. Justin Kipness justin_kipness@brown.edu Brown University
47. Joel Sachs joels@ucr.edu UC Riverside
48. Kristal Watrous kwatrous@uci.edu UC Irvine
49. Ellen Yeh ellenyeh@stanford.edu Stanford University

Email list:

emilyagu@usc.edu, tinkara.bizjak@slu.se, satya.spandana.boddu@emory.edu, julia.boyle@mail.utoronto.ca, christopheriancarlson@gmail.com, dcheam@ucmerced.edu, sfrail@stanford.edu, jacob.franci@gmail.com, agould@calacademy.org, hammert@uci.edu, jmacc003@ucr.edu, rowan.mclachlan@oregonstate.edu, pperez40@ucmerced.edu, bpipes@ucmerced.edu, iramalhodasilva@ucmerced.edu, nvillabo@uci.edu, cwilliams@sdzwa.org, williams.claire.e@gmail.com, jae.wang@mail.utoronto.ca, wangpu@grinnell.edu, mlwarren@stanford.edu, heidi.abresch@umontana.edu, cbivins@ucmerced.edu, rcrus002@ucr.edu, jespinoza5@sfsu.edu, ajouk001@ucr.edu, , pgonzalezmoreno@ucmerced.edu, llanducci@ucdavis.edu, jmolina2@pace.edu, mmunguiafigueroa@ucsd.edu, eneff@calacademy.org, amauryp@stanford.edu, rquaal1206@gmail.com, eroman@calacademy.org, ab.salinas28@gmail.com, asimonse@fiu.edu, dlsbardellati@ucdavis.edu, zee@olemiss.edu, farroyo@mail.fresnostate.edu, fernandabatistel@ufl.edu, sarahcoh@sfsu.edu, cfrank3@ucmerced.edu, nisma004@ucr.edu, nish@ucmerced.edu, justin_kipness@brown.edu, joels@ucr.edu, kwatrous@uci.edu, mum55@psu.edu, ellenyeh@stanford.edu, cbertrand2@ucmerced.edu

Funding generously provided by:

GORDON AND BETTY
MOORE
FOUNDATION