It is with great pleasure that I present to you the Fall 2010 issue of Compass. The commitment and dedication of my fellow graduate students to both the Department of Forestry and Natural Resources and to communities both local and international is nothing but awe-inspiring. A handful of their research stories appear in the following pages.

Through their stories, I, along with the FNR Graduate Student Government and the Magazine Committee hope to instill within you a better understanding of ground-breaking research being conducted at Purdue University and foster a deeper appreciation for the natural world in which we are all citizens.

I challenge you to identify your own personal talents and use them to positively change the world around you, as the authors of the following stories have done. I would also like to encourage you to find more information about the department and current research activities by visiting www.fnr.purdue.edu.

Kimberly Robinson, Compass Editor

Dear Compass Reader:

Professor Peter Beidler of Lehigh University once explained: “I teach because, being around people who are beginning to breathe, I occasionally find myself catching my breath with them”. Faculty are blessed in large part because their jobs provide them an opportunity to interact with students who are intelligent, energetic, and enthused about making a difference in the world. Such enthusiasm is contagious, and a principal reason for publishing Compass is to expose readers to students who serve as excellent vectors of the enthusiasm bug. Interested in biodiversity? Read about student research on species of conservation concern such as eastern box turtles, eastern hellbenders, and Allegheny woodrats, or genetic signatures of vertebrates in biodiversity hotspots such as Azerbaijan and Hispaniola. Curious about how wildlife cope in agricultural landscapes? Check out the articles on fish health in ditches adjacent to concentrated animal feeding operations, and the landscape genetics of rodents inhabiting farmlands. Think that “silence is golden”? Think again, and read how soundscapes can affect our enjoyment of nature. Concerned about the vitality of forests and forest products? Read about advances in the war on emerald ash borer, and the characterization of a beautiful figure in aspen wood that is primarily under genetic control.

If the enthusiasm bug hasn’t bitten you yet, keep reading. You’ll catch it here, in the Compass!
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Figure, as a wood characteristic, can be used in wood identification and can add extra value to wood products. Understanding the heritability is important to the reliable production of figure-wood products that can meet industrial standards. With this in mind, colleagues and I have used microsatellites to identify a 25-year-old male hybrid aspen named “Grober,” as a *Populus x canescens* (*P. alba x P. tremula*). More than 80 “Grober” propagules from the original parent tree were sampled at various times (7, 11, 12, and 60 months) after growth under two environmental regimes (field vs. greenhouse) and following two treatments (pruned vs. unpruned).

All “Grober” propagules exhibited figure in all woody parts. By splitting the wood longitudinally, the figure was identified as an undulating pattern on the radial surface. These results were not observed in the control group, which included two *P. alba* genotypes (A502 and 4877), a second *P. x. canescens* clone (Ca-2-75), “Crandon” (*P. alba x P. grandidentata*), and “Sherrill” (*P. alba x P. grandidentata*). The control group showed only smooth radial surfaces with straight grain. Unlike the control group, “Grober” propagules clearly exhibited a constant spirality (2.62 ± 0.12 degrees/cm) to axial growth in 11-month-old plantlets. However, this spiral growth pattern was not apparent in wood produced by the mature, parent tree from which the plantlets were derived. In addition, veneers cut from the same 25-year-old “Grober” tree appeared to produce a type of figure that had not yet been named. Based on its appearance, we decided to call it Scatter Moiré.

To investigate chemical signals that may be involved in figure formation, 160 grafts were made between “Grober” and various non-figured aspens. After sampling this population, we concluded that graft-transmissible signals are not involved in figure formation in “Grober.” Additionally, “Grober” was crossed to A502 to test the inheritance of the gene responsible for figure formation. More than 2,000 seedlings were obtained. A sub-sample of this population revealed that 79 out of 377 seedlings exhibited figure. A Chi-square test led to the rejection of a 1:1 segregation ratio between figure and non-figure phenotypes, but not a 1:3 segregation ratio. Overall, these analyses showed that figure in “Grober” is under genetic control, but the inheritance pattern may not be simple.

“Veneers cut from the same 25-year-old “Grober” tree appeared to produce a type of figure that had not yet been named... we decided to call it Scatter Moiré.”
Evaluating Threats To Allegheny Woodrat Persistence In Indiana

by Tim Smyser

Allegheny woodrats (*Neotoma magister*) are a species of conservation concern throughout their Appalachian range as populations have declined dramatically over the last 40 years. Abrupt declines have been reflected in Indiana populations as well. By 2005, Indiana woodrat abundance had declined nearly 60% relative to abundance levels observed during initial population monitoring efforts conducted in the early 1980s. A number of hypotheses have been proposed as causes for declines within Indiana and throughout the woodrat’s range. These causes include: 1) habitat fragmentation, 2) reduction in winter food resources, 3) increased mortality as a result of infection by raccoon roundworm (*Baylisascaris procyonis*), and 4) loss of genetic diversity.

Together with Dr. Gene Rhodes and Scott Johnson, and our collaborator with the Wildlife Diversity Section of the Indiana Department of Natural Resources, we have been working to evaluate how such factors threaten the persistence of Indiana Allegheny woodrat populations. A major emphasis of our research has been the experimental translocation of woodrats to evaluate the local effects of raccoon roundworm exposure and inbreeding depression. In the spring of 2007 and 2008, we transplanted 68 woodrats from large, genetically diverse populations in Kentucky and Tennessee into Indiana habitats in order to re-establish four extirpated (or locally extinct) populations and supplement two genetically and numerically depressed populations. Monitoring the demographic and genetic response of these populations to the addition of genetically diverse individuals has allowed us to evaluate the effects of inbreeding depression on local population declines. Additionally, to evaluate the influence of roundworm-related mortality on Indiana woodrat populations, three of the six translocated populations were treated with the monthly distribution of anti-roundworm baits to passively deworm associated raccoon populations and minimize roundworm-related mortality. The change observed in woodrat abundance between the three treated and three untreated sites will help us to understand the influence
of raccoon roundworm on local population dynamics.

To assess the local threat to woodrat populations posed by exposure to raccoon roundworm, raccoon latrine surveys were conducted across all translocation sites in the fall of 2006 prior to the supplementation of woodrats. Raccoon latrine surveys consisted of the systematic collection of raccoon scat (i.e., feces) from the forested areas surrounding woodrat habitat. Using a fecal float technique, the presence or absence of raccoon roundworm eggs was documented for each collected scat. Following the initial assessment of raccoon roundworm exposure by site, the monthly distribution of deworming baits in the forested areas surrounding woodrat habitat was conducted in three of the six translocation sites. To evaluate whether the monthly distribution of baits was having the desired effect on raccoon roundworm exposure risk, raccoon latrine surveys were repeated in the spring of 2007, fall of 2007, and fall of 2008.

In the spring of 2007, 54 Allegheny woodrats were captured in Kentucky and Tennessee and transported to Indiana. Here they were released directly into suitable den sites, based on the presence of complex rock structure and a history of past woodrat occupancy. At the time of release, individuals were provided with an abundant supply of rat chow and nesting material to encourage them to settle at the release site. During the initial year of translocation, all released individuals were radio-collared which allowed us to monitor their survival and settlement locations over the first two months.

During the summer of 2007, we used live-trapping to further monitor the survival of the released individuals, as well as to evaluate rates of reproduction, population growth, and the retention of the genetic diversity that had been introduced in translocated founders. As part of our live-trapping monitoring efforts, between 35 and 60 traps were set within each woodrat population. Traps were baited with sliced apples and checked daily. Upon the capture of a woodrat, the sex and age of the individual was identified, and the captured individuals were tagged. Uniquely numbered ear tags were placed on each ear (which allowed us to identify the individual upon future recapture), and a small tissue sample was collected for DNA analysis.

After the initial translocation efforts of 2007, we determined that the numbers of individuals released into our supplementation sites were too small to obtain the desired restoration of genetic diversity. Accordingly, in the spring of 2008 we returned to Kentucky and captured an additional 14 individuals to release across our two supplementation sites. In the summer of 2008 and fall of 2009, we again used live-trapping to monitor the response of the translocated populations.

In general, woodrats reintroduced into habitats treated with deworming baits successfully established stable and self-sustaining populations. However, the reintroduction of woodrats into sites in which exposure risk to raccoon roundworm remained unmanipulated failed, and these sites eventually became

Raccoon deworming bait.
uninhabited. The results on supplementation sites are slightly more complicated. A positive response in woodrat abundance has been observed among both the treated and untreated supplementation sites. Woodrat abundance in the untreated supplementation site has been dynamic, but current levels of woodrat abundance are much greater than prior to our translocation efforts. Despite the fact that the second supplementation site was untreated with deworming baits, the survival rate for woodrats at that site remained high throughout our annual monitoring efforts over the last five years, suggesting the impacts of exposure to raccoon roundworm were minimal at that location. Early genetic data collected on offspring captured at both supplementation sites suggests the translocation efforts positively impacted local levels of genetic diversity and a genetic-rescue effect may be occurring.

While the threat posed by raccoon roundworm naturally varies across sites and over time, it appears that the management of raccoon roundworm exposure risk through the monthly distribution of deworming baits improves the probability of population survival. Although the reintroduced populations were established with genetically diverse individuals, these populations were founded with a small number of individuals and population sizes have remained small. Also, these populations remain isolated from one another with no movement of woodrats among populations. Therefore, for the long-term survival of Allegheny woodrats within Indiana, additional and continued management actions will need to be taken to ensure the maintenance of high levels of genetic diversity. To address this need, we have recently started a captive breeding program. In this program, individuals are removed from isolated populations in Indiana and paired with individuals collected from other populations in Indiana and Pennsylvania. In time, they and their genetically diverse offspring will be returned to Indiana populations in a way which will help restore and maintain healthy levels of genetic diversity across all Indiana woodrat populations.

“While the threat posed by raccoon roundworm naturally varies across sites and over time, it appears that the management of raccoon roundworm exposure risk through the monthly distribution of deworming baits improves the probability of population survival.”

Allegheny woodrat following release from live-trap.
INVESTIGATING THE ENVIRONMENTAL IMPACTS OF CONCENTRATED ANIMAL FEEDING OPERATIONS by Jessica Leet

Concentrated animal feeding operations (CAFOs) are becoming a standard method of livestock production. As the number of CAFOs increases, there is a growing concern regarding their potential environmental impacts. CAFOs produce large amounts of animal waste that contain nutrients, as well as natural and synthetic hormones. It has become common practice to apply this waste to agricultural fields as a cheap and effective fertilizer. Midwestern agricultural fields typically have subsurface tile-drain networks that facilitate transport of excess water from agricultural fields to a ditch network system. As a result, sediments, excess nutrients, pesticides, and hormones are also delivered to ditches receiving tile drainage from fields fertilized with manure and associated lagoon effluent from CAFOs. There have been very few studies measuring contaminant levels in these ditches and their effect on aquatic organisms.

My research is a portion of a larger effort led by Dr. Maria Sepúlveda and other faculty at Purdue, including Dr. Linda Lee from Agronomy and Dr. Reuben Goforth from our Department, to study potential environmental effects of CAFO waste application practices. In Dr. Sepúlveda’s lab, we have been evaluating the health of fish communities in the aquatic ditches adjacent to the agricultural fields that are fertilized with waste from the Purdue University Animal Sciences Research Farm CAFOs. We are studying two ditches in particular, Marshall and Box Ditch. Marshall Ditch runs adjacent to corn and soybean fields that are broadcast applied with beef and dairy CAFO solid waste. Box Ditch runs adjacent to fields which are subsurface injected with swine and poultry CAFO waste. Both of these ditches receive tile-drain runoff from their adjacent fields.

Using backpack electrofishing we collected fish from these two ditches as well as from a reference site, Ghost Creek. At each site we...
identified individual fish at the species level, measured their total length, and then released them. We sampled each of the three study sites ever six weeks from May to October in 2008 and 2009. The overall species richness (number of species) for both years was significantly lower at the agricultural sites, Marshall and Box Ditches, compared to the reference site, Ghost Creek. In both years, species intolerant of habitat degradation were present at Ghost Creek and notably absent at both Marshall and Box Ditches. Ghost creek is a forested tributary to the Tippecanoe River, and it appears to have more consistently available fish habitat compared to both the CAFO influenced agricultural ditches. These results and the environmental parameters for all the sites provide evidence that the fish community in Ghost Creek is healthier and more stable than those in Marshall and Box Ditch. This may account for some of the observed changes in species richness. However, further investigation is being conducted on the contaminant levels in the agricultural sites to determine whether or not pollutants are playing a role in the alteration of these fish communities. The concentrations of hormones, pesticides, and nutrients are being analyzed in Dr. Lee’s lab for both collection years. This will provide further insight into differences between agricultural ditches adjacent to fields fertilized with CAFO waste and our relatively pristine reference creek.

In order to evaluate potential impacts of CAFO effluents on fish reproduction, creek chubs (*Semotilus atromaculatus*) were collected at each of the three study locations. So far our data indicate that fewer sexually mature chubs were found at the agricultural ditches compared to the reference creek. Samples are still being processed to determine the ages of each fish collected. Gaining information on the age structure of each population will help us determine whether the number of underdeveloped gonads we are seeing is due to the abundance of juvenile fish in the Marshall and Box Ditch populations or possibly due to stunted gonadal growth.

Along with gonadal development I am also concerned with sex ratios of fish exposed to increased hormone loads from applied CAFO waste runoff. Hormones in water can lead to masculinization of females, feminization of males, or potentially changes in sex determination. Beef and swine from CAFOs are often treated with various chemicals, including treatment with synthetic hormones for growth promotion. Synthetic hormones, their metabolites, and natural hormones produced by the animals can be excreted into the animal waste that is then used to fertilize agricultural fields. This can be an important source of synthetic and natural hormones to the environment. There is much the scientific community still does not

Field station adjacent to Marshall Ditch at the Purdue University Animal Sciences Research Farm.
know about the movement of these chemicals through the environment and the potential effects of these hormone mixtures on aquatic organisms.

I am also conducting laboratory studies exposing fathead minnows \((Pimephales promelas)\) to hormone loads typically found in our agricultural ditches to see if hormones present in this water can alter sex ratios. I have conducted an on-site exposure of fathead minnow embryos to inflowing ditchwater at a field station located on Marshall Ditch. These fish were grown in the ditchwater until sex could be determined (which in this species occurs about seven weeks after hatching). I compared the resulting sex ratios with a control group of fathead minnows that had been raised under similar conditions in uncontaminated well water at the Purdue University Aquaculture Research Lab. In the population that was exposed to water from Marshall Ditch the sex ratio was 59% males and 41% females, compared to the control population sex ratio of 51% males and 49% females. I will be conducting various other experiments in which I expose fathead minnows in the laboratory to individual hormones found in Marshall and Box Ditch water, as well as hormone mixtures, to help determine if these hormones have the potential to alter sex ratios in fish at concentrations present in the environment. Altered sex ratios could have large effects on fish population structures, and possibly explain some of the differences seen between fish communities in our agricultural ditches and reference creek.

The number of CAFOs continues to increase every year. Thus, it is important to continually evaluate what we know about the impacts of CAFOs, so regulations and operational practices can be adjusted to ensure health and safety. My research is adding to growing knowledge about the impacts of CAFOs on aquatic organisms and communities. I will continue to evaluate the biodiversity of the aquatic ditches surrounding our CAFOs of interest, and further investigate potential effects of hormones on fish in these ecosystems.
Movement Patterns Of Two Rodent Species In Fragmented Landscapes

by Sara Anderson

The Midwestern region of the United States has undergone extensive changes in land cover over the last 200 years. Before settlement, 87% of Indiana was covered in forest, and after extensive conversion to agriculture in the northern part of the state, forests make up only 8% of the land today.

Human alteration of natural ecosystems in the form of habitat fragmentation changes the composition and configuration of the landscape and may shift the distribution of resources essential for the survival of individuals and possibly entire species. Habitat patches become isolated by a complex system of non-ideal landscape elements, which can inhibit movement of animals and ultimately lead to genetic structure, which is a difference in gene frequencies among populations. In time, negative consequences of isolation may be loss of genetic diversity or extinction of local populations (“extirpation”).

Many species are sensitive to habitat fragmentation and have disappeared from the small forest patches that remain. There are a few species, however, that have flourished under this new configuration of forest patches and agriculture, including raccoons (Procyon lotor), Virginia opossums (Didelphis virginiana), eastern chipmunks (Tamias striatus), and white-footed mice (Peromyscus leucopus).

The Upper Wabash River Basin in northern Indiana is a highly fragmented landscape that has been studied extensively by researchers in the Department of Forestry and Natural Resources at Purdue University, and it is the study area for my research. In order to elucidate the effects of landscape features (e.g., forest, croplands, roads) on movement patterns of white-footed mice and eastern chipmunks, I evaluated levels of genetic structure exhibited by these two species at multiple spatial scales. Low levels of movement between populations would result in a high degree of genetic structure among populations, and extensive movement between populations would minimize genetic structure.

After identifying individuals through molecular genetics techniques, I found no evidence of genetic structure in white-footed mice at the scale of the entire study area, but I did detect variable patterns of genetic structure at small spatial scales. Considering the short movements of mice and their ability to attain high population densities in very small forest patches, the most likely explanation for these patterns is that mice in this landscape have retained most of their genetic variation due to their large reproductive capacity. In other words, the random loss of genetic diversity over generations is relatively small, as most genes of one generation are passed on to the next generation.

I investigated the possible factors influencing the variability in genetic structure at small spatial scales in white-footed mice using both landscape composition and configuration variables. My results indicated that while treed
corridors facilitated gene flow at small spatial scales, roads seemed to impede gene flow. Additionally, complexity of forest patch shape and distance between patches were important components in detecting genetic structure of white-footed mice. These landscape configuration variables imply that accumulation of genetic structure likely occurs in two situations:

1) When a given area has a few small patches separated by large distances; and
2) Among larger patches that contain riparian areas and have complex shapes.

Eastern chipmunks exhibited evidence of five large sub-populations across the study area, and sample cells seem to be connected by a very low, but significant, amount of dispersal. Analysis at small spatial scales revealed high levels of genetic structure in eastern chipmunks, again with subpopulations connected by very low levels of dispersal. Genetic differences over large distances are expected, because neighboring animals tend to be more similar to each other than they are to animals far away. However, genetic structure appeared between subpopulations of eastern chipmunks that were separated by distances well within the species’ movement ability, indicating that factors other than distance were limiting gene flow.

To understand what might be driving the buildup of genetic structure, I evaluated the permeability of eight land cover variables to chipmunk movement. Forests, grasslands, and non-treed corridors consistently facilitated gene flow to the greatest extent, while there was some evidence that treed corridors and roads limited gene flow. Many biologists consider roads to be a limiting factor to animal movement through direct mortality and road avoidance behaviors.

White-footed mice and eastern chipmunks employ different strategies to survive in this fragmented ecosystem. In general, white-footed mice don’t appear to move between habitat patches, but retain genetic variability with high reproductive capacity in dense populations. On the other hand, eastern chipmunks show signs of low-level genetic exchange between sub-populations, a situation known as a metapopulation (i.e., population of populations). I had expected eastern chipmunks to be more connected at fine spatial scales than I observed, but isolating factors in the landscape may have inhibited movement between subpopulations to a large extent.

By utilizing multiple data sources, such as genetic, ecological, and landscape data, we can better understand how the environment contributes to the patterns of populations in an ecosystem.

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By utilizing multiple data sources, such as genetic, ecological, and landscape data, we can better understand how the environment contributes to the patterns of populations in an ecosystem. Using a single data source provides part of the story, but to clarify processes in these complex agriculturally fragmented systems, cross-discipline research is essential.
Determining The Basis For Emerald Ash Borer Resistance by Darla French

Ash trees (genus *Fraxinus*) are important to North America. There are approximately eight billion ash trees nationwide. Besides being fundamental aesthetic elements of nearly every city and suburban landscape, ash trees have high stumpage value and are an important commercial lumber species to the hardwood, furniture, and tool-making industries. In addition, ash trees serve as significant Native American cultural resources and play an integral part in the ecology of North America. It is difficult to determine the economic importance of ash trees because so much of their worth is from their social and cultural value rather than its market price; however, an estimated dollar value is close to $300 billion annually. Sadly, the ash-tree industry in North America is now in grave danger. Why? A tiny newcomer to the North American landscape: The emerald ash borer.

The emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)), is a jewel beetle native to Southeast Asia. In its native range, this metallic wood borer acts primarily as a secondary or opportunistic pest, mainly attacking weak or dying trees of two Asian ash species, Chinese and Manchurian ash. The life cycle of EAB includes larval, pupal, and adult stages, but it is the larval stage that is most damaging. After hatching, the larvae bore through the bark into the cambium layer, where they preferentially feed on phloem, the tissue layer located just inside the bark of the tree that conducts food from the leaves to the roots. This can lead to girdling (a circumferential discontinuity in the phloem), which starves the tree. The effects of girdling are not obvious until well after adults emerge, usually a few months later, but in some cases the following summer. By the time it is apparent that the trees are dying (usually three to five years after infestation), the adults have long since moved on to infest other trees, either by natural (short-range) or human-facilitated (often

*Emerald ash borer adults were caught by the IDNR in Huntington County and transported to Huntington Nursery, where they were released on one pair of enclosed green ash trees for subsequent infestation.*
long-range) dispersal.

In North America, the EAB is considered an invasive, exotic species. It is thought to have been inadvertently introduced to this continent via shipping materials unloaded in Michigan, probably sometime in the early 1990s. Since its discovery in Detroit in 2002, it has spread as far east as Maryland, as far south as Kentucky, as far west as Wisconsin, and north into Canada. All North American ash trees, regardless of species, age, and health status, are vulnerable to EAB infestation. This insect seems to attack and subsequently kill North American ash trees indiscriminately; this is unusual in light of its behavior in Asia. We can conclude from this observation that our North American ash trees are somehow completely susceptible to EAB infestation, whereas those ash trees native to Asia are not. But what is the biological mechanism by which EAB results in death of its host, and why are our North American trees susceptible to this insect while the Asian species are resistant?

The EAB is a relatively new insect to North America, so there are very few published reports on this devastating beetle. A literature search revealed a total of about 30 published papers, a few of which are from China and the rest have been published since the discovery of the insect in Michigan in 2002. One might wonder at first about the relative lack of information on this insect even in its native range, but this really is not surprising. In contrast to the destruction it has caused in North America in just a few short years, in its native range EAB is not problematic. To date, most U.S. research has focused on efforts of controlling or curbing the spread of EAB, including mass trapping, chemical treatment of individual ash trees, detection methods such as test trapping, and various visual screening and monitoring methods. Biological controls, including parasitoid wasps, are also currently being investigated. So far, none of these approaches has been particularly success. We currently lack the information about this insect and its effects on ashes that we need to effectively control it.

Through my research, I am attempting to determine why North American ash species are so markedly different from their Asian counterparts with respect to EAB susceptibility. The main objective of my work is to compare the metabolomic profiles of North American and Asian ash species. If we can determine that the difference is due to compounds produced by one species but not by another, we might be able to exploit those differences to engineer ash trees with elevated resistance to EAB. Seedlings of susceptible North Ameri-
can ash species (white, green, and black) are being grown in a greenhouse alongside resistant Asian ash species (Chinese and Manchurian) and two European ash species that have unknown resistance levels. Seedlings will be challenged with the compound methyl jasmonate, via a root drench, to simulate insect feeding; some will be left untreated to serve as controls. At various intervals post-treatment, a range of plant tissues will be sampled and subjected to chemical analyses. Resulting chemical compound profiles for each species group will be compared in an attempt to identify one or more compounds that may be responsible for resistance in the Asian ash trees.

A second objective of my research is to compare the transcriptomic profiles of infested and uninfested green ash trees via high-throughput sequencing in an attempt to determine which genes are turned on or turned off in response to EAB feeding. Green ash trees were planted in cages on property owned by Huntington Nursery (Huntington, IN) in summer 2009. In collaboration with the Indiana DNR, one pair of trees was subjected to infestation by introducing EABs into the cage in which these trees were housed; the cage surrounding the control trees protected them from infestation. The trees were harvested in early fall 2009. Messenger RNA was isolated from phloem of each tree and submitted to the Purdue Genomics Facility for subsequent high-throughput sequencing. The resulting transcriptome profiles are being compared to identify genes that show an altered expression pattern in response to EAB infestation. Once candidate genes have been identified, a set of molecular experiments will be conducted to investigate the function of various genes in the jasmonate pathway, which mediates a plant’s response to insect attack, in tissue harvested from methyl jasmonate-challenged seedlings.

Vince Burkle (Indiana DNR) releases emerald ash borer adults on one pair of enclosed green ash trees, while Darla French (Purdue University) looks on.

Vince Burkle (IDNR) screens last season’s EAB-infested logs, used as a source for EAB adults to use in our study, for larval galleries and adult exit holes. A gallery can be seen on the peeled log near Vince’s left foot.
Spatial Ecology, Population Size, and Health Status of Eastern Hellbenders
by Nick Burgmeier

While unbeknownst to many traversing the tributaries of the Ohio River in southern Indiana, whether floating lazily down the river by canoe or wading among the rocks, they may be very near an ancient creature few people see, but many “old timers” once feared. This reclusive creature goes by many names with varying origins, including: snot otter, devil dog, grampus, and Allegheny alligator. More commonly, it is referred to as the eastern hellbender (Cryptobranchus alleganiensis alleganiensis), a name which itself is of uncertain origin. Take one look at this two-foot-long, brown and slimy salamander, and the name “snot otter” will seem the perfect description.

Hellbenders are found throughout the eastern United States. They are long-lived, fully aquatic salamanders requiring cool, rocky, swift flowing streams and rivers with abundant large, flat shelter rocks for cover and a high dissolved oxygen content allowing for them to breathe through their skin. Unfortunately, these traits make the species particularly vulnerable to decimation by common land practices throughout its range. In the past several decades, much of its habitat has been degraded or destroyed as rivers became silted and contaminated due to agricultural run-off and channelization, while dams have reduced available habitat through decreased flows and the reduction of dissolved oxygen. Additionally, many negative myths surround the hellbender. It was once thought to be poisonous by local residents, and fishermen believed they would steal fish off of their line or eat all of the fish in an area. In reality they feed almost exclusively on crayfish. These beliefs led to widespread persecution in areas where hellbenders were commonly captured by fishermen. The direct killing of hellbenders resulted in local extinctions, because the species is slow to mature and has low reproductive output.

In Indiana, hellbender populations have been reduced to one tributary of the Ohio River in southern Indiana. Biologists with the Indiana Department of Natural Resources (IDNR) were becoming increasingly aware that this remaining population had been declining and that reproductive output was seemingly small, if occurring at all. This prompted the IDNR to implement a comprehensive research strategy aimed at gathering as much ecological information about the

“The steep decline of hellbenders over the past few decades is especially concerning given their potential as a bioindicator.”

Photo by Shem Unger

The face of a not so cuddly Eastern hellbender.
population as possible in order to implement a management plan based on the best, most complete science possible.

In order to accomplish this goal, our research has focused on several different aspects of hellbender biology. My research ranges from spatial ecology and molecular biology to overall physical health. Thus, the objectives of my work are to:

1) Estimate current population size and density in Indiana;
2) Compare demographic and genetic techniques of population estimation;
3) Determine home-range size, and habitat use and preference;
4) Establish baseline blood chemistry parameters, evaluate health and disease status, and enumerate blood-borne parasites;
5) Investigate the potential use of molecular techniques to check for a biomarker (vitellogenin) linked with endocrine disruption and as a possible means of gender determination; and
6) Evaluate physicochemical water quality parameters.

During the summer and fall of 2008 and 2009, we implemented an intensive mark-recapture survey (~1,500 person hours) across 35 sites (~11km). Individuals were captured, marked, and biological samples were taken before they were released back to their site of capture. The results of this study confirmed the IDNR’s initial suspicions that the population size had decreased far below that which was reported in the mid-1980’s. During a total six months of actual surveys, we only captured 78 individuals, all of which were adults. To put this in perspective, we captured 70 individuals across all age classes from a healthy population in Georgia in only three days. The density has also dropped to a level much less than that reported from most other “healthy” hellbender populations throughout its range. This paints a grim picture for the continued survival of the population, but provides managers with a solid number to work with when designing the goals of future projects. Using the results from the demographic data derived from this study, we’re also looking to compare classic mark-recapture population estimators with various genetically determined estimates of effective population size. This will allow fine-scale tuning of our estimates and provide a more realistic view of the status of the population.

During the same period as the previous study, we implanted 21 radio transmitters in hellbenders across eight sites. These individuals were monitored from June 2008 to October 2009 and the movements and habitat use of each were recorded. Using 1,193 locations, I estimated home-range size and habitat use for each individual. Though highly variable, our home-range estimates were considerably larger than those developed for other populations. However, habitat use was relatively similar, as individuals tended to prefer large flat rocks and gravel substrate. Due to the low densities...
at the sites, the home ranges may be larger as hellbenders expend less energy defending shelter rocks and may need to range further to encounter a mate.

In order to evaluate the health of the remaining population, a series of biological samples (i.e., blood, skin swabs, sperm, and tissue) were taken from each captured individual. Blood samples were used to determine baseline blood chemistry parameters and to check for parasites. Knowledge of baseline blood chemistry will allow for the evaluation of individual health of captured hellbenders and give some idea of the overall physical well-being of the population. Although parasites are common in many wild species, none were found in any captured hellbenders. Plasma collected from the blood was also used to check for potential endocrine disruption and as a means of gender determination for unknown individuals. Vitellogenin (VTG) is an egg-yolk precursor protein found in most egg-laying species and is typically only expressed in females. Thus far, positive readings in individuals of known gender have only been found in females, indicating the utility of VTG as a means of sex determination. A positive VTG result in a male individual would indicate endocrine disruption.

Skin swabs have also been taken from all individual to check for the presence of the infectious and often lethal chytrid fungus (*Batrachochytrium dendrobatidis*). This fungus has caused the decline of amphibian populations in central and South America. Unfortunately, we had a positive result in one individual within the Blue River. This was not entirely unexpected as the fungus has been found in many hellbender populations without producing noticeable affliction. Sperm was taken when available to ensure reproductive viability. Despite small sample sizes, all indications show that hellbender sperm were healthy and similar to other populations. Finally, tissue samples were taken as part of another study to develop genetic markers in order to determine genetic variability within and between populations, but also to develop the genetic population estimates for comparison to the mark-recapture method.

As a final measure, to determine potential stressors to the population, water quality at all radio-telemetry sites was evaluated to determine whether water quality might be a factor in continued hellbender population decline. Physical properties (e.g., dissolved oxygen, pH, temperature, specific conductivity), nutrients (e.g., nitrogen, phosphorus, ammonia), and 27 pesticides were tested for from November 2008 through July 2009. All physical properties indicated a high quality system with

*Many types of biological samples are taken from each captured individual including blood, skin swabs, sperm, and tissue.*
high dissolved oxygen levels and low specific conductivity. Nitrogen and ammonia levels were low, though phosphorus levels were slightly higher than recommended by the USGS. Atrazine, simazine, and metolachlor were three pesticides found in the system from late April through early July. Atrazine is one of the most widely used pesticides in the world and some reports implicate it as a potential endocrine disruptor. This is especially worrisome for hellbenders, as their long life spans and fully aquatic nature would allow continual exposure over long periods of time.

The steep decline of hellbenders over the past few decades is especially concerning given their potential as a bioindicator. Their decline suggests a potential problem with the overall system. Moreover, their extinction would mean the loss of an ancient creature which has persisted in this region for millions of years. Our findings provide a considerable amount of information of value for both basic ecology and practical management. It will serve to drive management recommendations and will be instrumental in the development of a successful translocation plan if this step is deemed necessary. This research represents an important first step towards developing a comprehensive management plan aimed at not only preventing the extirpation of the species from Indiana’s waterways, but to the eventual restoration of the species’ previous range.

“Though highly variable, our home-range estimates were considerably larger than those developed for other populations.”

Radio-telemetry is used to track hellbenders to study home range size and habitat use.
Purdue University’s Human-Environment Modeling and Analysis (HEMA) lab, under the direction of Dr. Bryan Pijanowski, is conducting research on soundscapes found in Tippecanoe County, Indiana. The HEMA lab has made recordings of soundscapes on different Purdue properties that include forests, wetlands, agricultural, and developed lands. Results of this research will begin to identify how Midwestern soundscapes change with land use and how they vary over time. Understanding how soundscapes are affected by land use may also help guide conservation efforts. My research in the lab is focused on soundscape conservation being implemented by the U.S. National Park Service (NPS).

The NPS protects 392 management units, including national parks, monuments, battlefields, historic sites, recreation areas, lakeshores, and seashores. Within these parks are natural, cultural, and historic resources that are part of America’s heritage. When visiting a national park, such as Yellowstone, most visitors go to see wildlife and view amazing geologic features. But did you realize that contributing to the overall experience at national parks are the sounds that are present? A study conducted by the NPS found that experiencing natural sounds is a major reason why people go to national parks. Some sounds one might expect to hear at Yellowstone include geysers shooting water into the air, elk bugling, wolves howling, rivers rushing, and many different birds singing. A growing body of research indicates that the sounds heard during park visits influence the overall experience.

The NPS has determined that the acoustic environment, like wildlife, water, and scenic views, is a resource worthy of protection. All of the sounds present within an area are considered its soundscape. Many types of sounds can comprise a park soundscape, and they change with time and location within the park. A soundscape can be comprised of natural sounds from wildlife, wind, water, and other geophysical sounds. It can also have historic or cultural sounds, such as canons or rifles and Native American music. Park soundscapes have other human-generated sounds, including visitors talking, children laughing, vehicles on park roads, trains, off-road vehicles (ORVs), boat engines, aircraft flying...
overhead, and park-operation sounds (i.e., chainsaws, lawnmowers, building construction or repair, and road plowing). How pleasing and appropriate certain sounds are to an individual will vary based on factors such as visitor expectations and the duration and volume of the sounds.

Sounds that are disturbing or annoying are considered noise. The NPS is working to determine what constitutes noise in different parks and how much noise is appropriate to ensure a healthy soundscape that reflects the different parks’ values. Legislation and park policies have been developed mandating that the NPS address this issue. Beginning with its Organic Act of 1916, the NPS has been directed to preserve the resources and ensure the enjoyment of visitors for both this and future generations. This means that the impact of noise sources on park resources and visitors should be determined. Aircraft noise is one of the first noise sources to be addressed by the NPS. In 1987 the National Parks Overflight Act was passed directing the NPS to determine the effects of aircraft on the National Parks System. The Act also required the Grand Canyon to develop an aircraft overflight management plan. Research conducted by the NPS determined that low-flying aircraft had the greatest negative impact on visitor experience and park resources. The majority of these low-fliers are tour operations, also known as flight seeing aircraft. The findings culminated in the National Park Air Tour Management Act of 2000, a law that requires parks with air tours operating over, or just outside the park boundary, to develop a management plan.

The NPS Natural Sounds Program was established in 2000 to help parks develop air-tour management plans. The NPS works collaboratively with the air-tour operators, the Federal Aviation Administration, Native American tribes, and environmental organizations to develop these plans. Soundscape monitoring is one of the first steps in developing alternatives, but it is also used to inform wildlife management, transportation planning, and recreation studies. Monitoring the soundscapes requires deploying automated acoustic recorders. Some recording devices can moni-

Aircraft noise is one of the first noise sources to be regulated in parks. This picture shows an air tour helicopter flying over Haleakala National Park, Hawaii.
tor sounds continuously for extended periods. Audio recordings are analyzed by the Natural Sounds Program or park managers to determine acoustic baseline conditions. Some acoustic characterizations identify different types of sounds that are present, the percentage of time that motorized vehicles are audible, and the volume of motorized sounds. These automated acoustic recorders permanently capture the soundscape conditions that currently exist, creating a database of our acoustic heritage that can be analyzed in other ways in the future.

For the first phase of my research, I conducted in-depth phone interviews with NPS resource managers at different parks. The first objective was to determine if park managers thought the National Park Air Tour Management Act was mitigating air tour impacts. Parks experience different types of aircraft overflights. High-elevation commercial flights occur over most parks. Some parks were under flight paths for major international airports. Other flight types include: military, air tours, emergency services, park operations, general aviation, and other government agencies. Out of all types, air tours were considered by the managers to have the greatest negative impact on park visitors and resources. Parks with air tours that operated over the park were questioned about developing an air tour management plan. While many parks expressed interest in having an air-tour management plan, none have been completed for any of the parks. The planning process has proven to be difficult. Just over 100 parks have air tours, so the NPS has their work cut out for them.

A secondary objective of the phone interview process was to identify other noise-related issues in parks. Park managers reported many different noise sources that diminish the value of park experiences. Some noises are very park-specific, such as logging or mining operations outside of park boundaries. Yet, most park managers indicated that the noise source of greatest concern was aircraft. The second-ranked noise source of greatest concern was vehicular traffic on park roads. Some parks are actively working to mitigate soundscape impacts. A few are monitoring and conducting research to develop soundscape management plans. These plans will identify desired future soundscape conditions at different locations in the parks. Other parks no longer allowed personal vehicles in certain parts of the park. Instead, visitors take buses to view the unique landscapes. The buses used in one park are electric and extremely quiet. The resource manager reported that the noise from
cars used to permeate to the remote and undeveloped backcountry, but that now it is much quieter.

This initial research spurred my current efforts. I am studying the reasons that some parks are implementing soundscape conservation efforts more readily than others. Recently, the Natural Sounds Program distributed a survey to every park unit. The survey asked managers to identify important components of their parks’ soundscapes and measures they are taking to minimize noise impacts. I am analyzing the results and will be contacting some of the parks for follow-up interviews. This research will identify ways to improve soundscape conservation efforts in our national parks.

Park soundscapes are unique resources that contain natural, cultural, and historical sounds. The ability to hear these types of soundscapes is diminishing as development and transportation systems expand. The national parks should protect the opportunities to experience these sounds. If you are interested in learning more about national park soundscapes, you can go to: www.nature.nps.gov/naturalsounds/.

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**Here’s A Chance To Decide For Yourself!**

![Yellowstone and Grand Canyon landscapes](Photo by Joe Dumyahn and Sarah Dumyahn)

**What is your initial reaction to viewing these Yellowstone and Grand Canyon landscapes?**

... Now imagine the sounds of flowing water, wind, and birds...

**Does your reaction change if you imagine the natural sounds of these landscapes mixed with the sounds of cars on nearby roads and aircraft engines overhead?**
Most people claim to never have heard of a country named Azerbaijan, but if you watch movies, chances are good that you have. Once featured in the James Bond film, *The World Is Not Enough*, Azerbaijan is often exploited for its involvement with the crude oil industry. The reign of the Russian Empire ended in 1917, and the Former Soviet Union (FSU) was dissolved by 1990, but the environmental legacy of the FSU lives on in the petroleum-driven city of Sumgayit, Azerbaijan. Sumgayit was developed into the dominant industrial sector for the refinement of crude oil destined for the Soviet Union. While the development was beneficial for the Azeri economy, the persistent regional disputes and legacy of slack environmental control has greatly affected the region as well as its nearly 30,000 refugee inhabitants.

Sumgayit recently was recognized as one of the world’s 10 most polluted cities by the Blacksmith Institute. Considerable attention was given in the popular press to the environmental catastrophe in Sumgayit and its potential to impact the venerable Caspian Sea. Environmental studies have been conducted in Sumgayit since 1995 including assessments of contaminant levels in soil, sediment, water, and wildlife tissues. In particular, these studies have focused on the impact of genotoxic chemicals on wildlife populations using biomarkers and molecular genetics. Those studies have shown that chemical contaminants, including polycyclic aromatic hydrocarbons (PAHs); heavy metals such as mercury; pesticides, including lindane, DDT and its breakdown products; and PCBs are widespread but are especially concentrated in the sediments of streams and wetlands, and in the tissues of aquatic vertebrates. Biomarker studies of turtles and frogs have documented genotoxic impacts on somatic tissues. A population genetic study of frogs has shown evidence of an ecological sink based on gene flow patterns, as well as regional and site-specific reduction of genetic diversity, and evidence of induced genetic mutations in Sumgayit. Those studies have also shown the occurrence of mitochondrial heteroplasmacy, a condition where the mitochondria possess at least two distinct haplotypes. This form of heteroplasmacy was represented by two different control-region sequences in a given individual.

The data presented by previous wildlife studies have shown the following: presence of contaminants in the sediments, correlations...
between biomarkers and contaminants, and that Sumgayit is an ecological sink and a source for new mutations in marsh frogs. My research tested these conclusions formed under the assumption that there were not any pre-existing confounding historical factors (i.e., glacial cycles producing genetic diversity). To effectively eliminate the effects of historical factors, my research utilized an organism with a known history in Azerbaijan. Eastern Mosquitofish were introduced into Europe in the early 1900’s to combat malaria and were introduced into Eastern Europe in the 1930’s, roughly the same time that Sumgayit was extensively developed into an industrial powerhouse for the Soviet Union. Mosquitofish were taken from ponds, roadside ditches, or other wetlands in Azerbaijan as well as Portugal, Italy and the United States. There were five sites sampled within Sumgayit that were identical to the sample sites for the marsh frogs from previous studies. Mitochondrial control region amplifications were sequenced and analyzed at both regional and worldwide scales. Heteroplasmic individuals were discovered in varying proportions in a similar regional pattern to that of the marsh frog. Heteroplasmy was confirmed by cloning heteroplasmic individuals and sequencing the individual colonies and a simple branching network was constructed to show the network of haplotypes. The results from the regional analysis of mosquitofish contrast with the results of the regional analysis of marsh frogs. Sumgayit is an ecological sink for marsh frogs, but is an ecological source for mosquitofish (both studies showed increased levels of heteroplasmy in Sumgayit). The two studies revealed that historical factors are not responsible for the increased levels of heteroplasmy and how different organisms can display a common anomaly but react differently.

“Historical factors are not responsible for the increased levels of heteroplasmy and different organisms can display a common anomaly but react differently.”

Despite the industrial contamination in Sumgayit, Azerbaijan is one of few diversity hot spots in the world. Its proximity to the Greater and Lesser Caucus Mountain Ranges creates a continuum of habitats that nourish a wide variety of flora and fauna.
increased health problems to exposure and environmental contamination. With such a profound presentation of environmental contamination and genetic perturbations exhibited in Sumgayit, what role does a simple mitochondrial heteroplasmy play in organisms inhabiting the industrial sector of Sumgayit?

While codons are the focus for genomic DNA and even coding regions of the mtDNA, secondary structures (DNA strands folding upon themselves to produce various active sites for replication processes) are the focal points for the mitochondrial control-region. Control-region sequences for Eastern Mosquitofish, Gambusia holbrooki, and Western Mosquitofish, Gambusia affinis, were submitted to a Web-based server that predicted secondary folding structures for DNA. The two folding temperatures used for predictions mimicked those of winter and summer conditions that individuals would likely encounter in Azerbaijan. Secondary structures and sequences of both species (once considered conspecific) as well as both types presented in heteroplasmic individuals were compared to the known associations and impacts of mitochondrial sequence perturbations in humans using an interactive mtDNA display called MitoWheel 1.2 The results of the folding predictions showed that the heteroplasmy was associated with the termination associated sequence (TAS) for the mitochondrial control region (the structure that ceased mitochondrial genome replication, which plays a vital role in cellular metabolism).

My research built upon the work completed by many others who conducted research in Azerbaijan attempting to show the extent of the damage done by the pollution in the city of Sumgayit. The legacy of the Soviet Union is one that is still being dealt with today despite the almost 20 years of effort by foreign and local scientists. Continued work that increases our understanding of how organisms are affected by environmental contamination is needed, as well as an increased awareness of the unknown environmental tragedy of Sumgayit, Azerbaijan.

After collecting DNA, turtles that were collected from the “WTP” site were released.
Determination of Historical Demography of a Region by Studying Multiple Bird Species. By Anna Fahey

With rapidly changing conditions (i.e., climate change and habitat destruction by humans), conservation of Earth’s biosystems has become an important aspect of scientific research. Maintaining a high level of biodiversity is not only important for the environment but also for human agriculture, health, industry, and leisure. Biodiversity has the ability to benefit mankind through improvement of crops, discovery of medicines, enhancement of water quality, and the identification of additional industrial materials. Minute changes in the environment can cause considerable damage to an ecosystem (e.g., bald eagle response to DDT). The management of threatened species is often related to ecosystem preservation, which, in turn, helps to protect local biodiversity.

Avian biodiversity appears to be a good indicator of general biodiversity for several reasons. Birds are found in almost all regions of the world and they are sensitive to environmental changes. Birds are also one the best-recognized and recorded taxonomic categories. High levels of bird endemism correspond with elevated endemism in other organisms. Seventy-six percent of Endemic Bird Areas are found in tropical regions, and birds with restricted ranges (endemic island birds such as the Eastern and Western Chat-tanagers of Hispaniola) are at a greater risk of extinction. Ranges can be further constrained by vegetation and altitudinal variation on islands, resulting in habitat variation in confined ranges.

Tropical ecosystems (i.e., Southeast Asia and the West Indies) contain some of the highest levels of biodiversity on the planet because of their warmer, wetter, and less seasonal climate, yet many aspects of their evolution still remain unknown. The West Indies, the tropical islands located between North, Central, and South Americas, offer a unique laboratory because they are isolated allowing for endemic species to occur, yet they are close enough to continental land masses that their populations can interact. As a result of many breaks and connections between the landmasses, the larger islands (Hispaniola, Cuba, Puerto Rico, and Jamaica) have complex geographic histories. The islands are also diverse in both plant and animal biota and are considered a hotspot for biodiversity. These different characteristics make the West Indies an ideal place to study.
the evolutionary histories of various species. Of the islands in the West Indies, Hispaniola supports one of the highest numbers of bird species, from North American migrants to numerous endemic species. In a global survey of biodiversity, Hispaniola has been ranked as the highest priority for protection. The critical ranking of the island is due to the large amount of endemic species with limited ranges and destruction of >90% of the forest habitats (<1% of historical forests remain on Haiti’s side of the island). Besides deforestation, another cause of Hispaniola’s ranking is its inability to enforce environmental laws, due to a lack of money and manpower. A final conservation concern is the lack of education regarding national environmental ethics to encourage the citizens to protect the land.

Hispaniola has a variety of habitats suitable for different species of birds. The environment includes grasslands and agriculture lands, as well as wet and dry forest types. The island consists of three parallel mountain ranges that run east to west and reach altitudes greater than 2,500 m. The mountains, deep valleys, rivers, and lakes create natural barriers to some bird populations and promote speciation. Hispaniola also has 10 offshore islands that supply habitat and breeding grounds for permanent and migrant birds. In addition to geographical features, Hispaniola has had a noteworthy climate history, which would be evident through the avian fauna story. The island has a hurricane season from June to November that can have detrimental effects. All of these aspects contribute to the fact that Hispaniola is an important biodiversity hotspot and should have a high priority for protection of its unique ecosystem.

Our goal was to determine demographic histories for multiple bird species in one region. We were able to obtain ~800 blood samples from 30 different species from the Dominican Republic already collected by our collaborators Robert Ricklefs (University of Missouri-St. Louis) and Steve Latta (National Aviary). These specimens were collected from the Aceitillan sector of Sierra de Bahoruco National Park in dry forest, pine forest, montane, and desert habitats. The Sierra de Bahoruco National Park is the most important park for avian conservation on the island of Hispaniola. By sequencing a common gene, we have been able to accomplish a demographic census of the 13 different species that are well represented in our data (>20 samples). These species include Bananquit (Coereba flaveola), Common Ground Dove (Columbina passerina), Prairie Warbler (Dendroica discolor), Palm Warbler (Dendroica palmarum), Cape May Warbler (Dendroica tigrina), Greater Antillean Bullfinch (Loxigilla violacea), Green-tailed Ground Tanager (Microligea palustris), Stolid Flycatcher (Myiarchus stolidus), Black-crowned Palm Tanager (Phaenicophilus palmarum), Ovenbird (Seiurus aurocapillus),
Hispaniolan Spindalis (*Spindalis dominicensis*), Red-legged Thrush (*Turdus plumbeus*), and Broad-billed Tody (*Todus subulatus*).

For our analyses, we have chosen to sequence a mitochondrial gene (mtDNA), NADH dehydrogenase 2 (ND2). MtDNA characteristics, such as rapid evolution and small effective population size, make it a good marker for species identification and evolutionary studies. The ND2 gene in particular is excellent for evolutionary studies because it is a long and evolving gene, meaning there will be considerable variation between sequences. The sequence data allows us to reconstruct demographic histories and gain insight into evolutionary forces shaping the avian fauna. If the histories of the species are harmonious, then this would imply that environmental impacts (e.g., climate) shaped them in the same way. On the other hand, distinctive histories would mean that there was a species-specific force (e.g., disease or biological characteristics) affecting the history of that species.

One prediction for what we would anticipate is a difference between migrant and year-round residents. We expected that migrant species would exhibit evidence of population expansion due to the increase in their breeding territory (with the retreat of glaciers in North America) while resident species would show stable population numbers. Though our analyses are not complete, thus far the null model of population expansion cannot be rejected for most of these species. The one exception was the Stolid Flycatcher for which evidence shows a stable population. Further investigations of life history characteristics and additional statistical tests should give us a better understanding of differences between species.

A multi-species demographic analysis such as this has never been done before. By looking at a combination of many bird species’ histories we can get a more holistic view of the record of this tropical ecosystem. Sequence analyses can be used to determine endemic species that should be a concern for conservation. We will also be able to understand if any species already of concern (i.e., Key-West Quail Dove, Hispaniola Highland-tanager, Bicknell’s Thrush, etc.) can be defined by certain habitats and distributions or discover if a population is in decline. It is important that we protect the tropical biodiversity on Hispaniola and the endemic avian fauna found there that may be suffering due to environmental changes and human impacts.
Eastern box turtle (Terrapene carolina carolina) populations in Indiana are in trouble. This species of hardwood forest-dwelling turtle is experiencing population declines throughout most of their range in eastern North America, likely due to habitat fragmentation, road mortality, and collection for the pet trade. It is a species of special concern in Indiana, and it is not difficult to imagine how the state’s cornfields and roads can take their toll on populations of forest-dwelling, slow-moving creatures.

Serious declines in population numbers may be challenging the future of this species in at least two important ways. First, fewer individuals in any population can reduce genetic variation, which, in turn, leaves the population without the genetic ability to adapt to changes in their environment. These changes could include: novel diseases, fragmented forests, climate change, and/or lower population densities.

This last force is the second important challenge faced by box turtles. It is not exactly known how box turtles find each other for mating, but experiments, personal observations, and their natural history traits all seem to suggest that it may simply be chance encounters that result in matings. If box turtles have evolved to mate only when they bump into each other, reduced population density means fewer chance encounters and fewer matings. This is compounded by naturally low reproductive success in this species, meaning that many matings do not result in surviving offspring.

In Indiana, the most pressing concern for the future of our box turtles is likely to be habitat reduction and fragmentation. Anyone who has ever seen Indiana in an aerial photograph knows that forest fragmentation is common, and anyone who has driven roads after a summer rain knows that roadkills happen. What effects do habitat destruction and fragmentation have on box turtle genetics? Are their
population sizes really declining? If genetic diversity decreasing? Can populations survive despite road mortality and reduced habitat?

To begin to address these questions, I, along with my advisors Drs. Gene Rhodes and Rod Williams, have developed a set of genetic markers specific to eastern box turtles that we will use to measure population genetic parameters. These markers have enough power that they can even give each individual turtle a DNA fingerprint, much the same way a human’s genetic fingerprint might be used in a court of law to connect a suspect to a crime. However, the development of these markers is just the beginning. We may also be able to tell if something like a highway is a barrier for turtles.

For an example, imagine a forest divided by I-65 and two groups of turtles living in the forest, one group on each side of the interstate. Now imagine a third group of turtles, the same distance from each of these groups, but separated from only one of the initial groups by the interstate. If the two groups of box turtles present on the same side of the interstate are from the same genetic population, but the remaining group of turtles on the other side of the interstate is not, it would suggest that the interstate, itself, creates a barrier in which individual turtles can not cross to mate. The isolated turtle population is forced to remain separate from each of the other populations, and, as such, these populations are unable to pool their genetic diversity. As a result, all three of the populations are smaller - perhaps too small to survive. Such knowledge of box turtle population genetics and the location of possible barriers to turtle movement and mating can have implications for forest management, for planning how to deal with climate change and, perhaps, when deciding where to build a new Interstate.
XIAOXIAO LI

There is strong demand to increase the accuracy of pre-existing land use land cover maps especially for dramatically changed and complicated urban landscapes. High spatial resolution digital orthophotographs are widely used to accurately classify urban environments but they include a mixture of information and “noise”. The results of delineation of urban landscapes exhibit uncertainty due to the spectral similarity of urban land-cover types. Object-based classification methods can solve these problems faced by pixel-based methods but they themselves can face the problem of over-or-under segmentation. In my research, a combined pixel and object-based approach is used to classify one-meter resolution digital orthophotographs via: 1) Pixel-based algorithms to facilitate distinction of vegetation types, building roofs, roads, and shadows; 2) Road data and modified digital terrain models (DTM) as thematic layers for the Object-based image analysis (OBIA) for road and building extraction; 3) Nearest-neighbor and fuzzy membership function methods to classify the images by incorporating information on spectral signature, texture, and spatial location of image-objects; and 4) A road segment growing framework that specifies multi-feature information of image-objects and hierarchical relationship as a criterion to identify and connect segments into integrated roads. Manually digitized polygons of land-cover categories and existing building and road maps are used as reference data for accuracy assessment.

ANDREA CURRYLOW

Habitat alteration through direct and indirect anthropogenic episodes such as deforestation and global climate change is becoming increasingly frequent. An understanding of how these changes affect habitat use and selection by native fauna is vital to the preservation of diversity. However, temporal scales are often overlooked when considering habitat selection, and confound management for the required habitats. Long-lived species whose biology dictates their persistence in changing environments need be studied to address these issues. The Eastern Box Turtle (Terrapene carolina carolina) is a long-lived and geographically widespread forest animal, yet numerous populations are experiencing precipitous declines. This species’ ability to endure in a range of available habitat and its close ties to temperature fluctuation make it ideal for study of habitat use and selection amid anthropogenic disturbances. For three consecutive years we studied the behavior of 23–43 adult Eastern Box Turtles using radio telemetry to determine seasonal habitat selection with habitat alteration. This experimental design using radio telemetry data and direct observation of a wild turtle population prior to and in response to anthropogenic habitat alteration is the first of its kind. Ultimately, the results of this research can influence management decisions to enhance habitat on forested lands.

CECILIA HENNESSY

Terrestrial animals often move across the landscape in search of mates, food, or new territory. However, roadways may create effective barriers to movement. In order to investigate the scope of the problem, my research involves trapping five species of mammals (raccoons, opossums, gray squirrels, fox squirrels, and chipmunks) on opposite sides of interstate highways in Indiana, employing mark-recapture techniques, and taking genetic samples. Using 12-14 microsatellite markers per species, I will create a genetic profile of each individual animal. Comparing these genetic profiles with statistical software will enable me to investigate the population genetics of each site and determine if there is a history of population differentiation that corresponds with the presence of the highway. If so, then this is evidence that the highway is a barrier to gene flow and, thus, movement. The range of species may provide a continuum of population-level effects, because some species may be more successful when crossing the highway than others. In order to identify the species that are most likely to successfully cross the interstates, I will also be employing camera traps at the entrances of culverts on the study sites. I will also be investigating the scale of population differentiation for raccoons and possibly opossums, combining the genetic data from the interstate sites.
with genetic data from ongoing research projects on these species. By the end of my sampling process, we will have genetic information from raccoons and opossums across the state of Indiana, which will allow me to investigate the landscape genetics of these wide-ranging species.

**Phillips Christian Perry**

I am working with several partners to examine the links between fish populations in northern Indiana’s glacial lakes and the catchments draining into those lakes. We’ve use Geographic Information Systems (GIS) as a tool to delineate catchment polygons at three specific scales: 100-meter buffer catchments around each lake, “local” catchments which drain directly into each lake without first draining into a stream, and “tributary” catchments which drain into a stream prior to draining into each lake. This information is being shared with an organization called the Midwest Glacial Lakes Partnership (MGLP) which includes eight states (Illinois, Indiana, Iowa, Michigan, Minnesota, North Dakota, South Dakota, and Wisconsin) all of which are delineating catchment polygons using coordinated methods. We have completed work on the catchments in Indiana and are now in the process of expanding a geodatabase which links specific information about fish populations of each lake directly to the polygons related to those lakes. For this, we are relying on a multi-decadal dataset from the Indiana Division of Fish and Wildlife which includes extensive records on standardized fish surveys across the northern lakes. We are also generating some of our own data and indices for the geodatabase. Using this exhaustive geodatabase, we have already begun to do some analysis on the lake morphometric variables and catchment variables which may contribute to explaining differences in fish populations. For example, initial results indicate that the ratio of lake perimeter to surface area may have an important role in explaining the difference in catch per unit effort (CPUE) of bluegill across 101 lakes. In other early results, we have found that the ratio of lake perimeter to surface area may also be important in explaining variation in species richness across the lakes. As we progress in this line of research, we will begin to look at how the percentage of various land cover classifications within each lake catchment might also broaden our understanding of how landscapes affect the fish populations within them at multiple scales.

**Ying Wang**

I joined Dr. Paula Pijut’s lab last fall because of my great interest in genetic engineering and molecular plant biology. I work on the genetic transformation of black cherry (*Prunus serotina*) for flowering control and insect pest resistance. Black cherry is a valuable hardwood species in the eastern US and Canada for its high-quality wood for furniture, cabinetry, and veneer. However, it is susceptible to several insect pests and its defense response greatly reduces its wood quality. Therefore, genetic engineering of black cherry trees with pest resistance genes could be a promising way to protect them from wood damage caused by pests. But before that, transgenic black cherry trees with reproductive sterility must be obtained because of the potential for dispersal of transgenic pollen and seeds and its unknown impact on the environment. Our goal is to produce transgenic black cherry plantlets containing both a flowering-control gene and pest-resistance gene in the near future.

**Valerie Clarkston**

The Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*) is an endemic subspecies to the Prince of Wales Island and a few surrounding islands in southeast Alaska. Southeast Alaska has experienced extensive industrial-scale clearcut logging for the past 40-50 years, necessitating that biologists understand...
the predation risk of this endemic subspecies in the current anthropogenic and evolutionary novel mosaic of clearcut, second growth, and old-growth habitats. From April to August 2010, we will conduct our study in the east central section of the Prince of Wales Island, Alaska (55°79'-55°98'N, 132°67'-133°13'W). We are interested in quantifying predation risk in three different types of managed timber stands and two unmanaged forest habitats: clearcuts, thinned second growth, un-thinned second growth, peatland mixed conifer, and old growth, respectively. For each stand, we will locate the center point and place a flying squirrel taxidermy model along with an infrared camera on the ground or on a tree limb/bole. Habitats will be sampled simultaneously and we will leave models/cameras at a location for 1 week before relocating them to a different set of replicates. This process will be repeated 11 times for a total sampling period of 12 weeks. Every day we will check the condition of the models and the battery life of cameras. For each model, we will use predator approaches as the response variable for predation risk and we will use a known-fates model to estimate survival. We will compare survival across habitat type using the Cox proportional hazards model. Based on this subspecies biology, we predict higher predation risk to occur in timber managed stands than in old-growth habitats because in the former there is less vegetation available for cover and gliding.

**JIM BEASLEY**

My research is focused on understanding how the fragmentation of native habitats, which characterizes much of the world today, has altered various aspects of wildlife ecology. Specifically, I am using mark-recapture, radio-telemetry, and genetics to determine the extent to which habitat fragmentation influences raccoon population dynamics, disease prevalence, and movement behavior in a fragmented agricultural landscape in northern Indiana. As a part of this research I have used radio-telemetry to collect over 11,000 locations on 125 raccoons over the last several years. From these data I observed that raccoons maintain much smaller home range sizes in agricultural landscapes than raccoons inhabiting other rural landscapes, and home range sizes decreased as forest patch quality increased. In addition, as part of mark-recapture experiments I also have tagged and collected disease and genetic samples from >2,300 raccoons distributed throughout 80 forest patches in northern Indiana. These data have demonstrated that spatial variance exists among forest patches in numerous aspects of raccoon demography, but that a few local and landscape level habitat characteristics explain a large proportion of the variance in demography among local populations. Collectively, the results of this research will greatly expand our understanding of how wildlife species are impacted by forest fragmentation. In particular, these data will enhance our understanding of crop damage and songbird nest predation patterns, and ultimately will be important in the parameterization of models characterizing the spread of infectious diseases throughout fragmented landscapes.

**PENG ZHAO**

The genus *Juglans* (*Juglandaceae*) has about 20 species which are referred to as walnuts. Butternut (*Juglans cinerea L.*) also called white walnut, oilnut, or lemonnut is an important tree species formerly valued for its nuts, wood, and wildlife mast. Unfortunately, butternut trees are in danger of being lost in natural forests from butternut canker, a fungal disease. The presence of *Juglans x bixyi* and other interspecific hybrid trees are difficult to identify by using conventional morphological characteristics. In addition, black walnut (*Juglans nigra*), which is named American walnut, is also a valuable tree species for its timber of high quality. Molecular markers (genetic markers) are an important tool in tree genetic research. This tool has multiple functions such as identifying ambiguous species to marking specific loci, locating the gene of interest,
etc. We used different molecular markers like RAPD, SCAR, CAPS, Chloroplast, ITS region, \textit{trnF-L} region, \textit{and mitochondria} markers to identify the hybrids from butternut and Japanese walnut (\textit{Juglans ailantifolia}). We also used microsatellite markers (SSR) to study the black walnut genotype, paternity, and maternity. Now, in our study, these molecular techniques are being used in parentage analysis. In the present study, we have presented data with markers \textit{trnF-L}, ITS, 16R-2B, 22-5, 14R-1, 15R-8, 39-6, 40-1, and 3-9. With these markers, we are able to distinguish between butternuts and hybrids in cases where morphological identification is impossible or inadequate. Some of the markers have already been used to identify non-hybrid trees in national forest, to establish seed orchards, and to further butternut breeding efforts by the US Forest Service and cooperating institutions.

**William Beatty**

The Virginia opossum (\textit{Didelphis virginiana}) is a generalist in every sense of the word, and over the past century, has thrived in Midwestern landscapes transformed by agriculture. However, opossum populations in local areas are highly dynamic due to apparently high mortality in young of the year and the seemingly transient movements of adults. Experimental data indicate opossums are excellent scavengers, and this species is thought to have limited predators in the Midwest. Thus, it is unclear how potential limiting factors such as food availability, interspecific competition, and predation influence the movement behavior and population structure of this species in agricultural landscapes. Primary goals of this project are to 1) examine movement patterns and habitat selection in opossums in a fragmented agricultural landscape, and 2) identify patterns of local and landscape level genetic structure in opossum populations in Indiana.

**Michael Wellman**

Shovelnose sturgeon are a long-lived, late maturing species of fish that inhabit the Wabash River here in Indiana. Currently, they support a commercial harvest for their roe to supply the caviar market but important population information still remains unknown. The Wabash River near Lafayette is identified as important spawning grounds for this migratory species but little is known about how they specifically traverse into and out of the area.

Dr. Reuben Gorforth and I are currently working to identify how the Wabash River population of shovelnose sturgeon utilize both the area directly around Lafayette as well as the river as a whole. Sturgeon, 30 in total, were implanted with hydroacoustic tags in the spring of 2009. These tags allowed for both manual and passive tracking of the fish throughout the river over the spring and summer.

**Nicholas Marra**

I’m a second year graduate student in Dr. De-Woody’s lab, which I joined in May 2009 after a year of rotations through the PULSe program. My research is on the evolution of genes expressed in the kidneys of Banner-tailed Kangaroo Rats (\textit{Dipodomys spectabilis}). Kangaroo rats are well known for their ability to survive in the desert without drinking water. They are able to conserve the water by staying in burrows for
the hottest parts of the day and by producing highly concentrated urine. In order to prevent the loss of excess water in their urine, they have very efficient kidneys. I am looking to see whether the pressure to conserve water has resulted in signatures of strong selection on various genes expressed in the kidney. Additionally I am seeking to see whether there are differences in the genes that are expressed in the kidneys of this species when compared to those of a related species, *Heteromys desmarestianus* that lives in more mesic areas of Costa Rica. I will employ 454 sequencing to identify differences in gene expression and to find differences in the sequences of genes such as aquaporins (which encode proteins for water channels that should be involved in retaining water from urine). From there I will use conventional sequencing to look at evolution of these genes across other members of the Heteromyid rodents to see if the patterns of this comparison hold in comparing desert adapted species to those from wetter environments.

**Suman Maity**

My work is defined under the broad area of ‘Eco-toxicology’; in particular, the state of aquatic organisms interacting with different perturbations in the environment is my focal point. Our research effort at Professor Marisol Sepúlveda’s Lab, is exploring the probable cause(s) of declining population of *Diporeia* in the great lakes. Historically, *Diporeia* has been a major food source for a number of important fish species (Lake white fish, Yellow perch). Although in the past, *Diporeia* population did experience occasional fluctuations in population abundance but since early nineties, there had been a precipitous trend that has been concomitant with the establishment of the relatively new invasive species of Dreissenid mussels in the Great Lakes region. This has prompted the hypothesis that the expansion and establishment of the exotic mussel is causing the slow demise of the *Diporeia*. At the same time there had been a number of competing hypotheses which range from starvation-induced physiological stress to spread of a new pathological element among the affected *Diporeia* population. In our study, we are conducting an “in-house” exposure study on *Diporeia* under various stressors thought to be at work in their natural habitat and then comparing them with the field samples from wild *Diporeia*, collected over a widely dispersed area in the Laurentian lakes. We are using metabolomics to find out some of the answers to this puzzle. So far our research has detected some inherent response patterns specific for a particular stressor. Working ahead, our goal would be to elaborate those discerning pattern for the predictive purpose to serve as an early indicator of the population level stress response in the wild.
Ecological restoration specialists and plant producers face many challenges when trying to cultivate native plant species for their projects. Germination requirements for native plant species are often provided without supporting peer-reviewed research. Understanding germination requirements allows for increased success with current and future restoration projects, assures the persistence and viability of populations, and ultimately increases the biodiversity of that ecosystem. We tested germination requirements for four native species: *Castilleja cocinea*, *Lithospermum croceum*, *Pedicularis canadensis*, and *Sisyrinchium atlanticum*. Selection of these four species was based on the frequency with which they were identified as ‘difficult to establish species’ amongst 19 restoration specialists and plant producers who were surveyed prior to this experiment. A range of experimental treatments were conducted on these species’ seeds. The treatments were intended to test the effect of the following germination: 1) temperature, 2) light, 3) scarification, 4) smoke, and 5) stratification. Our results suggest that the probability of establishing *Castilleja cocinea*, *Lithospermum croceum*, and *Sisyrinchium atlanticum* species from seed in Upper Midwestern tallgrass prairies would be maximized by using the proper timing and method of planting. *Castilleja cocinea* germinated best when the seeds were either exposed to light and had a stratification period of 60 days or exposed to smoke with a germination temperature of 15 °C. Therefore these should be broadcast seeded in the fall to allow light penetration and adequate stratification or treated with smoke prior to broadcast planting in the early spring. *Lithospermum croceum* germinated the best at 15 °C following a 60 day stratification period. Therefore, these should be broadcast during the fall to allow adequate stratification and proper germination temperature levels. *Sisyrinchium atlanticum* germinated the best for the 15°C germination temperature. Because stratification did not seem to enhance germination significantly, we predict that early spring planting would be sufficient for this species. *Pedicularis canadensis* produced no conclusive results to suggest proper planting methods.
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