

OLIVIA DEANGELIS, Pomona College

Interspecies estradiol variation across wild primates: is diet a driver?

Mentors: Michael Wasserman, Tessa Steiniche, Emily Chester, and Danielle Chusyd

Estradiol, a hormone in the estrogen family essential to physiological processes like reproduction, is conserved across vertebrates. Yet concentrations of estradiol vary between species. Why did this interspecies hormone variation arise? This meta-analysis focuses on estradiol variation across wild primates and approaches this question from the perspective of diet. Primates consuming high concentrations of phytoestrogens, naturally occurring estrogen mimics, may have evolved to compensate for these compounds by increasing overall circulating concentrations of endogenous estradiol. The relationship between fecal estradiol concentration and the proportion of folivory to frugivory in a species' diet was assessed. Estradiol concentrations were collected from 14 species across the literature. It was hypothesized that predominantly folivorous primates would have higher estradiol than frugivorous primates. Although the findings were not statistically significant under a linear model ( $p\text{-value} = 0.1957, R^2 = 0.3083$ ), the general trend opposes the hypothesis and suggests that folivorous primates may have lower estradiol than frugivorous primates. We discuss implications for primate ecology and evolution, and consider how fluctuating phytoestrogens in plant food sources due to changing climate could potentially interfere with primate physiology and fecundity.

KAITLYNNE EGGLESTON, King University

Short-term melatonin administration alters aggressive behaviors in male Siberian hamsters

Mentors: Greg Demas and Amanda Han

Seasonally breeding animals undergo physiological and behavioral changes across the seasons in response to environmental cues, such as photoperiod and temperature. Furthermore, the pineal gland hormone melatonin serves as the biochemical cue for photoperiod, and as one of the essential hormones in animals that experience day/night shifts. Our lab has previously shown that Siberian hamsters housed in short winter-like photoperiods, or given prolonged, short-day like injections of melatonin, display elevated aggression compared with control hamsters housed in long summer-like days. Although prolonged melatonin injections have been shown to increase aggression, the short-term effects of melatonin remain unknown. To assess the direct effects of melatonin on aggressive behavior, a resident-intruder paradigm was used to compare control hamsters to one-time melatonin injected hamsters. C-Fos, a proto-oncogene that is expressed in activated neurons, will be examined by immunohistochemistry to determine the brain regions involved in melatonin's actions on the neuroendocrine circuit. We predicted that because hamsters would not have sufficient time to undergo the seasonal changes, then a single injection of melatonin would not affect aggression. In contrast, it was found that melatonin did increase attack number and duration compared to the control. There was little difference between chase duration and latency to first attack. These findings suggest that melatonin may have immediate effects on aggression, suggesting it may act directly on neural circuits that regulate aggression.

ETHAN GUARDADO, Purdue University

How body mass interacts with behavioral and physiological thermoregulatory mechanisms in wild songbird nestlings

Mentors: Kim Rosvall, Mary Woodruff, and Susanna Tsueda

Animals are experiencing more frequent and intense heat waves. Behaviorally, animals can combat heat stress by evaporative cooling, often by panting. Physiologically, animals can also upregulate heat shock proteins (HSPs) to combat heat-induced cellular damage. However, prolonged HSP upregulation may itself cause damage and could be a trade-off with other fitness-related traits. Further, larger bodies retain heat, so individual mass or total mass in a litter or brood may impact thermoregulatory mechanisms. We investigated how HSP gene expression interacts with panting and mass in Tree Swallow (*Tachycineta bicolor*) chicks. We experimentally heated nests using air-activated Uniheat warmers to 4°C above control nests for 4 hours during the afternoon when chicks were 12-days-old (heat, n=25 nests; control, n=21 nests). We video-recorded and quantified chick behavior, including rate of panting, during the 2nd hour of treatment. When measuring HSPs, we focused on the hippocampus because this brain area mediates exploration and migration. Using qPCR, we quantified hippocampal HSP90AA1 relative gene expression for the median mass chick in each nest. Heat significantly increased both panting and HSP upregulation. In heated chicks, mass was positively correlated with HSP90AA1 gene expression. Additionally, in control nests total brood mass was positively correlated with panting. However, panting was not correlated with HSP abundance or average chick mass. Our findings suggest that heat waves may increase selective pressure on chicks to reduce mass and mothers to reduce brood size in order to avoid taxing thermoregulation.

MYA KNAPPENBERGER, Ursinus College  
Replay of Incidentally Encoded Episodic Memories in the Rat  
Mentors: Jonathon Crystal and Cassandra Sheridan

Replay of episodic memory is defined as the ability to sequentially recall the flow of past events (Crystal, 2021). Rats can replay episodic memories (Panoz-Brown et al., 2018; Sheridan et al., in preparation). Rats can also use incidentally encoded information about a single event to answer an unexpected question (Zhou et al., 2012). However, it is not known if rats can replay incidentally encoded episodic memories to answer an unexpected question using multiple events. To address this problem, we provided rats with opportunities to learn two tasks. One task gave rats a list of scented plastic lids in an encoding context. A memory assessment context presented two odors from the encoded list (one item was the third to last listed odor (correct choice) and a foil item from a different ordinal position in the list). Baseline accuracy in the replay of episodic memories was approximately 81%. In the second task, rats foraged in an 8-armed radial maze with baited food under unscented lids. To engineer incidental encoding followed by an unexpected question, we plan to allow rats to forage for food with scented lids replacing unscented lids. Next, rats will be confronted with a memory assessment with two odors that were recently encountered in the maze. If the rats can replay incidentally encoded information, then the rats will select the third to last odor that was encoded in the maze at their baseline level of accuracy. Alternatively, accuracy will be at chance (50%).

MACKENZIE RIVERA, Mount Saint Mary College  
The Effect of Physical Enrichment on Social Behaviors in Mice  
Mentors: Laura Hurley and Lauren Brunner

Enrichment in laboratory animals is an important aspect in increasing animal welfare as well as being a more reflective model of human problems. Although different methods of enrichment have been implemented in many laboratories, there is a gap in knowledge on how levels of enrichment effect social behaviors, specifically those used in courtship. During courtship, mice communicate through a series of vocalizations including ultrasonic vocalizations (USVs) in males (mostly) and broadband vocalizations (BBVs) in females. USVs produced by males are important for these interactions because during courtship the number of syllables, or calls, produced increases, as well as mounting attempts. Female mice greatly influence the production of USVs by different variables including their presence alone. This previous knowledge supports that communication through vocalizations and behaviors are an important aspect of mice social competency and sexual motivation. In order to assess the effect of physical enrichment on courtship behaviors we used two different groups of socially isolated CBA/J mice, one physically deprived (SD/PD) and the other physically enriched (SD/PE). We then employed a direct interaction model to evaluate USV production and physical courtship behaviors. In literature, there is also a lack of research on the preference of certain aspects of environmental enrichment that enhance species-specific behavior in CBA/J mice in their home environment. Because of this, we also investigated enrichment preference through observing the SD/PE group in their home cage. Through the home cage observations, we found that mice preferred the hut provided more than any other enrichment, but this seemed to decrease over time. In direct interactions, the mice that were physically enriched began to call sooner as well as had a higher average number of total USVs. It was also observed that mice that were physically enriched had a lower latency to mount, mounted more, and were rejected less by the female mice when compared to the physically deprived group. These findings suggest that mice have a preference for which enrichment they interact with, and support physical enrichment may have an effect on social behaviors but further studies with larger group sizes would be worth conducting.

AUDREY SEQUEIRA, Brandeis University  
Effects of Larval Diet and Density on Brain Morphology and Gene Expression  
Plasticity in *Spea bombifrons*  
Mentors: Cris Ledón-Rettig, Sarah Lagon, and Dante Nesta

Brain morphology is well known to be influenced by environmental stimuli, through a phenomenon known as plasticity. However, little is known about how various diet types and social environments interact to impact larval brain morphology. In order to study this, a two-way factorial experiment was performed with *Spea bombifrons*, in which tadpoles were given either a live prey diet or a detritus diet, and either a high or low social density. Tadpoles were raised for 12 days, after which brains were dissected, imaged, and measured to obtain volumes of the following regions: telencephalon, diencephalon, optic tecta, cerebellum, and hypothalamus. A gene expression assay was performed on whole brains, using BDNF (brain-derived neurotrophic factor) primers and qPCR to assess whether larval environments impact neurological gene expression. We found that diet significantly impacted whole brain volume and individual brain regions, but trade offs between brain regions were not found. Social density did not affect brain volumes, except for marginally within the optic tecta. qPCR found no significant differences in gene expression across groups, indicating that BDNF levels are not largely impacted by diet and social environment. We postulate this has to do with the role of BDNF in neuronal upkeep - it is favorable for the protein to function in a wide range of environments to maintain the function and survival of the larvae. Morphometric results indicate that diet significantly impacts brain size as a whole rather than individual brain regions, whereas social density may selectively impact optic tecta size.

ASHLYN THOMAS, George Mason University

Investigating the relationship between early life environment, testosterone, and immune function

Mentors: Cris Ledón-Rettig, Sarah Lagon, and Dante Nesta

Resource polyphenism is a type of plasticity – i.e., response to environmental cue – that allows organisms to capitalize on alternate diets. For instance, Spadefoot toads exhibit a resource polyphenism that allows their tadpoles to exploit live prey items and is induced through density and diet cues. However, little is known about how this developmental switch influences hormones and the potential influence of these hormones on an individual's fitness. Here, a two-part investigation was conducted to determine if early life social environment (high vs low population density) and diet (detritus vs shrimp) impacted testosterone levels, and whether testosterone levels could impact immune responsivity during the tadpole stage. Social density was found to have a significant impact on testosterone levels where low density animals exhibited heightened testosterone levels. Further, functional manipulation of testosterone levels with the aromatase inhibitor Fadrozole revealed that testosterone significantly depressed tadpoles' swelling responses to an injection with a vehicle, but not to a specific immune challenge (phytohemagglutinin injection). However, severe swelling reactions only occurred in PHA injected animals from the control group, whereas none occurred in the Fadrozole treated group, suggesting that the depressive effects of testosterone on immune responses may have been masked by our small sample size. Overall, our results indicate that testosterone levels can be influenced by early life environment, but further experiments are necessary to determine whether these modified hormone levels can affect an organism's immune responsivity.

ARIEL TYSVER, Indiana University

Anthelmintic Treatment Effects on Coccidia Shedding in the Dark-eyed Junco  
(*Junco hyemalis*)

Mentors: Ellen Ketterson, Katie Talbott and Sarah Wanamaker

Direct manipulation of the amount of one parasite, such as nematodes, in a host often has an impact on the other parasite species populations within the host, but this has not been well documented in the Dark-eyed Junco (*Junco hyemalis*, Junco). Both nematodes and the protozoa Coccidia are common parasites found in the gastrointestinal tract of these birds, so we used them as a model of the parasite interaction within the host. To assess the dependency of Coccidia population shedding on the population of nematodes in the host, we treated our Juncos with Ivermectin, a common dewormer intended to decrease nematode load. We also looked to assess if there are any differences in this relationship between males and females. Three treatment groups were used: males treated with Ivermectin, males treated with the control dextrose, and females treated with Ivermectin and samples were collected before treatment and after each dose for every bird. All fecal samples collected were analyzed using a modified fecal floatation method to isolate the Coccidia oocysts in the feces and determine the number of oocysts per gram of feces using microscopy. Although we did not find the expected increase in Coccidia shedding in treatment birds based on a previous study on mice, there was a significantly larger decrease in bird mass in the treatment group males compared to the control group males. This suggests that even if Ivermectin does not have a significant effect on Coccidia shedding in Juncos, it still may have an effect on the overall parasite community and the fitness of the host.



AEJAH VAN DYKE, Tuskegee University

The Effect of Enrichment in the Indirect Interactions of Male Mice

Mentors: Laura Hurley, and Lauren Brunner

Laboratory animals are often used as models for human diseases/behavioral disorders. However, while these animals are kept in sterile environments, we as humans live in rich and diverse surrounding. In this study, I explored the social behavioral effects of physical and social enrichment in the indirect interactions of male *Mus musculus* (house mice). My project focused specifically on two groups: Socially deprived/physically enriched mice (SD/PE), and Socially enriched/physically enriched (E). Each mice underwent five 30 minute home-cage observations prior to the trials, one 15 minute split cage assay and two 30 minute sociability assays. In the home-cage observations, time spent with each type of enrichment were analyzed. In the split cage assay, amount of and duration of ultrasonic vocalizations produced were analyzed. In the sociability assay, the time spent in each chamber, and the time spent sniffing the female stimulus in contrast to the empty wire cage was analyzed. In the home-cage observations, I discovered the mice preferred the hut enrichment the most. In the split-cage assay, the group E mice produced a significant more amount of baseline USVs than SE/PD mice. In the sociability assay, the SE/PD group mice spent more time in the empty chamber during playback and after playback than E mice. These findings suggest physical enrichment may have an effect on the sociability of male mice.

SHIARA VEGA NATAL, Universidad de Puerto Rico

Does circannual rhythm independently from photoperiod: a *Junco hyemalis* experiment

Mentors: Ellen Ketterson and Devraj Singh

Literature amongst different taxonomic groups suggest circannual timing is an ubiquitous occurrence. Much less is known about regional internal timing variation (latitudinal variation) on populations of the same species. Most animals are known to use the photoperiod (daylight length) as a calendar for when a precise biological event, such as reproductive cycle, molts and migration sequences, should occur given changes in temperature and humidity levels vary throughout the year and thus do not present a reliable source for timing. In order to test if the circannual rhythm can operate independently of photoperiod, two populations of male *Junco hyemalis*, one non-migratory (*J. h. carolinensis*) and one that migrates up north during its breeding phase but overwinters in sympatry with the resident population (*J. h. hyemalis*) were placed in a colony room under permissive constant dim lights (~3 lux) environmental conditions in the absence of the entraining cue (photoperiod) for eighteen(18) months. Reproduction (cloacal protuberance volume (CPV) and migratory (fat score (FS), body mass (BM) and body condition (BC) readiness indicators were collected at an interval of 4 weeks for the duration of the experiment. In addition, birds were bled for baseline testosterone(T) and GnRH challenged testosterone every 4 weeks. Lack of photoperiodic entrainment appears to delay the reproductive and migratory cycles for both populations as inferred by the CPV and body mass fluctuations throughout the eighteen months providing enough evidence to confirm the existence of an endogenous circannual rhythm. Furthermore, migratory birds started their second gonadal development cycle faster than resident birds even when housed under the same environmental conditions suggesting that the endogenous clock differs amongst individuals of different populations.