

**Department of Biology
Undergraduate Research Symposium
April 16, 2021
2-5pm**



Symposium on Zoom

Register: <https://tinyurl.com/36uarjsc>

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Thank you all for attending the 2021 Undergraduate Research Symposium in the Department of Biology. The Symposium is held annually by the Undergraduate Research Assistantship Program (URAP) Committee to highlight the excellent research being conducted by undergraduates in our department. We are incredibly proud of all of our undergraduate researchers, especially this year with the COVID-19 pandemic. We would like to thank all of these undergraduate researchers as well as their graduate student, post-doctoral, and faculty mentors. We would finally like to acknowledge that the University of Florida sits on the Indigenous lands of the Seminole and Timucua Peoples.

Your 2021 URAP Committee

Adania Flemming, Claudia Garnica, and Jessie Pelosi

Cover images were contributed by Department of Biology graduate students.

From upper right by row:

Jessie Pelosi

Bethany Zumwalde

Elise Richardson

Aditi Jayarajan

Ashley Meade Kusel

Lauren Kunselman

Arik Hartmann

Min Zhao

Lindsey Riibe

Poster Presentations

2:00-3:15pm

Ecotype variation of stomatal size-density tradeoff in switchgrass (*P. virgatum*)

Brian J. Branstetter, Raiza Castillo-Argaez, Claudia Garnica-Diaz, Grace P. John

Galliform lysozyme C evolution

Dawson Houghtaling, Rebecca Kimball

Assessing the Behavior of Ticks Harboring Human Pathogens

Brittney Jabot, Elise Richardson, Carl N. Keiser

Preliminary Comparisons between Cachexia in Patients with Esophageal and Pancreatic Cancers

Lam Le, Miles Cameron, Sarah Judge, Andrew Judge

Multi-month Ranavirus outbreak leads to mass-mortality and disease in two threatened amphibian species in Florida

Arik M. Hartmann, Max L. Maddox, Robert J. Ossiboff, Ana V. Longo

The Effect of Herbivore Interactions on Plant and Seed Development

Rebecca Molina, Phil Hahn

The Effect of BMAL1 on Pulmonary Hypertension

Amy Nguyen, Elnaz Ebrahimi, Andrew J. Bryant

Effects of systemic immunity on diurnal stomatal movement of *Arabidopsis thaliana*

Marral Pourmoghadam, Lisa David, Sixue Chen

Cell Size Variance in Switchgrass (*Panicum virgatum*) is Related to Ploidy, not Ecotype

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Stomata size-density tradeoff is scale-dependent in southern oaks

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Pancreatic tumor burdens elicits progressive respiratory muscle pathology and dysfunction

Enrique J. Trevino, Andrew C. D'Lugos, Daria Neyroud, Chandler S. Callaway, Sarah M. Judge, Andrew R Judge

Oral Presentations

3:30-5:00pm

3:30-3:45pm

Histone methyltransferase inhibitor chaetocin strongly and specifically affects metal responsive genes

Elijah Abraham, Juan Piloto, Cheng-Wei Wu, Ranjala Ratnayake, Hendrik Luesch, Keith P. Choe

3:45-4:00pm

Quantifying biomarkers of age and stress in amphibians: Do all tissues exhibit similar telomere length estimates?

Alexandria Hill, Zuania Colón-Piñeiro, Ana V. Longo

4:00-4:15pm

Investigation of myrosinase system at single-cell type using guard cells against *Pseudomonas syringae*

Lin Lu, Gedrick Mangual, Sixue Chen

4:15-4:30pm

Considering coral commensal crabs: A phylogenetic review of gall crabs (Family Cryptochiridae > *Opecarcinus*) and their hosts

Garret O'Donnell, Gustav Paulay

4:30-4:45pm

Intranasal and Intramuscular Naloxone Dispensing Across Age and Sex

Matthew Pham, Henry William Young II



Ecotype variation of stomatal size-density tradeoff in switchgrass (*P. virgatum*)

Brian J. Branstetter¹, Raiza Castillo-Argaez¹, Claudia Garnica-Diaz¹, Grace P. John¹

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Stomata are the pores on the leaf epidermis that regulate gas exchange via dynamic behaviors and/or anatomical traits. A tradeoff between stomatal size and density (SS-SD) is commonly seen in plants and has been linked to stomatal speed, photosynthesis, and water loss. Yet SS-SD has not been extensively studied within this species and genetic drivers for shifts along this axis are not resolved. Switchgrass (*Panicum virgatum*), a C₄ bunchgrass species and potential biofuel crop persisting over a wide geographic range throughout which several ecotypes have diverged, presents an ideal study system in which to test hypotheses for adaptive shifts along or against SS-SD. This study examines the stomatal anatomy of representatives of upland ecotypes with rapid life history and long-lived lowland ecotypes and their offspring in a common garden environment to inform future breeding of switchgrass lines. We used epidermal impressions of the abaxial and adaxial surfaces of over 100 switchgrass samples representing 4 unique F₀ genetic lines and their F₁ crossed offspring. We found a negative correlation for stomatal area versus density for both abaxial and adaxial data across all ecotypes but was stronger for abaxial data. This emphasized a SS-SD tradeoff independent of ecotypes. Post-hoc tests illustrated that amongst ecotypes, the lowland AP13 shows significant differences for stomatal area, such that AP13 possesses few and large stomata, more strongly expressed on the abaxial, relative to adaxial, surface. This suggests this is a dominant phenotype because the F₁ hybrid containing an AP13 allele also shows few, large stomata on the abaxial side. This has likely resulted from an adaptive shift towards improved water use efficiency (minimization of water loss while maximizing CO₂ conductance) in hot, dry lowland environments.



Galliform lysozyme C evolution

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The evolutionary history of species tends to follow a species-centric model of branching where phylogenies are built based on morphology and genetic similarities of modern species. Coalescence theory suggests that this is not always accurate, as more recent studies have shown that gene trees and species trees may not follow the same lineages. This happens through a multitude of factors, including horizontal transfer and lineage sorting—high population size and short branches allowing wide variation in gene transfer within a species over time. The lysozyme C character distribution in Galliform birds is paraphyletic, with including Odonophoridae and Numididae sharing three specific amino acid substitutions. Enzyme studies show that the mutation of just one of these risks the viability of the developing embryo. The accepted phylogeny of Galliformes would suggest that these groups evolved this gene separately, though the nature of this gene makes it highly unlikely. Avian lysozyme C (referred to as LYZ) may be an example of deep coalescence resulting in monophyly for the gene despite paraphyly of species. Here, we analyze the trees formed using the coding and non-coding regions of the LYZ gene in galliform species to find the most likely gene trees and species trees. We also explore site likelihoods of alternative topologies to identify which tree each part of the gene is most likely to represent.



Assessing the Behavior of Ticks Harboring Human Pathogens

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Tick-borne pathogens threaten both public health and animal health. As climate change impacts the ranges for tick inhabitation, this threat grows. The expansion of the range of the lone star tick, *Amblyomma americanum*, which historically inhabits the southeastern United States, poses a potential increase in tick-borne pathogen transmission. This study aimed to evaluate the impacts of habitat type and pathogen infection on host seeking behavior (questing). Tick collection happened at xeric hammock and successional hardwood habitat types at the Ordway Swisher Biological Station using a tick drag. Questing assays were then completed to record average height quested and total time spent questing. Then each tick's DNA was extracted, sequenced for *Rickettsia amblyommatis* utilizing PCR with *Rickettsia*-specific primers via Genewiz. *Rickettsia amblyommatis*, a member of the Rickettsia spotted fever group, was found in 28.6% of ticks collected. The prevalence in successional hardwood forest habitat was 28% and in xeric hammock habitat was 32%. Ticks collected from xeric hammock habitats spent double the amount of time questing as ticks from successional hardwood forest. Additionally, infected ticks spent less time questing than uninfected ticks. The study results show that habitat type and infection status influence tick questing behavior, which can have consequences for human disease risk.

Keywords: *Amblyomma americanum*, questing, *Rickettsia amblyommatis*



Preliminary Comparisons between Cachexia in Patients with Esophageal and Pancreatic Cancers

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Background: Cancer cachexia is a devastating condition affecting several cancers. Clinically, pancreatic ductal adenocarcinoma (PDAC) is most studied. Weight loss is, however, a common symptom in several other gastrointestinal cancers, most notably esophagogastric adenocarcinoma (EGA). Herein, we hypothesize that anthropometric changes associated with cancer cachexia in PDAC and EGA are comparable, despite the diverse pathophysiology of both tumors.

Methods: Clinical data was abstracted from the medical record in accordance with IRB protocols. Surrogate, blood-based markers of cachexia were compared, together with anthropometric measurements from routine computed tomography (CT) scans. All patients underwent surgery for potentially curative, biopsy proven EGA or PDAC. Muscle biopsies were obtained from one patient with EGA.

Results: Both patient groups commonly demonstrated signs of cachexia, including body weight loss exceeding 5%, low skeletal muscle index (SMI) and low muscle radiation attenuation (MRA). SMI was comparable between EGA (41.4 cm²/m²) and PDAC (43.9 cm²/m², $p = 0.4$). MRA was similarly comparable: (32.5 HU v. 32.2 HU, $p = 0.9$). Curiously, we identified that patients with EGA have lower bone mineral density (115 HU) compared to those with PDAC (142, $p = 0.0233$). Hemoglobin (12.3 g/dL v. 12.5 g/dL, $p = 0.3$), albumin (3.84 g/dL v. 3.87 g/dL, $p = 0.8$) and platelet count (273 v. 260, $p = 0.5$) were likewise similar between both cancers. Hematoxylin-and-eosin-stained cross-sections of rectus abdominis, diaphragm and intercostal muscle reveal no overt inflammatory pathology as seen in PDAC, though there is evidence of fiber degeneration and regeneration, supported by occasional centralized nuclei.

Conclusion: EGA and PDAC are unique cancers that both cause severe wasting disorders. While non-invasive imaging techniques suggest equal degrees of muscle atrophy, it cannot be assumed that both tumors function in similar ways at the mechanistic level. EGA cachexia deserves further attention clinically and in pre-clinical models.



Multi-month Ranavirus outbreak leads to mass-mortality and disease in two threatened amphibian species in Florida

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A severe and multi-month outbreak of Ranavirus (Rv) in a natural community of amphibians contributed to a mass die-off of gopher frog tadpoles *Lithobates capito* and clinical signs of disease in striped newts *Notophthalmus perstriatus* in a pond in Florida. Hemorrhagic disease, renal, splenic, and hepatocellular necrosis was diagnosed from pathological examination of dead and moribund gopher frog tadpoles. All amphibian species present were evaluated for the frog virus 3 (FV3) group of ranaviruses via quantitative PCR. *L. capito* (P = 100%) and *N. perstriatus* (P = 100%) and southern cricket frogs, *Acris gryllus* (P = 77%) were infected with FV3 ranavirus. During a subsequent survey we observed ongoing mortality of *L. capito* tadpoles, and severe Rv-related necrosis in *N. perstriatus*. High susceptibility and mortality in *L. capito* suggest that Rv can rapidly affect population structure through disruption of recruitment. Ongoing die-offs indicate that temporally spaced breeding events enable Rv to persist in environments through pathogen transfer between different age classes. Though no mortality was observed in *N. perstriatus*, high infection loads and progressed signs of disease suggests that Rv infections can result in mortality for the species.

Keywords: disease sampling, salamanders, Frog virus 3, gopher frog, striped newt, tadpole, amphibian mortality



The Effect of Herbivore Interactions on Plant and Seed Development

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Within plant-herbivore interactions, insects are the greatest cause of seed production loss. Some species of plants have developed defenses to mitigate these losses, with common techniques involving the overproduction and underproduction of their seeds. *Baptisia alba* is a perennial legume that is commonly predated by a weevil species, *Trichapion rostrum*. In our study, we aimed to see which *Baptisia alba* sites and plants were the most affected by weevils as well as to see the defenses they lacked against them. We also sought to understand the general impact that weevils had on *Baptisia alba* seeds. To test our hypotheses, *Baptisia alba* plants were gathered and sorted from four sites in Wisconsin. Notches were used as the comparative factor against weevil presence when testing susceptibility in individual plants. Notches represented how many seeds could be produced in a given pod at a time. Our results confirmed a significant relationship between weevil presence and seed damage, but almost no variation between sites, suggesting that more varied environments need to be brought into our sample. We also found that the relationship between notches and weevil presence showed a stronger correlation, but not enough for it to be significant. These illustrate the pervasive nature of weevils and the need for different factors to be accounted for in plant susceptibility to better predict plant defenses from herbivores.



The Effect of BMAL1 on Pulmonary Hypertension

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Pulmonary hypertension (PH) is a chronic disease defined by pulmonary arterial pressures greater than or equal to 25 mmHg. When PH arises with interstitial pulmonary fibrosis (IPF), the prognosis of IPF is severely worsened, quality of life is significantly decreased, and mortality risks are greatly increased. The hospital expenses that result from patients with PH total to about \$2 billion annually. There are currently no targeted therapeutic solutions to Group 3 PH and IPF. Brain and muscle ARNT-like 1 (BMAL1) and circadian locomotor output cycles protein kaput (CLOCK) are circadian transcriptional factors that amplify target genes as a heterodimer. Immune functions are under circadian control which include trafficking of immune cells, host-pathogen interactions, and activation of adaptive and innate immunity to reduce unnecessary energy expenditure unless required at specific times of day. Specific circadian contribution to PH is unknown, therefore we chose to explore the role of BMAL1 as a transcription factor by globally over-expressing the gene (BMAL1 OE) and deleting the gene in myeloid-specific cells (mBMAL1 KO) in mice. It was found that BMAL1 expression is altered by hypoxic stimulus in vivo and BMAL1 OE was protective against PH in hypoxic conditions. In mBMAL1 KO models, PH was worsened. PD-L1 expression was examined for potential therapeutic targets.



Effects of systemic immunity on diurnal stomatal movement of *Arabidopsis thaliana*

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Plants respond to pathogenic bacteria by eliciting long-distance mobile signals that travel to uninfected systemic tissue so that the remote tissues can “prime” itself for potential pathogen attack. This process is called Systemic Acquired Resistance (SAR). Here we investigate the effects of SAR in *Arabidopsis thaliana* elicited by the pathogenic bacteria *Pseudomonas syringae* pv tomato DC3000, on guard cells, the cells that form and control stomata movement of leaf surface. We found that the SAR immune response in uninfected remote tissue led to reduced stomatal apertures, which may provide protection against pathogen invasion. SAR immune deficient mutant non-expressor of pathogen response 1 (npr1) did not exhibit the narrowed stomatal apertures in response to “priming” by SAR signals. Later experiments were conducted to test the integrity of the priming effects on the diurnal cycle of the stomatal apertures of the wild type primed and control leaves. Leaf surface images were obtained by light microscopy and stomata apertures were measured from three biological replicates of hourly timepoints throughout the day to discover the overall effect of SAR signals on stomatal movement when the guard cells are also receiving diurnal signals. Experiments testing the NAD and NADP cofactors as potential signals that alter stomatal movements were also conducted. Elevated concentrations of these two cofactors led to stomatal aperture closure, enacting another immune response to protect the plant from pathogenic bacterial invasion.



Cell Size Variance in Switchgrass (*Panicum virgatum*) is Related to Ploidy, not Ecotype

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Genome doubling events, known as polyploidization, are a common cause for genome size increase within a plant species and have been shown to increase rates of growth and climate adaptation. Across species, genome size serves as a strong predictor of cell size with downstream implications for physiological processes, though this has not been tested in the context of polyploidy. Switchgrass (*Panicum virgatum*), a C₄ grass and biofuel candidate, has undergone two polyploidization events resulting in a dominant 4x variant and a less common 8x variant. Given the broad geographic range and ecotypic divergence across climatic niches (Upland, Lowland, Gulf, and Atlantic), switchgrass is an ideal system to test the relationship between cell size, ploidy, and climate. We used a common garden transplant experiment to test for differences in climatic niche and leaf anatomy by ecotype and ploidy and related these relationships to differences in yield. We found that leaf anatomy differed by ploidy but not ecotype. Specifically, the 8x variant had larger vasculature and smaller chloroplasts than the 4x variant. This shift likely confers an increase in metabolic rates, with downstream implications for phenologically adjusted yield for some ecotypes. The absence of a consistent yield increase across all ecotypes suggests possible gene by environment interactions. Understanding the mechanisms by which polyploidy impacts ecotypic variance holds implications for the growth and cultivation of switchgrass as a biofuel.



Stomata size-density tradeoff is scale-dependent in southern oaks

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Stomata are pores on the leaf surface that control both photosynthetic uptake and water loss. There is a well-described trade-off between stomatal size and density (SS-SD). Movement along this axis has been linked to climate in fossil leaves and extant species both across and within species. We tested the robustness of the SS-SD tradeoff to examine its linkage to climatic niche in an adaptive context across southern oaks. We quantified variance in epidermal anatomy for 9 closely related, co-occurring oak species representing three phylogenetic sections in a common garden. Based on geographic species occurrence, hydrological niche was species, rather than group-specific, while thermal niche was group-specific. We indeed found evidence for a fundamental SS-SD tradeoff. Yet, this relationship was only significant when data were considered independent of species or group. Individual trait divergences were significant at the species, but not section, level. “Stomatal syndromes” as established by multi-trait principal components (e.g., few-bigger cells vs. many-smaller cells), did not differ by section. However, the distribution of variance along principal components was qualitatively different, such that *Virentes* and *Quercus* varied primarily along an SS-SD axis (PC1), while Lobatae varied along a group-specific stomatal aperture height-guard cell width tradeoff (PC2). Our study suggests that, for southern oaks, movement along the SS-SD axis is phylogenetically independent and that divergence in stomatal anatomy occurs at the species level, likely due to community-scale niche partitioning or environmental conditions at smaller geographic scales.



Pancreatic Tumor Burden Elicits Progressive Respiratory Muscle Pathology and Dysfunction

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Cancer cachexia is a multifaceted condition affecting a large majority of cancer patients, with pancreatic ductal adenocarcinoma (PDAC) patients presenting the highest prevalence. Cachexia is characterized by progressive whole-body and skeletal muscle wasting, including vital cardiorespiratory muscles. There are currently no medical treatments for cachexia, largely due to a limited understanding of the underlying mechanisms leading to the progression of the disease, especially with respect to cardiorespiratory muscles. The purpose of this study was therefore to characterize the time course of diaphragm muscle pathology and dysfunction in a preclinical model of PDAC.

C57Bl6/J mice (male, 10 weeks old) received orthotopic injections into the pancreas of either PBS (SHAM, n=6) or mouse pancreatic cancer cells (KPC, n=30). Mice were monitored daily and euthanized at predetermined time points corresponding to 8 (D8), 10 (D10), 12 (D12), and 14 (D14) days post cancer cell inoculation or when mice met IACUC-mandated tumor endpoint (i.e. 15-18 days post tumor cell inoculation (END), body condition score < 2). In-vivo diaphragm function was assessed via M-mode ultrasonography immediately prior to euthanasia. Hemispheres of the diaphragm were harvested and subjected to immunohistochemical analyses.

In-vivo diaphragm (excursion amplitude) and respiratory function (respiratory rate, minute ventilation) were significantly impaired beginning at D12 versus Sham (-21%, -47% and -59% respectively). Additionally, significant diaphragm muscle fiber atrophy occurred at D12 (-16%), D14 (-17.6%), and END (-24.1%). Collagen remodeling, indicated by collagen hybridizing peptide reactivity, was increased by 113% at D12 versus Sham. Muscle area occupied by extracellular matrix was significantly increased by 52% D12 and persisted through END (+78%). Infiltration of CD45+ leukocytes significantly increased versus Sham at D8 and steadily increased through END. The abundance of infiltrating leukocytes significantly correlated to minute ventilation ($r=0.80$, $P<0.001$), muscle fiber size ($r=0.67$, $P<0.001$), collagen remodeling ($r=0.55$, $P=0.002$), and muscle area occupied by extracellular matrix ($r=0.82$, $P<0.001$).

Overall, this is the first study to characterize the time course of cachexia development and progression in the orthotopic KPC model. The current findings demonstrate an early and profound pathological remodeling of the diaphragm in response to pancreatic tumor burden, characterized by muscle fiber atrophy, extracellular matrix remodeling, immune cell infiltration, and ultimately respiratory dysfunction. These data help define the early cellular events underlying the development of muscle pathology and the associated respiratory dysfunction, providing an important translation tool for therapeutic investigations.



Histone methyltransferase inhibitor chaetocin strongly and specifically affects metal responsive genes

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A gene pair termed *numr-1/2* (nuclear-localized metal-responsive) was previously shown to encode an SR-like protein with an RNA-recognition motif that promotes longevity and growth in cadmium. We recently used a genome-wide screen to demonstrate that *numr-1/2* is activated by disruption of RNA metabolism. To investigate *numr-1/2* regulation and identify modulators of nucleic acid metabolism, we screened over 40,000 compounds and extracts from commercial and natural product libraries for *numr-1/2p::GFP* activation. We identified six *numr-1/2* inducers; three (floxuridine, 5-fluouracil, and actinomycin D) have well-characterized effects on DNA and RNA confirming that *numr-1/2* is regulated by changes in nucleic acid metabolism. Fungal toxin chaetocin was the most potent and least toxic *numr-1/2* inducer. RT-qPCR demonstrates that chaetocin induces *numr-1/2* and another stress-responsive SR-like protein gene (*W03G1.5*) over 50-fold within 45 minutes without affecting expression of canonical heat shock, osmotic stress, endoplasmic reticulum stress, mitochondrial stress, or detoxification response genes. Chaetocin does not activate other metal-responsive genes and actually reduces expression of metallothionein gene *mtl-2* and fluorescence of *mtl-2p::GFP* consistent with repression of *mtl-2* transcription. A time-course experiment demonstrated that induction of *numr-1/2* precedes decreases in *mtl-2* by 1-2 hours. We find that *numr-1/2* is required for chaetocin to repress *mtl-2* fully, consistent with NUMR-1/2 negatively regulating *mtl-2* transcription.

Chaetocin is a promising anticancer candidate in cell culture and *in vivo* models; it was first shown to inhibit histone lysine methyltransferase (HMT) SU(VAR)3-9 and later shown to affect thioredoxin reductase. Robust activation of *numr-1/2* in *C. elegans* provides an opportunity to harness genetic tractability to gain new insights into chaetocin bioactivity. Using a *trxr-1/2* double mutant, we find that chaetocin induces *numr-1/2* independently of thioredoxin reductase. *C. elegans* has over 35 predicted HMTs; we are now testing HMT mutants for induction of *numr-1/2*. Our results raise the possibility that histone methylation dynamically regulates stress-responsive genes in terminally differentiated somatic cells. This work was supported by NSF grant IOS-1452948 and a UF Research Opportunity Seed Fund Grant to KPC, CWW, and HL.



Quantifying biomarkers of age and stress in amphibians: Do all tissues exhibit similar telomere length estimates?

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Telomere length is a biomarker of age and stress in vertebrates. Telomeres are regions of repetitive nucleotide sequences that protect the terminal regions of chromosomal DNA. These caps become shorter with age, leaving the chromosomes more vulnerable to degradation. One challenge in determining telomere length is acquiring representative samples that are non-lethal in small animals. For this study, we focused on optimizing a quantitative polymerase chain reaction (qPCR) protocol to measure the relative amplification of telomeres to GADPH, which is a housekeeping gene that is constitutively expressed across cells and tissues. Our goal is to determine if individual traits such as body size and weight are associated with differences in telomere length. To measure the telomere length from the frogs collected, we first measured the snout-to-vent length (SVL) and weight of each individual. Then, we dissected the frogs to harvest samples from various tissues (blood, spleen, liver, blood, skin, muscle, and skin swabs). We extracted DNA, measured the concentration, and diluted DNA all samples to 20ng. We ran the qPCR reactions for the genes telb and GADPH. Our results showed that the liver and the blood had the highest amplification values in telb and GADPH. This information will help us in determining which tissues are best for getting the most reliable and reproducible results. We are in the process of optimizing the qPCR conditions for the rest of the tissues, including non-invasive skin swabs.



Investigation of myrosinase system at single-cell type using guard cells against *Pseudomonas syringae*

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Guard cells are specialized plant cells that control the movement of gases between the plant and the environment. They are also known to play a vital role in plant defense against pathogens and pests. Found within these guard cells, are enzymatic components known as glucosinolates and myrosinases. In the presence of pathogens and pests, this enzyme-substrate interaction is triggered resulting in a reaction called the 'Mustard Oil Bomb'. This 'bomb' is what triggers the movement of guard cells to close the stoma (opening) of the cell. This as a result, will halt gas exchange and prevent the entrance of foreign invaders.

This study will focus on what role myrosinases play in the closing of stomatal apertures. Model plant *Arabidopsis thaliana* has a mutant species, *atvam3*, that exhibits an overproduction of TGG1 and TGG2 myrosinases enzyme. The goal is to map out the phenotypical differences between *atvam3* mutant and Wild Type (*col-0*) guard cells. This will give us insight as to how an overproduction of myrosinases will affect the rate of initiation of the 'Mustard Oil Bomb'.

Current knowledge leads to the assumption that since there is a greater concentration of enzyme (TGG1 and TGG2 myrosinases) versus substrate (glucosinolates), then there will be an even faster rate of enzymatic reaction. The 'Mustard Oil Bomb' will activate at a faster rate, therefore, initiating the closing of stomatal apertures at a faster rate. A rapid closure rate of stomatal apertures can be an advantageous defense mechanism against invaders.



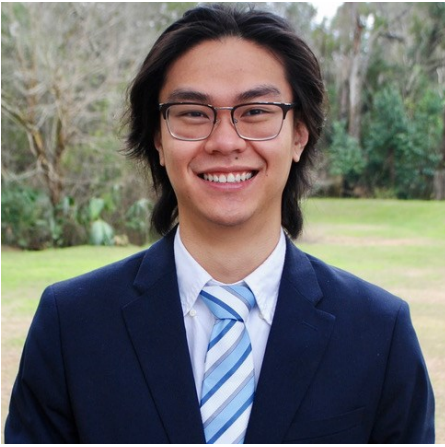
Considering coral commensal crabs: A phylogenetic review of gall crabs (Family Cryptochiridae > *Opecarcinus*) and their hosts

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Reefs are well established hotspots for marine biodiversity. Contributing largely to this diversity are symbiotic commensals and parasites. Brachyurans in the family Cryptochiridae are obligate symbionts with scleractinian corals. Cryptochirid biology is intrinsically symbiotic, and this life history strategy has led to immense observed diversity. The genus *Opecarcinus* represents an interesting case study for the family in terms of host specificity and geographical distribution. Past examinations of the genus have indicated that genetic diversity in the group may be more complex than previously assumed. This study aims to further explore this clade not only through phylogenetic analysis but through geographic and morphological means as well. Examination of gall crabs as commensal organisms raises several questions about its patterns of diversity. The hypothesis that species in the genus diversified via allopatric speciation leads to the prediction that sister species of *Opecarcinus* may exhibit allopatric ranges that reflect the geography of speciation that led to them. The hypothesis that species in the genus diversified via host speciation leads to the prediction that sister taxa in *Opecarcinus* will be on different coral hosts. Here, we find that *Opecarcinus* and its relatives do not exhibit clear indicators of either allopatric or host speciation patterns. While complete results are still pending, initial findings indicate that this group exhibits either novel or undescribed methods of speciation and that found diversity is cryptic and widespread.



Intranasal and Intramuscular Naloxone Dispensing Across Age and Sex Matthew Pham¹, Henry William Young II²

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Introduction: According to the CDC, around 450,000 people have died due to opioid related overdose from 1999-2018. In 2015 alone, 33,091 out of 52,404 total overdose deaths involved an opioid. The CDC reports that 30% of all opioid fatalities include heroin while the rest includes prescription opioids. While other studies have focused on opioid prescription patterns from 2007-2012, we focus on naloxone dispensing during 2012-2017 that may reflect efforts in curtailing the opioid overdose epidemic. Additionally, current studies have compared the efficacy of intranasal (IN) and intramuscular (IM) naloxone formulations, but there is a paucity of data reflecting dispensing trends specific to IN and IM naloxone. The CDC Guideline for Prescribing Opioids for Chronic Pain recommends prescribing naloxone when there is a history of substance use abuse, daily use of opioids 50 morphine milligram equivalents (MME), and other risk factors for risk of overdose are present.

Objective: Although data has already been reported from 2012-2018 of naloxone dispensing trends, we are interested in the patient characteristics of those that were prescribed opioids 50 MME. We are also interested in the patterns of the different type of naloxone formulations prescribed. The push to increase naloxone's accessibility reflects existing barriers in effectively preventing opioid overdose deaths.

Methods: Data on all naloxone formulations dispensed in the United States from 2012 to 2017 were collected from the IQVIA National Prescription Audit (NPA). The NPA represents 91% of all prescriptions dispensed in the United States from retail pharmacies. Variables including naloxone formulation, strength, date of prescription, duration of prescription, amount prescribed, specialty of the prescribing physician and patient sex and age will be analyzed using chi squared analysis, fisher exact test, and multiple logistic regression. Prescriptions will be aggregated by specialty, and the rates of prescriptions of each naloxone formulation will be compared for each year from 2012 to 2017.

Results: The total number of naloxone dispensed was (21,129.30) in 2012 and (387,000) in 2017 (table 1), reflecting a 1440% increase. Females were more likely to be prescribed naloxone than males (figure 2), except in the younger age groups ('20-24 and '25-34). In 2017, the odds that a male was given a naloxone prescription per high dose (50 MME) was 1.21 times (table 1) the odds for a female ($P < 0.001$). The odds that a male was given a naloxone prescription per very high dose (MME > 50) was 1.11 times (table 7) the odds for a female ($P < 0.001$). The odds for a male to be prescribed nasal naloxone was 0.971 times the odds (table 5) for a female ($P < 0.001$), while the odds that a male was prescribed intramuscular naloxone was 1.021 times the odds for a female ($P < 0.009$). Those in the 20-24, 13-19, and 25-34 age groups were more likely to receive either IN or IM naloxone compared to 45-54 reference group. The odds of prescribing nasal naloxone (table 4) were highest for pediatrics (11.77x; $P < 0.001$), addition medicine (4.67x; $P < 0.001$), psychology (4.29x; $P < 0.001$), psychiatry (3.43x; $P < 0.001$), and genetics (2.42x; $P < 0.001$) using internal medicine as a reference group.

Conclusion: The odds of a male receiving a naloxone prescription was 1.21 times the odds for a female. Varying IM and IN naloxone dispensing patterns by age, sex and specialty suggests further research on its impact on increased at-risk groups.

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