2024 GRADUATE ORGANIZATION OF BIOLOGICAL SCIENCES RESEARCH SYMPOSIUM

SCHEDULE OF EVENTS

9:30-10:45 Oral Presentations, Session 1

10:45-11:00 Trail Mix Bar

11:00-12:30 Poster Presentations

12:30 – 1:30 La Masa Empanada Buffet

1:30 – 2:45 Oral Presentations, Session 2

3:00 – 3:15 Closing Remarks and Award Ceremony

* Oral presentations in Alumni Fireside Lounge

** Poster presentations in Union Ballroom West (B)

BIOLOGICAL SCIENCES

ORAL PRESENTERS (Alumni Fireside Lounge)

SESSION 1 – *9:30 to 10:45*

9:30 Fatemeh Cheraghi
9:45 Madison A. Rittinger
10:00 Sage A. DeLong
10:15 Kimberly Mayer
10:30 Drew Little

SESSION 2 – *1:30 to 2:45*

1:30 Sara Seidita
1:45 Anish Chakraborty
2:00 Alexander Sweet
2:15 Sian-Yong Wei
2:30 Hossein Asgari

POSTER PRESENTERS (Union Ballroom West)

Nery M. Zamudio Bautista *(poster #1)*
Delaney Carolan & Noah Pison *(poster #2)*
Saswata Chakrabarty *(poster #3)*
Mohamed S. Fayez *(poster #4)*
Katelyn Flitcroft *(poster #5)*
Chandika RG *(poster #6)*
Ton Nu Bao Vy Huyen *(poster #7)*
Kristin Huelsbeck *(poster #8)*
Shreyashi Mitra *(poster #9)*
Madeline Opie *(poster #10)*
Andrew Pagel *(poster #11)*
Samuel Pruhs *(poster #12)*
Hassan Richardson *(poster #13)*
Florin Saitis *(poster #14)*
Chloe Skinner *(poster #15)*
John Joseph Srok *(poster #16)*
Harleen Tewatia *(poster #17)*
Parnian Vakili *(poster #18)*
Olivia Valle *(poster #19)*
Arabella Voegeli *(poster #20)*
Gabriella Voit *(poster #21)*

ORAL PRESENTATION ABSTRACTS (organized by time)

9:30 - 9:45

Using *Caenorhabditis elegans* to test the impact of 14-3-3 on Raf protein levels and localization.

Fatemeh Cheraghi and Claire C De La Cova*

Noonan Syndrome (NS) is a member of a group of human developmental disorders termed RASopathies that are associated with increased Ras signaling. NS affects 1 in 1,000 individuals and is characterized by cardiac abnormalities, short stature, and facial dysmorphia. Gain-of-function mutations in RAF1, a member of Raf family kinases, are found in ~5% of NS patients. Raf kinases contain three conserved regions. An N-terminal regulatory domain is responsible for Ras binding and kinase autoinhibition. A second region, termed CR2, is a phosphorylated motif that interacts with 14-3-3 proteins. Finally, a C-terminal kinase domain is responsible for catalytic activity. The CR2 motif and a second 14-3-3 site, located near the C-terminus, promotes 14-3-3 binding and kinase autoinhibition. In NS patients, a large number of RAF1 mutations are clustered at or around the CR2 14-3-3 binding site. Previous studies showed that although many NS mutations result in increased kinase activity, others do not. We use *Caenorhabditis elegans* to assess the impact on Raf activity, protein abundance, and localization when either of the conserved 14-3-3-binding sites is mutated. We used CRISPR/Cas9 techniques to generate alleles that disrupt the CR2 and the C-terminal 14-3-3 binding sites. Mutation of the CR2 site did not cause obvious phenotypes. In contrast, mutation of the C-terminal site resulted in a vulvaless phenotype characteristic of Raf loss of function. In a double mutant lacking both CR2 and C-terminal sites, the vulvaless phenotype was partially suppressed. These results are consistent with a model where the C-terminal site contributes to Raf activation, and the CR2 site contributes to Raf inhibition. In future studies, we will test how Raf levels and localization are affected by 14-3-3 binding. An animal model to assess endogenous Raf levels and localization will contribute to our understanding of Raf regulation by 14-3-3 proteins and mechanisms underlying NS.

9:45 - 10:00

How Pholcus phalangioides Cellar Spiders (Araneae: Pholcidae) Solve Prey Capture Problems

Madison A. Rittinger and Rafael L. Rodriguez*

Animals encounter and solve a myriad of problems daily. How animals solve problems can

be specific to the species or to the individual. Species-specific solutions likely evolved and are innate, while individual-specific solutions may be learned or even novel. *Pholcus phalangioides* cellar spiders have a species-specific solution to capturing prey; yet, how cellar spiders solve new or rare problems capturing prey may vary between individuals. We investigated the problem-solving capabilities of cellar spiders by analyzing variation, both within and between individuals, in the behavior they use to capture multiple prey simultaneously. Overall, there were three main solutions that cellar spiders utilized to solve this prey capture problem: keeping prey in situ; gathering prey in situ; and bundling prey. We found that there was individual variation in the solutions used to this problem, and that individuals did not always use the same solution across trials. This suggests that spiders solve new prey capture problems by integrating current information about their environment, which requires more complex cognitive processes than solving problems using innate or previously learned solutions. In conclusion, this research explores how the behavioral flexibility of animals such as web-building spiders, whose behavior is typically explained via 'hard-wired' motor routines or basic learning abilities, can help understand the range of cognitive abilities different brain architectures can afford and how animals evolve to confront problems that arise in their lives.

10:00 - 10:15

Vibrations speak louder than words: vibrational signals communicate different information under two pre-copulatory contexts in red milkweed beetles

Sage A. DeLong, Camille Desjonquères, Rafael L. Rodríguez, Lauren A. Cirino*

Substrate-borne vibrational communication is commonly used by herbivorous insects to communicate species-specific information. This form of communication is underexplored, and many discoveries of the form and functional significance of these vibrations have yet to be made. Here, we report on plant-borne vibrational communication in red milkweed beetles, *Tetraopes tetrophthalmus* (*Coleoptera*: *Cerambycidae*). Red milkweed beetles, like other cerambycids, commonly use stridulation to produce audible squeaks when in stressful situations and can also produce plant-borne vibrations when in contact with conspecifics. These beetles engage in male contests for territory with females which ultimately results in mating opportunities. We placed beetles into three behavioral scenarios: male-male (contests), male-female (courtship), or male-male-female (contests and courtship). We used laser vibrometry and found that red milkweed beetles communicate by producing plant-borne vibrational signals composed of low-frequency rumbles and high-frequency clacks during male-male contests and copulatory courtship. We then compared the spectral and temporal features of the vibrations between the two pre-copulatory contexts. We found that vibrational signals were shorter, had a higher dominant frequency, and a lower clack rate when beetles engaged in contests compared to

courtship behaviors. Our results show that beetles use plant-borne vibrations as context-dependent signals that communicate different information under these two pre-copulatory scenarios. Our data also suggests that context-dependent signals can help beetles gain access to mates.

10:15 - 10:30

Novel Link between Ire1 and MAPK Slt2 Pathways via Transcription Factor Rlm1 in Yeast *Saccharomyces cerevisiae*

Kimberly Mayer, Anish Chakraborty, Jagadeesh Uppala, Madhusudan Dey*

Accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) causes ER stress, which initiates a network of signaling pathways collectively known as the unfolded protein response (UPR). The UPR is compromised when yeast cells lack an ER-resident RNase Ire1, or a cytosolic protein kinase Slt2. Ire1, a conserved RNase, activates the UPR by cleaving an inhibitory intron from HAC1 mRNA in yeast *Saccharomyces cerevisiae*. The spliced HAC1 mRNA then translates a transcription factor that activates expression of protein-folding enzyme genes to mitigate ER stress. In contrast, the role of Slt2 in UPR is not clearly defined. Slt2, a conserved mitogen activated protein kinase (MAPK), is known to phosphorylate multiple substrates, including the transcription factor Rlm1. We observe that both the splicing of HAC1 mRNA and the expression of Hac1 protein were reduced when cells were exposed to ERnstress in yeast cell lacking Slt2 (slt2Δ) or its substrate Rlm1 (rlm1Δ). Interestingly, we also observe that Ire1 was activated immediately after exposing cells to an ER stressor, whereas Slt2 at is activated at a later stage (at least after 2 hours). These findings suggest that UPR has two phases: (I) an early phase occurring within two hours of ER stress induction and (II) a later phase extending beyond two hours. The early phase predominately operates through the Ire1 pathway. However, in the later phase, the Ire1 pathway is regulated by additional factors like MAPK Slt2. Furthermore, bioinformatics analysis predicts that Rlm1 can bind to the Ire1 promoter. Consistent with this prediction, we observe that a high-dose of Ire1 restored the diminished UPR in the slt2Δ or rlm1Δ strain. Together, our results provide compelling evidence that Slt2 activates the Ire1 signaling pathway by enhancing Ire1 expression through Rlm1 during the sustained UPR.

10:30 - 10:45

Context dependent signaling in *Enchenopa binotata*

Drew Little and Rafael Rodriguez*

In this experiment we placed male and female *Enchenopa* treehoppers in different social grouping to determine if individuals adjust their signaling behavior based on who is

present. What we found was that courtship in this species is a multi-stage process with males incorporating 7 additional signals after establishing contact with females as well as having unique signals only produced when competing males are present. We also found that females have a more involved cooperation in courtship with a previously undiscovered signal which is necessary for males to attempt mating.

1:30 - 1:45

Function of Spontaneous Signals in Enchenopa Treehoppers

Sara Seidita and Rafael Rodriguez*

Natural environments have varying degrees of noise that can disrupt communication signals. Many animals communicate with plant-borne vibrational signals that can easily be influenced by wind-induced noise. Enchenopa treehoppers are herbivorous insects that communicate with plant-borne vibrational signals and use male-female duetting in pair formation. In addition to signaling in direct response to males, Enchenopa females also sometimes produce duetting signals in the absence of recent male signals. We hypothesized that female Enchenopa treehoppers may attempt to compensate for vibrational duetting interference from wind noise by using spontaneous signals to re-establish communication with a potential mate. We presented females with plant-borne vibrational playbacks of attractive and unattractive male signals with and without wind generated by a computer fan. Females were more responsive with attractive stimuli than unattractive stimuli regardless of wind, and without wind regardless of stimuli. Females produced spontaneous signals more often in the presence of wind during a playback regardless of stimuli. Females also produced spontaneous signals more often without wind in the one-minute after a playback. We conclude that female Enchenopa treehoppers use their spontaneous signals to sustain and re-establish communication with potential mates, but not as a function of wind noise conditions.

1:45 - 2:00

Essential Kinases Pkh1 and Ypk1, Orthologues of Mammalian Kinases PDPK1 and SGK1 Regulate Cytosolic Splicing of HAC1 mRNA by Modulating the Abundance of Ire1 Protein

Anish Chakraborty, Jagadeesh Kumar Uppala, Saswata Chakraborty, Madhusudhan Dey*

The unfolded protein response (UPR) is a cellular strategy to enhance the protein folding capacity of cells. A key player of UPR is the dual protein kinase/RNase Ire1, responsible for cleaving HAC1 mRNA in yeast cells or XBP1 mRNA in mammalian cells, thereby removing the inhibitory intron and de-repressing its translation. Hac1 and Xbp1 proteins serve as a

transcription factors, activating the expression of genes encoding protein-folding enzymes to alleviate ER stress. Recent studies indicate the significant involvement of additional kinases, including Slt2 and Tor2 (an essential kinase), in the UPR. These findings suggest that the multiple signaling pathways, involving essential kinases, operate concurrently to achieve an optimum UPR. To explore the potential role of essential kinases in the UPR, we investigated Hac1 protein expression in yeast cells carrying the temperature-sensitive (ts) allele of essential kinases. The levels of Hac1 protein were significantly reduced in yeast strains carrying the ts-allele of protein kinase Pkh1 (pkh1 ts) or its substrate kinase Ypk1 (ypk1 ts) when briefly grown at the non- permissive temperature (37 o C) in the presence of an ER stressor. At 37° C, the cytosolic splicing of HAC1 mRNA was also inefficient in both pkh1 ts and ypk1 ts strains. Interestingly, the Hac1 protein expression from the splicing independent HAC1 mRNA remained unaffected in the pkh1 ts strain. Additionally, a notable decrease in the Ire1 protein level was observed in both pkh1 ts and ypk1 ts strains. Furthermore, over-expression of Ire1 enhances the cellular response to ER-stress of the ypk1 ts strain. Together, our results demonstrate that the Pkh1-Ypk1 signaling pathway plays a regulatory role in cytosolic splicing of HAC1 mRNA by influencing the abundance of the Ire1 RNase.

2:00 - 2:15

Where did she go? : Changes in social context alter calling strategies of Eastern Gray Treefrogs

Alexander Sweet, Olivia Feagles, Gerlinde Höbel*

How a species reacts to a change in social context can be critical in understanding its overall behavior, especially during courtship displays. In lekking species this is of utmost importance as behavioral adjustments in response to social context can have great ramifications on an individuals mating success. Here, we explore how male Eastern Gray Treefrogs (*Hyla versicolor*) change their calling strategies in response to a change in social context. Male Eastern Gray Treefrogs emit advertisement calls consisting of a series of short pulses (ranging from 5-30), and those displaying more pulses (longer duration call) or a faster call rate are considerably more attractive to females. However, males have finite energy and are limited to one high energy call trait (duration or rate). We recorded individual males across two social contexts and take into consideration environmental change. We first recorded lone, naturally displaying males in the pond, then united each male with a female to form a mating pair (amplexus) for an hour, then recorded the males again in response to amplexus interruption. We also accounted for changing environmental factors such as temperature throughout this process. The results show that male frogs before amplexus invest energy into emitting longer duration calls, but after amplexus separation invest energy into a faster call rate instead. Even though the call strategies shift

from prioritizing longer to faster, they maintain similar energy outputs (i.e. no change in duty cycle). We also found a significant effect of male ID, indicating a unique individual element to calling behavior despite the significant change in strategy. Temperature also had a significant effect on male calling, but not in a manner that compromised the significance of social context.

2:15 - 2:30

Isolation and characterization of anti-fungal metabolites from a biocontrol bacterium targeting *Botrytis cinerea*

Sian-Yong Wei, John Joseph Srok, Ching-Hong Yang*

Botrytis cinerea, a necrotrophic fungus, poses a significant threat to agriculture, infecting hundreds of dicotyledonous and a few monocotyledonous plant hosts, causing gray mold and bunch rot. Chemical control is one of the vital strategies in controlling *B. cinerea* infection in the field. However, the emergence of fungicide resistance in *B. cinerea* underscores the urgent need for new anti-fungal solutions. Exploring environmental sources for anti-fungal metabolites has become an important strategy in the fight against fungal disease. In the present study, the bacterial strain JS239 was isolated from a blue-green citrus root weevil. The crude metabolites from JS239 were extracted by using ethyl acetate. The extracts exhibited a potent inhibitory effect on *B. cinerea* growth. Through flash chromatography with silica-gel column, the metabolite mixtures were separated, and bioactive fractions were identified through inhibitory assays against *B. cinerea*. These were then analyzed using gas chromatography-mass spectrometry (GC-MS), matched against a mass spectral library. This process led to the identification of two compounds in the bioactive fraction. Future research will focus on identifying the major bioactive metabolite and evaluating its efficacy in gray mold disease control. This study illuminates the potential of compounds derived from JS239 strain as novel anti-fungal agents, paving the way for innovative strategies to manage fungicide resistance in plant pathogens.

2:30 - 2:45

On the function of the male front legs in *Sepsis* (*Diptera*: *Sepsidae*): evolutionary insight from behavioral detail

Hossein Asgari and Marjan Seiedy*

Male genitalia and the non-genital structures that contact the female during copulation diverge more rapidly than many other traits. We tested two hypotheses that attempt to explain the function of these structures and the causes of their evolution: the sexual conflict and the female choice hypotheses. We derived predictions from them in terms of

the nature of behavioral and morphological male-female interactions that they envision. We tested these predictions with two closely related species of sepsid flies: *Sepsis fulgens* and *violacea.* Traditionally, the species-specific structures of male sepsid fly front legs have been viewed as mechanical devices for clamping the female wings for mating. We found that the wings were neither sexually dimorphic nor species-specific. The general regions of the wing bases contacted by the species-specific structures of the male front legs contained numerous campaniform sense organs. Males dynamically stimulated females by moving their legs in rhythmic, stereotypic, and species-specific ways. Such copulatory courtship movements have never been described in sepsid flies. We conclude that male front legs are stimulation devices rather than species-specific mechanical clamps. These results support the female choice hypothesis, and emphasize the power of attention to behavioral detail to promote discovery.

POSTER PRESENTATION ABSTRACTS (organized by time)

POSTER 1: Rare Coexistance During Coevolution Between Phage U136B and its Host, *Escherichia coli*

Nery M. Zamudio Bautista and Alita R. Burmeister*

Natural selection can drive a population to adapt to a coevolving partner. In some cases, coevolution can end when one of the partners goes extinct. In laboratory experiments, bacteria and phage have been observed to undergo many cycles of coevolution, with some ending in extinction. During these cycles, phages select for phage resistance in bacteria, which can occur at the expense of other traits, including antibiotic resistance. In a previous study, an evolution experiment was conducted for 10 days with *E. coli* and phage U136B. During that experiment only 5 of the 10 phage populations survived to day 10. It was also found that phage infection selected for tolC mutant bacteria that had increased antibiotic sensitivity. However, it is not yet known how long phage U136B and *E. coli* can coevolve with each other and what, if any, mutations occur that allow for their sustained coevolution. It is also unknown whether phage extinction alters the subsequent evolution of antibiotic sensitivity of the bacteria. To address these questions, we are conducting a coevolution experiment with phage U136B and BW25113. Our preliminary data shows that 3 of 20 populations of phage survive to 10 days, and only 1 of 20 populations persist to 26 days. In my proposed graduate work, I will study how phage U136B and *E. coli* BW25113 evolved over time, including identifying their evolved mutations, and quantifying changes to antibiotic resistance.

POSTER 2: Role of MMP28 in early placodal development using *Xenopus*

Delaney Carolan, Noah Pison*, Nadège Gouignard*

Matrix metalloproteinases (MMPs) are mostly known for their extracellular matrix remodeling activity and their function in wound healing, cell proliferation, and migration. They are involved in pathological and physiological events including cancer, tissue homeostasis, and embryogenesis; however, our understanding of their molecular function remains incomplete. Cranial placodes (CP) are embryonic cells that will give rise to most of the head pair sensory organs (inner ear, lens, olfactory system) and the facial nerves. Defects in the CP development program are responsible for numerous diseases such as Branchio-oto-renal syndrome, anosmia, and deafness. In *Xenopus laevis*, MMP28 is expressed in the pre-placodal region, a domain that emerges in the anterior dorsal ectoderm at the neurula stage. At the tailbud stage, MMP28 expression is maintained in the epibranchial placodes that give rise to facial nerves. Previous experiments in the lab have shown that placodal MMP28 controls the development of the neighboring cranial neural crest. However, the role of MMP28 during the CP development itself was not investigated. Here we focus on understanding MMP28 function during CP development using *Xenopus laevis* as a model system. We performed loss of function experiments using morpholino oligonucleotides to transiently knockdown MMP28 expression. Embryos were then collected at neurula and tailbud stages, and whole mount in situ hybridization was performed. We used a set of CP markers to evaluate the effect of MMP28 knockdown on the development of the placodes. Our results showed that the plan placodal marker (Six1) and the epibranchial marker (Pax8) were downregulated at neurula stage. The olfactory marker (Dmrta1) showed downregulation at both neurula stage and tailbud stage. And finally, the otic placode marker Sox9 was also strongly affected by the lack of MMP28 at tailbud stage. Taken together our data showed that MMP28 plays a key role in placodal development and potentially their derivatives.

POSTER 3: Auto-inhibitory Intra-domain Interactions of Phosphoinositide-dependent Kinase-1 (PDPK1) are Released by Phosphorylation of the Respective Domains

Saswata Chakrabarty, Jagadeesh K Uppala, Anish Chakraborty, Madhusudan Dey*

Human PDPK1 is a master protein kinase, which plays a crucial role in cell growth and differentiation by phosphorylating and activating multiple protein kinases, including Akt/PKB, S6K and SGK. PDPK1 contains an N-terminal region (NTR) spanning 75 amino acids, a central kinase domain (KD), and a C-terminal region (CTR). The CTR

contains a pleckstrin homology (PH) domain which is shown to bind and inhibit the PDPK1-KD. However, the specific role of the PDPK1-NTR remains elusive, and the mechanisms underlying the activation of PDPK1-KD from its inactive state are not fully understood. Human PDPK1 rescues the temperature-sensitive growth in a yeast strain carrying a temperature-sensitive (ts) allele of the protein kinase Pkh1 (pkh1 ts). We systematically deleted residues from both the NTR and CTR of PDPK1 and tested the ability of truncated proteins to rescue temperature-sensitive growth of the pkh1 ts strain. Our deletion analyses identified inhibitory regions within both the NTR (residues 28 to 50) and CTR (residues 445 to 556). Subsequent yeast two-hybrid assay demonstrate that the identified inhibitory regions bind to PDPK1-KD. Furthermore, examination of the mass spectrometry databases revealed the autophosphorylation sites within these inhibitory regions, particularly residues T33 and T37 within the NTR and T513 within the CTR. Interestingly, we observed that substituting these residues (T33, T37 and T513) with the phospho-mimetic equivalents abolished the interaction between the PDPK1-KD and the NTR or CTR. The results collectively suggest that autophosphorylation at residues T33, T37 and T513 release the auto-inhibitory intra-domain interactions within PDPK1. By means of genetic screening, we have detected certain mutations (S258A, G468R, E507A, T518A) that augment the activity of PDK1, along with variations (P39A & S241A) that diminish its function. The identification of these mutations was accomplished by creating a library of mutants. Significantly, the G468 and T518 were located on the same exon 13 of the PDK1 gene. Furthermore, the G468R or T518A mutations caused a disruption in the connection between the kinase and PH domains of PDK1. This gain of function mutation necessitates more investigation to determine its involvement in the over-activation of subsequent pathways that contribute to the development of diseases.

POSTER 4: Exploring the Molecular Mechanisms of Evolutionary Trade-Offs between Phage Resistance and Antibiotic Resistance

Mohamed S. Fayez and Alita R. Burmeister*

Bacteriophages are a potential alternative to chemical antibiotics for control of bacterial pathogens. One such phage, U136B, uses the antibiotic efflux pump protein TolC and lipopolysaccharide (LPS)as receptors when infecting the K12 strain of *E. coli*. When K12 bacteria evolve resistance to thisphage, they often do so through mutations that either inactivate TolC, and therefore reduce antibiotic resistance, or alter the LPS. However, it is not yet known how other *E. coli* strains interact with phage U136B, and if those strains follow the same evolutionary dynamics via TolC and LPS mutations. We are investigating these dynamics using phage plaque assays, receptor gene knockouts, and genetic complementation with a panel of four E. coli host strains: K12, B, uropathogenic *E. coli*

(UPEC), and enterohemorrhagic *E. coli* (EHEC). Here, we confirm U136B's host range on K12, B, and UPEC and reveal that it cannot infect wild-type EHEC. We also find that U136B is unable to infect a tolC knockout of E. coli B, but that infection can be restored through genetic complementation with tolC. Interestingly, we find that U136B has limited infectivity on EHEC mutants with altered O-antigen, suggesting that O-antigen serves as a U136B resistance factor. Our preliminary data also suggests that LPS O-antigen truncation, via an rfaJ knockout, may allow infection of rare U136B host-range mutants. In our proposed research, we aim to knock out the tolC and LPS-related genes and perform complementation assays in UPEC and EHEC. We will also use experimental evolution to test how phage resistance and antibiotic resistance evolve in these different strains when under selection by phage U136B. This work will reveal how host genetic background influences the evolution of phage resistance and corresponding changes to antibiotic resistance.

POSTER 5: Cell Specific Mechanisms of Fibroblast Growth Factor Signaling in *Caenorhabditis elegans*

Katelyn Flitcroft, Claudia S. Rodriguez Torres, Nicole B. Wicker, Michael J. Stern, Claire C. de la Cova*

Fibroblast Growth Factor Receptors (FGFRs) belong to the Receptor Tyrosine Kinase family and are necessary for animal development and a wide range of cellular functions. Ligand binding to FGFRs induces dimerization and activation of the intracellular tyrosine kinase domain. This leads to activation of multiple signaling proteins and pathways, including the GTPase RAS and the kinases RAF, MEK, and ERK. The *Caenorhabditis elegans* FGFR, EGL-15, is expressed in the sex myoblast and the hypodermis, where it is needed for sex myoblast migration and fluid homeostasis, respectively. Previous studies demonstrated that SEM-5, an adaptor protein required for RAS activation, interacts directly with the EGL-15 C-terminal domain (CTD). This interaction is required in the sex myoblast, as an EGL-15 mutant with a truncated CTD, which we term egl-15ΔCTD, does not permit cell migration. In the hypodermis, the EGL-15 CTD is not required, as the same mutant can still maintain fluid homeostasis. To quantify these results, we used ERK activity as a metric to assess the impact of the egl-15ΔCTD mutant. We hypothesized that ERK activation by the EGL-15ΔCTD mutant would be reduced in the sex myoblast yet unchanged in the hypodermis. To measure ERK activity, we used an in vivo, fluorescent biosensor termed the ERK Kinase Translocation Reporter (ERK-KTR). The ERK-KTR is a substrate of ERK and its phosphorylation state is monitored through nuclear/cytoplasmic localization within the cell. In the egl-15ΔCTD mutant, we observed that ERK activity was significantly reduced in the sex myoblast. However, ERK activity was moderately but significantly increased in the hypodermis. Our results suggest that EGL-15 uses cell specific signaling mechanisms,

relying on its CTD for signaling in the sex myoblast, and a CTD-independent mechanism for signaling in the hypodermis.

POSTER 6: A Versatile Low-Density Genomic Panel for Studying White-Tailed Deer Populations and Chronic Wasting Disease Monitoring

Chandika RG, Anais K. Tallon, David Navarro, Julie Blanchong, Peter Euclide, Caitlin Ott-Conn, Daniel Walsh, Randall DeYoung, Emily K. Latch.*

Chronic Wasting Disease (CWD) is a prion disease that affects white-tailed deer populations in North America. The disease is highly contagious and has been identified in 32 US states and four Canadian provinces. Effective mitigation of CWD transmission requires knowledge about deer connectivity and disease susceptibility. This study aims to use Genotyping-in-thousands by sequencing (GT-seq) to develop a custom SNP panel to assess genetic population structure, relatedness, and CWD risk across 11 US states. Our multistate collaboration facilitates integration of data across states, increasing capacity to look more holistically at white-tailed deer and CWD. We collected 1,969 samples from CWD+ and CWD- deer in collaboration with 11 US states (Minnesota, Idaho, Tennessee, Pennsylvania, Iowa, Indiana, Wisconsin, Mississippi, Texas, North Carolina, and Michigan). We designed a custom GT-seq panel with 578 loci, including anonymous loci distributed throughout the white-tailed deer genome, sex identification markers, and ~50 genes reported to be associated with CWD. Our GT-seq panel can efficiently inform genetic diversity in white-tailed deer populations across all 11 states, accurately characterize population structure and relatedness, and track the frequency of CWD-associated genes. In light of our findings, we discuss the utility of this custom SNP panel for targeted applications in CWD management, including assignment of individuals to populations of origin. By developing a single robust panel in a collaborative approach, this joint project will facilitate cross-jurisdictional surveillance and management to advance CWD control and maintain healthy, sustainable wild white-tailed deer populations.

POSTER 7: RejuArgo A: Pioneering a Sustainable Solution to Combat Fire Blight in Apple and Pear Orchards

Ton Nu Bao Vy Huyen, Jian Huang, Ching-Hong Yang*

Erwinia amylovora, the cause of fire blight, poses a significant challenge for apple and pear farmers, negatively impacting the agricultural economies of major regions including the United States, Europe, the Middle East, New Zealand, South Korea, and China. Despite extensive research and control efforts, effectively managing this persistent disease has proven difficult. Traditional methods, mainly the use of antibiotics such as streptomycin and oxytetracycline, are being compromised by the development of antibiotic-resistant

strains of *E. amylovora*, especially in California, Michigan, New York, and Georgia. This resistance not only reduces the effectiveness of these treatments but also concerns the potential spread of resistance genes to human-affecting pathogens and the broader ecosystem. Consequently, the need for alternative strategies is urgent. Our research has identified a promising candidate: RejuArgo A (RAA), a novel antimicrobial agent produced by the Pseudomonas soli T3-07 strain, isolated from Wisconsin soil. RAA has shown superior effectiveness in suppressing *E. amylovora* strains resistant to streptomycin. Tests in both greenhouse settings and field trials have confirmed RAA's ability to control fire blight effectively, achieving results comparable to streptomycin. The widespread use of the same antibiotics in plant, animal, and human health reduces the effectiveness of these treatments and jeopardizes the long-term sustainability of these drugs, highlighting the importance of finding alternative antimicrobial methods in agriculture. RAA represents a viable, environmentally friendly option for fire blight management, encouraging a shift away from traditional antibiotics toward more sustainable agricultural practices.

<u>POSTER 8:</u> Invaders eat their greens: the crustacean Hemimysis anomala grazes not only on zooplankton but also on algae

Kristin Huelsbeck and John Berges*

The mysid *Hemimysis anomala* (the 'bloody red shrimp') is a native of the Ponto-Caspian region that was first reported in the Great Lakes in 2006. We know it eats zooplankton; however, little is known about feeding rates or preferences for phytoplanktonic algae. Our goal was to determine feeding kinetics on two different phytoplankton species and also natural Lake Michigan communities of phytoplankton. Animals were collected from Lake Michigan and either: kept in aquaria for experiments, or immediately frozen to determine phytoplankton pigment in the gut. To determine grazing rates, animals were held in beakers and fed various concentrations of either the green alga *Chlamydomonas reinhardtii*, the diatom *Navicula sp.*, or concentrated Lake Michigan phytoplankton samples. Grazing rates were calculated from removal of cells (flow cytometry counts before and after) and ingestion of algae was estimated by measuring presence of alga pigments in the gut (extraction in solvent and fluorescence measurement). Hemimysis consumed *C. reinhardtii* at a maximum rate of about 12,000 cell ml^{-1} h^{-1} $animal^{-1}$, which half-saturated at a concentration of 130,000 cell ml^{-1} , and Navicula sp. was consumed at lower rates (925 cell $ml^{-1}h^{-1}$ $animal^{-1}$, which half saturated at 40,000 cell ml^{-1}), with evidence of inhibition at high concentrations. When fed natural phytoplankton samples, it was difficult to accurately estimate cells consumed, but gut pigments were much higher (325 µg chl a and phaeopigment h^{-1} $animal^{-1}$) than for the cultures (50-70 µg chl a and phaeopigment $h^{-1}animal^{-1}$). Samples collected directly from the field had gut pigment comparable to those fed natural phytoplankton assemblages. There was little evidence for differences in grazing

rates among adults and juveniles. Hemimysis are not necessarily carnivorous but feed significantly on algal cells, which could change predictions of their effects on Lake Michigan food webs.

POSTER 9: Revolutionizing citrus disease management: Evaluating RejuAgroA as an alternative to antibiotics in disease control

Shreyashi Mitra, Jian Huang, Manda Yu, Ching-Hong Yang*

Citrus is the most important fruit crop in the world that is grown commercially in more than hundred countries. Currently, the citrus industry is under significant challenges from two devastating diseases Huanglongbing (HLB) and Citrus canker. This has led to substantial losses in terms of production and economic loss. Despite extensive research efforts, effective control measures for HLB and citrus canker are still lacking. Recently, the use of clinical antibiotics, streptomycin and oxytetracycline, for disease control has been approved by the EPA which has shown to have improvement in tree health. The use of antibiotics raises concerns about the emergence of antibiotic-resistant bacteria, environmental contamination, and the looming health risks to humans because of antibiotic residues in the fruits. This study evaluates the potential of RejuAgroA (RAA), a natural metabolite from *Pseudomonas soli*
strain T3-07 in managing HLB and citrus canker. Foliar application of RAA at 20ppm has shown to significantly reduce *Candidatus Liberibacter asiaticus* (CLas) the causative agent of HLB and promote new growth in the infected trees. Notably, initial field trial also demonstrated that applying RAA via foliar spray at concentrations ranging from 20 to 30 ppm could reduce HLB severity to a degree comparable to the effects of Streptomycin administered at a dosage of 1160 ppm. Moreover, trunk injections of RAA show promising results in reducing CLas populations within a mere 7-day post- treatment. In the case of citrus canker, caused by Xanthomonas citri subsp. Citri, greenhouse and field trials of RAA revealed its potency to reduce the incidence of disease, demonstrating effectiveness comparable to that of streptomycin. These findings indicate that RAA could serve as a sustainable and effective substitute for antibiotics in managing HLB and citrus canker, contributing to the resilience of the citrus industry and ensuring the environment and consumer health.

POSTER 10: Which Vole is Which: DNA-based species identification for Wisconsin's three *Microtus* species

Madeline Opie and Emily Latch*

Accurate species identification is necessary to implement conservation strategies in the wild. When traditional morphology-based species identification is challenging due to phenotypic plasticity, overlapping characteristics, or the species are otherwise cryptic,

DNA-based species identification may be more suitable for the system. Of the three species of Microtus in Wisconsin, two are listed as threatened at the state level. Both *M. ochrogaster* and *M. pinetorum* have stable population levels at the national level but are along the northern edge of their ranges in Wisconsin. Small and vulnerable populations of *M. ochrogaster* and *M. pinetorum* are limited to isolated patches in the southwestern corner of the state. A primary challenge in conservation efforts is the overlap in distribution and habitat with the third and more common species, the meadow vole (*M. pennsylvanicus*). The three species have similar morphology, but *M. ochrogaster* and *M. pennsylvanicus* are nearly identical morphologically. To distinguish between the three species in the field, biologists rely on overall appearance, however, small differences in an individual organism's morphology can result in misidentification in the field. We evaluated two approaches for genetic species identification of the three species: a length polymorphism in the avrpr1a gene and novel, system-specific DNA barcoding in the COI gene. We evaluated methods using tissue and less invasive samples (fecal, whisker, and hair) that will minimize the need for handling animals in the field. A robust genetic species identification method will allow the Wisconsin Department of Natural Resources to gather accurate monitoring data and improve conservation efforts for *M. ochrogaster* and *M. pinetorum.* In addition, our methods for the development of system-specific DNA barcodes can be applied to similar systems that encounter challenges in morphology-based species identification.

POSTER 11: Modeling a Human Noonan Syndrome Mutation in *Caenorhabditis elegans* Raf

Andrew Pagel, Fatemeh Cheraghi, Claire C. de la Cova*

The MAP Kinase (MAPK) pathway allows animal cells to communicate signals relating to cell proliferation, differentiation, and apoptosis. Extracellular growth factors initiate signaling by binding to receptor tyrosine kinases (RTKs). The small GTPase Ras is one downstream effector of RTK signaling, and stimulates a kinase cascade comprised of Raf, MEK, and the MAPK ERK. Mutations in the human RAF1 gene are linked to Noonan Syndrome (NS), a developmental disorder involving heart defects, short stature, and cognitive deficits. Causative mutations associated with NS have been identified in three distinct conserved regions of the RAF1 protein: a 14-3-3 binding site, the kinase domain, and the C-terminal domain. These mutations have been shown to be gain-of-function and increase ERK signaling. However, the mechanisms by which mutations alter signaling and affect protein-protein interactions in NS are unknown. Using the nematode *Caenorhabditis elegans*, our lab has generated mutations in *C. elegans* Raf equivalent to those seen in the human RAF1 gene of NS patients. We have used gene editing to insert green fluorescent protein (GFP) at the N-terminus of the Raf gene. This endogenous GFP- tagged Raf, along

with the transparent nature of *C. elegans*, allows us to observe Raf protein levels and localization with confocal microscopy. During my SURF (Support for Undergraduate Research Fellows) experience, I generated the NS mutation L648V. I am planning to investigate how L648V affects Raf protein levels, localization, and ERK activity in the *C. elegans* germline and vulval precursor cells. Our long-term goal is to gain a better understanding of Raf function and to develop treatments that normalize Raf activity in human NS patients.

POSTER 12: Shrimp Munch on Microplastic Lunch: Quantification of Microplastics in a Lake Michigan Invader, the crustacean H*emimysis anomala*

Samuel Pruhs and John Berges*

The opossum shrimp, *Hemimysis anomala* is a Ponto-Caspian native that invaded the Great Lakes waterways in the mid 2000's. Microplastics harm wildlife by leeching harmful chemicals, and accumulate in natural water, however, we know little about their effects in Great Lake foodwebs and nothing about their ingestion by zooplankton. We quantified microplastics in *H. anomala*, sampled from Lake Michigan, using the fluorescent stain Nile Red, epifluorescence microscopy and the image analysis. Animals were sampled from a Lake Michigan breakwall using lighted funnel traps. Some were immediately frozen to examine microplastic gut contents, while others were kept in aquaria for control experiments. To validate the methods, animals were allowed to graze on polyethylene microspheres (45-53 μm diameter) for three hours, while control animals were maintained in filtered water. Animals were digested to remove organic matter (which could interfere with staining) using Fenton's Reaction (30% H_2O_2 and 7.2 mM Fe(II)SO_4), collected on filters, and stained with Nile Red (1 μg/mL in 55% DMSO). Samples were visualized under epifluorescence microscopy (40 x magnification, excitation 490 nm, emission 520 nm) and images quantified using ImageJ and custom-designed software, calculating quantity, area, and feret (largest linear length) of microplastic particles. When fed microspheres of known size, the method correctly quantified the plastic particles from the gut contents, validating the method. Microplastic particles were found in all animals, varying from 4 to 101 particles and 3 to 7240 μm in diameter. These data support the idea that *Hemimysis anomala* ingest microplastic particles and could serve as a model organism to examine the effects of microplastics in the Great Lake food web.

POSTER 13: Environmental Conditions that may Predict Observations of the Critically Endangered Grenada Frog.

Hassan Richardson, Billie Harrison, Emily Latch*

The Grenada frog (*Pristimantis euphronides*) is a montane species endemic to the island of Grenada in the Lesser Antilles. P.euphronides is listed as critically endangered on the IUCN Red List due to limited range and habitat connectivity, disease, and interspecific competition. Our previous research explores the potential links between the presence of *P.euphronides* and its competition, mainly the introduced Johnstone's Whistling Frog (Eleutherodactylus *johnstonei*). Other resident species (*Anolis spp.*), seasons, weather conditions, and temperature were also separately compared to *P.euphronides* observation frequencies as they may reveal notable correlations. Considering these environmental conditions, nonetheless, it remains difficult to determine where suitable habitats still support *P.euphronides.* Histograms facilitated initial research with visualizing correlations of *P. euphronides* observations and these variables. Additionally, passive bioacoustic monitoring data aided with understanding *P. euphronides* call frequencies in comparison to call *E. johnstonei* call frequencies. Since most data had non-normal distributions with overdispersion, we used Spearman's rank analysis (for non-parametric variables) and Quasi-Poisson linear regressions to visualize correlations. Results show that there may be some notable relationship between temperature and number of *P. euphronides* observations. Broadly, our research explores various factors that can influence endangered species populations, and should be largely considered when managing and protecting threatened and endangered species. Further research remains necessary to find additional correlations, to guide appropriate conservation efforts towards the critically endangered Grenada frog.

POSTER 14: Reconstituting Full-Length Mitochondrial Fission Protein 1, Fis1, and Assessing Its Inhibitory Role in Pancreatic Cancer

Florin Saitis and Blake Hill*

Mitochondrial fission is the process by which mitochondria divide and is necessary for organelle segregation into daughter cells during cytokinesis. Excessive mitochondrial fission has been linked to increased tumor growth in many cancers, including pancreatic cancer. Pancreatic cancer is one of the deadliest cancers in the U.S., with a 5-year survival rate of 10%. The goal of this project is to advance a novel therapeutic route against pancreatic cancer by targeting mitochondrial fission proteins. Inhibition of mitochondrial fission, either genetically or pharmacologically, blocks oncogenesis. Dynamin-related protein 1 (Drp1) performs mitochondrial fission and is recruited to sites of fission by mitochondrial fission protein 1 (Fis1). By inhibiting Fis1, which is three times more upregulated in pancreatic cancer than Drp1, mitochondrial fission is restricted. We have developed pep213, a novel peptide inhibitor, that inhibits Fis1 and prevents mitochondrial fission. However, pep213 was designed to block a version of Fis1 that lacks its transmembrane domain that anchors it to the outer mitochondrial membrane (Fis1ΔTM),

and it has not been tested in vitro against Full-length Fis1. We hypothesize that inhibitors designed against Fis1ΔTM will have an enhanced affinity for membrane-anchored Fis1. To this goal, Full-length Fis1 was successfully reconstituted in a detergent micelle. We then tested the hypothesis that Cy5-Drp1 would have an enhanced affinity for Full-length Fis1. Using microscale thermophoresis, it was determined that Full-length Fis1binds with Drp1 at a similar binding affinity as Fis1ΔTM. The Fis1 cytoplasmic domain is destabilized in the presence of detergent micelles and appears to adopt more than one single conformation. Nuclear magnetic resonance (NMR) also revealed chemical shift perturbations of residues in the Full-length Fis1 regulatory arm region compared to Fis1ΔTM. Pep213 was also analyzed using NMR and circular dichroism. We then tested how inhibited Fis1 impacts pancreatic cancer cell proliferation by using cellular proliferation assays and discovered that pharmacologic inhibition of Fis1 by pep213, and not a control peptide, in mouse pancreatic cancer cells reduced cell proliferation by 75%.

POSTER 15: Bacterial Strain Specificity in the Evolution of Mucoid Phage Resistance

Chloe Skinner and Alita Burmiester*

Phage-resistance mutations occur rapidly in bacteria, preventing phage infection. One mutant phenotype we study, called mucoid, is also a virulence factor that may limit the ability of phages to be used as therapeutic alternatives to antibiotics. In our previous work, mucoid mutants evolved from Escherichia coli K-12 were selected for using phage U136B. These isolates show resistance through mutations in genes encoding the Rcs Phosphorelay pathway. This pathway is activated through a stress response at the cell membranes and wall, resulting in mucoid capsule regulation. However, we do not yet know if and how other E. coli strains can evolve mucoid resistance. To test this, we are investigating the variation of the mucoid frequency in different laboratory strains with two separate phages, U136B and P1. We used plate-based selection experiments and screened for the mucoid phenotype using E. coli B and K-12 with phages P1 and U136B. We also tested cross resistance of our U136B-resistant, K-12 mucoid mutants on P1 phage using the cross-streak method. We found that mucoid mutants of E. coli K-12 appear under P1 selection (4/28 replicates, 14%) but at a lower frequency than under U136B (35/110 replicates, 32%). However, mucoid mutants that are resistant to U136B are also resistant to phage P1. Strikingly, mucoid mutants of E. coli B do not appear under selection by either phage (0/45 P1 replicates, 0/57 U136B replicates). Overall, these results show that mucoid resistance depends on both the host strain and the selecting phage.

POSTER 16: Combating Crop Diseases

John Joseph Srok and Ching-Hong Yang*

Amidst escalating global demands for agricultural outputs, the efficacy of traditional chemical pesticides is on the decline, primarily due to the emergence of pathogen resistance. Furthermore, these traditional chemical pesticides pose toxicity risks and contribute to environmental pollution. Notably, fungal pathogens account for over 70% of all plant disease cases worldwide, underscoring the urgent need for sustainable disease management strategies. Biocontrol agents represent an environmentally friendly and sustainable alternative to mitigate fungal diseases in crops. Leveraging the natural antagonistic relationships between microorganisms, these agents offer a strategic approach to disease management that reduces reliance on chemical fungicides, thus minimizing ecological footprint and safeguarding biodiversity. In this study, we demonstrate that the production of 2-phenylethanol by *Lelliottia nimipressuralis* exerts inhibitory effects on the growth of the fungal pathogen *Colletotrichum dematium*, which is the causal agent of anthracnose. *Colletotrichum* species impact a wide array of economically significant crops, spanning fruits, vegetables, ornamentals, cereals, legumes, and turfgrass, causing substantial agricultural losses and affecting food security. The *L. nimipressuralis* strain was identified through the screening of crude extracts derived from insect-associated bacteria. Advanced analytical techniques, such as Nuclear Magnetic Resonance (NMR) and Gas Chromatography-Mass Spectrometry (GC-MS), applied to bioactive fractions obtained via liquid chromatography, confirmed 2-phenylethanol as the inhibitory compound. This study constitutes the first identification of 2-phenylethanol production by *L. nimipressuralis*. Given the established antifungal properties of 2-phenylethanol, our findings suggest that *L. nimipressuralis* hold significant promise as a biocontrol agent against anthracnose in crops, offering a sustainable alternative to chemical fungicides.

POSTER 17: Resistance to Beta-Lactam Antibiotics in Mucoid Mutants of Escherichia coli

Harleen Tewatia and Alita Burmeister*

In *Escherichia coli*, the mucoid phenotype is a virulence factor that can produce harmful effects related to biofilm formation. Some mucoid mutants are additionally known to have increased resistance to the antibiotics cefsulodin and amdinocillin. We previously isolated and characterized mucoid mutants with variable resistance levels to the bacteriophage U136B. To determine if these mutants also have increased antibiotic resistance, we measured MICs using broth microdilution and efficiency of plating assays with cefsulodin and amdinocillin. We observed increased antibiotic resistance of the mucoid mutants compared to wild type and tolC mutant controls. In future work, we will test resistance to a broader panel of clinically relevant antibiotics. Overall, this work will address whether the

phage- selected mucoid phenotype poses a risk to phage therapy in the form of increased antibiotic resistance.

POSTER 18: Using *Caenorhabditis elegans* to Test the Impact of Cardiofaciocutaneous Syndrome Mutations

Parnian Vakili, Fatemeh Cheraghi, Claire C. de la Cova*

Cardiofaciocutaneous (CFC) syndrome and Noonan syndrome (NS) are collectively termed RASopathies, a group of genetic disorders characterized by dysregulation in the RAS signaling pathway. CFC syndrome presents with facial dysmorphisms, short stature, cardiac anomalies, and developmental issues. CFC syndrome is predominantly associated with mutations in the BRAF gene, which encodes a protein kinase that activates the kinases MEK and ERK. BRAF protein contains a regulatory region with a Ras-binding domain and a cysteine-rich domain (CRD), and a catalytic region with a kinase domain. The most common BRAF mutation found in CFC is the missense Q257R, which alters the CRD. The Q257R mutation leads to increased kinase activity, which in turn activates MEK and ERK. Currently, the effects of CFC mutations on BRAF protein levels, localization, and protein interactions are unknown. We previously generated mutations in the *Caenorhabditis elegans* RAF gene that were modeled after those found in NS. The aim of this project was to examine the molecular consequences of the Q257R CFC syndrome mutation in *C. elegans* RAF. Our strategy employed CRISPR/Cas9 gene editing to induce mutations in *C. elegans* that model those observed in human patients. We generated the mutation M194R in the *C. elegans* RAF gene, which is similar to the Q257R mutation observed in the human BRAF gene. In future studies, we will use these mutants to investigate how RAF protein levels and localization are altered. This work will establish a new mutant model for understanding RAF dysregulation in CFC.

POSTER 19: Understanding the Regulation of Antibiotic Secretion in *Xenorhabdus szentirmaii* by the Quorum-sensing Regulator LsrF

Olivia Valle, Ritisha Dey, Kimberly Mayer, Steven Forst, Shama Mirza, Madhusudan Dey*

Antibiotic-resistant bacteria have become an increasing threat to public health worldwide. With the rapid rise in resistance to many commonly used antibiotics there is a pressing need to identify new antibiotics from new sources to combat this issue. One promising source is *Xenorhabdus szentirmaii*, a symbiotic bacterium residing within the intestines of the soil nematode, which naturally produces non- ribosomal peptide (NRP) antibiotics. Our research reveals that these NRPs are synthesized by 16 non-ribosomal peptide synthetases (NRPSs) encoded from the *X. szentirmaii* genome. Understanding the fundamental biology

of these NRPSs is important for their potential commercial application. To identify genes regulating the NRPSs in X. *szentirmaii*, we isolated total RNAs from the log phase (when no antibiotics are produced) and the stationary phase (when antibiotics are produced) of the cell growth. Total RNA was then subjected to RNA-Seq analyses. Two top hits from these analyses are the quorum- sensing regulators lsrF and lsrG. Interestingly, we observed that the lsrF mutant of X. *szentirmaii* produces less antibiotics compared to its isogenic wild-type strain. These findings suggest that LsrF plays a significant role in the regulation of antibiotic secretion in X. *szentirmaii.*

POSTER 20: Are you a lefty, a righty, or ambidextrous? Assessment of brain lateralization using handedness assays in treefrogs

Arabella Voegeli, Olivia Feagles, Gerlinde Höbel*

Brain lateralization is common across the animal kingdom and thought to be beneficial because eliminating repetitive functions across hemispheres increases cognitive capacity. However, lateralization may be detrimental in certain scenarios as it can delay reaction times or impair movements requiring bilateral coordination. Given that different species have variable lifestyles and locomotive habits, the prevalence of brain lateralization is not entirely ubiquitous. Here, we investigate brain lateralization using two handedness behavioral assays (snout-wiping and righting responses), consequently recording side-biases in reflexive motor responses. We also investigate the consistency of handedness patterns across six closely related treefrog species. Results from the snout-wiping assay provide evidence of ambidexterity (i.e., no lateralization) across all species, however, some species show left-bias during the physically more demanding righting response assay. Wiping and righting tasks may be more reflexive and not require strong brain lateralization. Lateralization may mostly occur in tasks that require higher order cognitive processing, such as predator avoidance or prey capture.

POSTER 21: Biomechanical Regulation of Zebrafish Brain Morphogenesis

Gabriella Voit, Kleida Doci, Savannah Makowski, Jennifer Gutzman*

Brain morphogenesis is a delicate process involving the coordination of biochemical and biophysical forces. Disruptions in these processes often lead to structural defects during development. While significant work has been conducted to understand biochemical pathways, there remains a gap in our understanding on the biomechanical mechanisms. Our research aims to elucidate the biomechanical mechanisms and gain insight into the mechanosensory proteins that mediate brain morphogenesis. We are examining the highly conserved zebrafish midbrain-hindbrain (MHB). The MHB forms from a sharp basal tissue fold in the neuroepithelium and requires the basement membrane protein,

laminin-111. We hypothesize that mechanosensory proteins such as talin mediate forces via the mechanotransduction pathway aiding tissue morphogenesis. Talin undergoes conformational changes based on forces in the cell and once activated binds to integrins located on the basal edge of the cell. We also hypothesize we can use vinculin, a protein that binds to talin, as a tool to study the force in a region-specific manner. Overexpressing vinculin protein tagged with green fluorescent protein (GFP) in both WT and laminin-111 mutant embryos allows us to visualize vinculin localization. We have found enrichment of vinculin localization at the basal edge of the cell in wildtype and higher vinculin-GFP signal in laminin-111 mutants. This study aims to investigate how cells mediate forces via mechanosensory proteins during key developmental events and develop tools to detect region specific forces within cells. These findings are important in furthering research in tissue engineering and regenerative medicine.