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Graduate Explorers:

Discovery is a key component of the land-grant mission at Purdue University. In the Department of Forestry and Natural Resources (FNR), a focus of discovery efforts is to develop and disseminate information associated with the protection, management, and sustainable use of terrestrial and aquatic ecosystems.

How is this information developed? As amply demonstrated in the pages of this inaugural issue of Compass, research by graduate students is the engine that powers the department’s discovery efforts. A principal goal of Compass is to enable graduate students in FNR to share their work, and in the process convey to readers the passion, struggles, and perseverance involved in addressing important questions related to natural resources.

Graduate students in FNR are instrumental to progress in natural resource sciences. Indeed, many of the notable accomplishments that we take pride in as a department are based on the creativity, critical thinking, and hard work of our graduate students. Graduate student research can lead the department in a diverse array of new and exciting directions. The 85 current graduate students in our program pursue intriguing lines of inquiry in the fields of fisheries and aquatic sciences, forest biology and silviculture, forest measurement and assessment/GIS, genetics, natural resource social science, wildlife science, and wood products and wood products manufacturing technology. Student projects highlighted in this issue are as varied as prairie restoration, land-use collaborations, vertebrate mortality on Indiana roads, and effects of water-borne endocrine disruptors.

I am excited and proud to share Compass with you. Explore and enjoy!

Rob Swihart
Professor and Head
Prairie Restoration Ecology
BY MEGAN BENAGE

Work and Play at Purdue
BY KRISTIN FLORESS

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BY AMY ROSS-DAVIS AND KENLI SCHAAF

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BY SONIA MAE JOHNS
How to Build Habitat:
Prairie Restoration Ecology at the Purdue Wildlife Area

BY MEGAN BENAGE

It is fall and I am just beginning graduate study in the Department of Forestry and Natural Resources (FNR) at Purdue University. One of my first tasks is to begin collecting data. My project is evaluating the ecological impact of land restoration by monitoring the abundance of small mammals, birds, bats, and frogs. The first animal group that I will sample is small mammals. On one hot afternoon where summer is trying to stretch itself into autumn, I set small live traps at equally-spaced distances apart. In response to summer’s reach, fall harvest is coming. I have to pre-bait and finish my trapping session before the tractors come. Pre-baiting allows the animals time to get accustomed to finding goodies in traps and makes them more likely to enter a trap when it’s set. Night falls too quickly before my field assistant and I can finish finding all of my traps, so we hunt around for them with a flashlight. No trails are made in the lodged prairie grasses yet, so it is slow going. We finish and head tiredly home, mentally preparing for tomorrow.

4 a.m. on a cold, sunny morning and it’s time to check my traps. I have been trained to handle small mammals, but this is my first time handling them alone and my nerves are a little shaky. My first few traps are empty, but the next one isn’t. It’s judgment time and I have to be prepared for what might be inside. I tilt the door down a fraction of an inch. All I can see is a tail. It’s not a large tail so some of my fears are released. I open the trap; the mouse peers out at me as I try to get him to go in my handling bag. I restrain him a little while I try to maneuver him. He wiggles just a little, and I ungraciously yelp and drop my grip, he hops easily out of my grasp and scampers off. The next trap goes better and I begin to improve how I handle the small critters: Peek in trap, restrain gently, use gloved hand, use pliers to tag unmarked animals, read marked animal’s tags by gently pushing back ear, record sex, age, weight, and overall condition. Just a day in the life of a prairie restoration researcher.

Land restoration is a growing topic in research and in practice as many habitats become degraded or disappear completely. Although the definitions tend to vary, it is essentially a process involving the change of land from its current condition to a state in its previous history. Restoration efforts can also provide additional native habitat to replace those that are rapidly disappearing. This can occur on a small scale of hundreds of acres to a large scale of thousands of acres of land. As an up-and-coming topic, restoration of land to native conditions is gaining force across the world as a popular method to increase biodiversity. Therefore,
it should not be surprising that a restoration effort is in full practice just around the cornfield here at Purdue University.

FNR manages several off-campus properties where classes, research, and general recreation occur. One of these properties is the Purdue Wildlife Area (PWA). It is the current site of our main restoration effort, which involves the conversion of agricultural land and invasive brush to a prairie and savannah complex. PWA includes a 256-acre tract of land including a 96-acre wetland complex. It was in agriculture when purchased by the University in 1960, but was quickly planted with various exotic shrub and tree species as part of research aimed at quantifying the wildlife benefits of each species. When the research ended, mowing between segments of the property ceased and various aggressive exotic woody species quickly spread. Thirty-five years later, the FNR department began looking for ways to combat the spread of these invasive species. The passing of the 2002 Farm Bill, which increased the Conservation Reserve Program (CRP) land limit to 39 million acres, created an influx of funds available for farmers to take part in restoring environmentally-sensitive cropland back to a natural, uncultivated state. This bill created more interest towards engaging in a restoration effort and the department began conducting research on projects related to land restoration.

Restoration sometimes involves controversy over how the historical state of a piece of land is defined. Our decision to convert PWA to a prairie and savannah complex was based on the fact that the area was originally (pre-European settlement) within the prairie/forest transition zone of Indiana. These habitats are among the most threatened, both regionally and globally. Since a loss of habitat may lead to a loss of biodiversity, conserving threatened areas is of great ecological importance.

Our project is achieving the restoration of native species diversity through control of invasive woody species with mechanical and herbicide treatments. Mechanical methods involve employing a Kershaw mower (similar to a bulldozer only with considerably less compaction) to level vegetation to a manageable height and composition. Herbicide treatments involve broadcast spray, cut-surface, and stem applications to decrease the number of invasive woody species present at the site. Prairie habitats are being restored, following control of the woody species, using different intervals of herbicide application as well as different seed mixes and planting rates to achieve a healthy balance of prairie forbs and grasses.

One of the major criticisms of restoration projects is that they involve the disturbance of landscapes. These disturbances can sometimes have detrimental effects on wildlife communities. This is where my
research enters into the picture. I am evaluating the faunal responses to restoration. This is an integral part of restoration research because it can distill criticisms and provide accurate data about the status of wildlife populations. I am measuring response by calculating population abundances for frogs, small mammals, birds, and bats in varying habitat sites. My sampling sites occur in invasive late successional brushland, restored prairie, early successional brushland, and agricultural land (pre- and post-harvest).

Our restoration project comes with many benefits, both biological and otherwise. The most significant benefits include 1) provision of a blueprint for restoration processes that can be utilized by the professional and the individual, 2) the enhanced recovery of lost and disappearing ecosystems’ flora and fauna, and 3) gaining information regarding fauna populations pre- and post-restoration.

It’s now my second trapping session, and my technician and I are surveying invasive brush and early-successional habitat. The brush is a wall of honeysuckle and is dark inside even at noon. I’ve never had to crawl for four hours at a time. I try to daydream, but that only gets your eyes poked by overgrown honeysuckle. I open a trap and I’ve caught a shrew! I won’t mark him because shrews are highly susceptible to stress fatalities from capture and I want to make sure he survives. My excitement dies away. This shrew is vicious and apparently willing to fight anything, including my thumb. I try to determine his sex so I can at least record that. It’s hard to keep him in place as he bites his way around my hand. I can see his teeth gnashing at me as I try to release him. He responds by releasing a white substance onto my hand. I later learn that shrews are equipped with several different glands that secrete odors for communication and defense. Blarina species have a particularly strong gland on the belly used to secrete an aromatic defense deterrent. This works wonders on me and I try more fiercely to remove the shrew from my body. He is too interested in the fight and refuses to let go. After some prodding, I manage to remove him from all of my appendages. However, his deterrent remains on my hands and clothes. All of this was thoroughly amusing to my technician.

The day is not over after my unfortunate shrew incident. It’s afternoon and I am checking my early successional brush plots and I’ve caught the typical number of animals: 13 deer mice, 10 white-footed mice, one meadow vole, and one shrew. I approach a closed trap expecting more of the same. I open the trap to see what I’ve caught this time… and a bird flies into my face. I had to take a personal time-out for ten minutes. They never tell you exactly what to expect when you engage in wildlife research.

Key partners in this project include BASF, Zach Lowe, Dr. Harvey Holt, Dr. Harmon Weeks, and Dr. George Parker. Thanks also to the many volunteers that assisted in this project, without their efforts my project would not be possible.

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Work and Play at Purdue

BY KRISTIN FLORESS

When considering institutions for higher education, the importance of research and recreation opportunities is often a factor. There are a variety of opportunities for both work and play near Purdue University’s main campus in West Lafayette, Indiana, especially for those who enjoy spending time outdoors. Whether your interests involve hiking on park trails, conducting research in areas like a restored prairie, or hunting and fishing in the woods and waters, there is a place for everyone within a close drive of campus.

Graduate students in the Department of Forestry and Natural Resources at Purdue have a variety of study sites available to them. Purdue lands near the West Lafayette area include Martell Forest, the Purdue Wildlife Area (PWA), and Ross Biological Reserve (RBR). Martell consists of 425 acres of native forests and plantations. Located only eight miles from campus, it is highly utilized by many within the department for research and also serves as a nearby site for short hikes, picnics, and other events. White-tailed deer hunting is popular in autumn, and the Wabash River and Wildcat Creek are great fishing getaways for many students.

Nathan Lichti, a Ph.D. student in the department, is studying the effects of forest fragmentation on blue jay movements and how this affects acorn dispersal patterns. In addition to working at Martell, he is also using PWA and RBR. According to Nathan, “The PWA is primarily early seral/shrub that is in the process of being restored to prairie oak and savannah.” Additionally, there are two wetland areas and a white oak/shagbark hickory stand of mature trees. An indoor animal care facility, mammal exclosures, and three radio telemetry towers are also located at PWA. The RBR, managed by the Biology department, is located adjacent to the Wabash River, and at least some of the stands of trees at the site have been mapped. You will also find steeper topography here, primarily because of its location near the river. This makes for challenging hiking opportunities!

Research facilities are not limited to those near Purdue. Researchers in the department have conducted studies at Davis Woods in Randolph County, home to an old-growth woodland with a button-bush swamp and plenty of bur oaks and swamp-white oaks. Purdue forestry research began at this site in the 1920s and continues to be conducted...
there today. Every ten years, a complete inventory of trees in the woodland has been completed. Dr. George Parker, Professor of Forest Ecology in the FNR department at Purdue, has been able to study the long-term dynamics of old-growth forests. Graduate student Ben Dolan created an ecological classification system for the Bluffton Till Plain in Davis Woods.

“It’s an amazing forest, with lots of wildflower diversity and huge overstory trees,” Dolan said. “It’s an unusual place because it’s surrounded by agriculture and most other nearby woodlands do not have large trees like Davis. It is one of the few old-growth forests in north-central Indiana.”

When students aren’t studying for exams or conducting research, there are a few state parks nearby for them to spend some time relaxing or exploring. The topography of Prophetstown State Park, a short 10-minute drive from West Lafayette, was shaped by glaciation and today prairie grasses cover the gentle hills. There is a paved biking/hiking trail, a 2.75 mile rough-terrain trail, and camping opportunities. Picnic areas and shelters are available for day-use as well.

A bit further from West Lafayette you can find Shades State Park. Located near Crawfordsville, Indiana, Shades has a variety of short hiking trails and opportunities for fishing and camping. A seasonal naturalist leads hikes and other programs during the summer months. If you have a bit more time on your hands, you can venture out to Turkey Run State Park, about an hour’s drive from the West Lafayette area. Although there are plenty of routes for more casual day hikers, the deep ravines also provide rugged hiking trails for those who want a challenge.

Purdue students have a lot of recreational and research land at their fingertips, and most are found within an hour’s drive of town. For those who seek a place off-campus to spend their time, the University’s land as well as Indiana state parks can offer a nice retreat from the everyday sites most students see. Take the time to explore!

Kristin is a second-year Ph.D. student majoring in Human Dimensions under the direction of Dr. Shorna Broussard and Dr. Linda Prokopy at Purdue. She can be reached at: kfloress@purdue.edu.
The expansion of humans into the wilderness is not a new phenomenon. Humans have been steadily intensifying their impact on the wild areas of the world, especially since the advent of large-scale automobile manufacturing in the early 20th century. Areas that formerly saw only sporadic horse and wagon traffic can now be accessed by automobiles. The United States alone has 3.8 million miles of public roads, which link essentially every local area in the nation. The 200 million vehicles traveling on these roads lead to a modern source of wildlife mortality: roadkill.

When most people think of roadkill, they picture dead deer along the roadside, and rightfully so; an estimated 1.5 million animal-vehicle collisions involving deer alone occur annually in the United States. Estimated damage to vehicles in these collisions exceeds $1.1 billion in total and approximately $1,500 per collision. However, many other ecological effects of roads on species, soils, and water have been identified, with effects varying in distance outward from feet to miles. These “road-effect zones” impact an estimated 15-20 percent of the United States. While roads are an important part of the nation’s infrastructure and can provide some ecological benefits such as maintenance of grassland plants in intense agricultural areas, they can also present several ecological problems such as acting as physical and biological barriers to many wildlife species. Some of the less visible wildlife impacted by roads are reptiles and amphibians.

Over the last decade, amphibian populations have been declining worldwide and these declines are often associated with some type of habitat modification. One such modification is fragmentation due to road expansion. In fact, even though amphibians may tend to avoid roads, a growing literature suggests that the greatest transportation impact on amphibians is roadkills. In Australia, the annual mortality of herps on roads is estimated at 5 million individuals. Road mortality is especially prevalent in areas where roads intersect wetlands. A study in Ontario, Canada observed over 32,000 individual animals (92% amphibians) killed along a 2.2mi stretch of road adjacent to a large wetland in only a four-year period.

The life histories and biological characteristics of many reptile and amphibian species make road mortality a genuine concern. These species generally move slower than mammals and birds, making them more susceptible to road mortality. When amphibians migrate across roads to
reach breeding ponds, mortality of breeding adults can reach 20-40 percent. With female ambystomatid salamanders producing on average over 1,000 eggs per individual and anuran egg numbers ranging from several hundred (in smaller hylids) to several thousand (in larger ranids and bufonids), the road mortality of migrating gravid females has the potential to remove hundreds or even thousands of new salamanders, frogs, and toads from the population. The impact of road mortality on populations of reptile species such as snapping turtles may be detrimental due to their inherent life-history traits such as low annual recruitment rates and delayed sexual maturity. These characteristics, combined with migrations during breeding season to nesting sites make it difficult for populations to absorb the loss of sexually mature adults. Additionally, many reptile species bask during the warmth of the day to increase their body temperatures and metabolisms. This thermoregulatory behavior can bring reptiles into a position of imminent danger.

My research is a result of the observation of tiger salamanders being killed on a two-lane highway (Indiana State Route 26) during their spring migration to breeding ponds near a university property, the Purdue Wildlife Area. Here in Indiana, the landscape consists primarily of a highly fragmented, agriculturally dominated matrix. There are over 93,000 miles of roads, with the Indiana Department of Transportation (INDOT) managing approximately 10,560 miles of these. The biological effects of this road network are not well-known; however, the combination of high habitat fragmentation and road density may have detrimental effects on many wildlife species, including reptiles and amphibians. Other than raccoon roadkill surveys (for population estimates) conducted by the Indiana Department of Natural Resources and deer/vehicle collision records kept by the Indiana State Police, there is currently no other annual roadkill survey or multi-species road mortality index for the state.

With support from the Indiana Department of Transportation and the Joint Transportation Research Program at Purdue, we developed a study to monitor vertebrate road mortality on a number of Indiana roadways. While my emphasis is on reptiles and amphibians, I am also collecting data on all roadkills I find. I have two key objectives: 1) identify, characterize, and evaluate roadkill sites throughout Indiana and incorporate them into a Geographical Information System (GIS) database to determine habitat

**In one year alone, over 7,000 frogs have been killed on a 1.1-mile stretch of road.**
entered into a mobile GPS/GIS system, and then they are marked or removed to avoid recounting.

Between March 2005 and March 2006, I recorded over 9,000 road mortality events across all of the survey routes. At least 62 species are represented among the mortalities and of these, 92% have been reptiles and amphibians, 6% mammals, and 2% birds. One route with a particularly high incidence of roadkill is Lindberg Road located within the city of West Lafayette. This road bisects the Celery Bog Nature Preserve and over 7,000 frogs (Rana sp.) have been killed on this 1.1-mile stretch of road since the beginning of the surveys.

Hopefully, my research will assist INDOT in making sound decisions when dealing with biological issues. Being able to identify potential roadkill “hotspots” will help officials decide which areas they should be concerned about and which areas to avoid when planning new road projects. By implementing various mitigation techniques during new road construction, engineers may be able to keep existing habitats connected and lessen the impacts of construction. Moreover, some modification could be retro-fitted to existing roads that have high incidences of roadkill.

Wildlife road mortality is a serious issue. Road mortality can have significant impacts on populations by altering age and sex ratios, and can lead to local extinctions. Moreover, reduced populations may not be able to range as far for resources, possibly affecting the distribution and abundance of the general plant and animal community. As scientists, we need to recognize these issues and recommend responsible solutions.
Spotlight on Fisheries Research

BY HOLLY PATRICK

Purdue University’s Fisheries and Aquatic Sciences (FAS) Program offers a number of opportunities to students who have an interest in water and the organisms that depend on it for survival. The small class sizes in this program and interactive practical labs give students an opportunity to gain valuable hands-on experience in areas such as aquatic sampling techniques and mapping and navigational skills. There are also some opportunities for undergraduate students in the program to obtain internships, employment, and volunteer experiences.

Long-term career aspirations of the student body enrolled in this program are variable. Some students aspire to have their own businesses, such as pond-consulting companies. Other students want to work for a fish hatchery or a state agency. Through these positions and other program-related activities, there is the potential for students to make professional contacts that often translate into future career opportunities. In addition, there are a number of clubs formed by FAS students to get involved with, such as the Purdue University student subunit of the American Fishery Society (AFS), the Recreational Fishing Club, and the Bass Fishing Club.

Research on the River

Thomas Bacula, a senior from Arlington, Illinois, has had a great experience in the FAS program at Purdue. Tom is conducting independent research which allows him to learn new skills and obtain credits for his work as well. Specifically, he is looking at population characteristics, stock dynamics, and reproductive biology of blue sucker in the Wabash River under a Ph.D. graduate student mentor. Tom and other volunteers spent time on the river on an electroshocking boat to capture blue suckers. Electroshocking is a method by which an electrical current is sent underwater and causes muscle relaxation for fish. The fish floats up to the water surface, and researchers can scoop up the fish with a long-handled net and place the fish in a live-well on board. The fish recovers within a few minutes, and length and weight data are taken for later analyses. Different fish structures are used to determine the age of each fish, including fin ray sections and otoliths (inner-ear bones). Tom spent much of his time counting the annuli (ear marks) on these structures to determine the age of

Photo by Andrew Muir

Tom Bacula (left), a senior at Purdue, is conducting undergraduate research on blue suckers in the Wabash River. Dan Daugherty (right), a Ph.D. candidate at Purdue, is one of Tom’s mentors for the project.
each fish, in much the same manner as the rings of a tree are counted. Eventually Tom hopes to become a non-game fisheries biologist, and his opportunities at Purdue have lead him in the right direction toward achieving this goal.

Mentoring Skills

Graduate students and professors have the opportunity to be a positive influence on the undergraduate student body through mentoring, teaching assistantships, and club activities. These interactions can have far-reaching positive outcomes, such as guiding a student through the difficulties of school work, broadening skills and knowledge of the field, and expanding personal backgrounds. The success achieved by the student is always a reflection of their mentors’ abilities to help them achieve their goals.

Tom received mentoring assistance on his independent research from Dr. Trent Sutton, Associate Professor of Fisheries Biology, as well as from Daniel Daugherty, a Ph.D. candidate in FAS. Both Dr. Sutton and Dan enjoy being involved with undergraduate research projects because it provides opportunities to apply their supervisory and advisory skills, while simultaneously providing opportunities to learn about different species and various sampling techniques. In particular, Dan feels that his involvement in undergraduate mentoring has helped him to become a more well-rounded researcher.

The Aquaculture Experience

Adam Charlton, a senior from Cambridge City, Indiana, is working in partnership with Robert Mollenhauer, a junior from Chicago, Illinois, as volunteers in the Purdue Aquaculture Research Laboratory (ARL). Adam and Bob are technicians for graduate student research being conducted in the FAS program. They are currently taking care of approximately 90 lake sturgeon and seven sea lamprey at the ARL. In between their classes at Purdue, both students have learned to care for lake sturgeon in a laboratory and test the water quality. They have also assisted graduate students in the plumbing, system design, and maintenance that go along with aquaculture research. Both Adam and Bob feel that their experiences at Purdue have been positive overall and that their teamwork and practical skills will help them in the future.
Human dimensions researchers are often interested in how individuals and communities interact with their environment and also how the environment is impacted by human activities. As the rate of land development in the United States increases, there are new challenges associated with land-use changes, such as maintaining biological conservation and community health and prosperity. Many natural resource professionals have called for a shift from traditional forms of individual property management to more collaborative forms of management that recognize the interconnectedness of individual decisions across space and time. Collaboration has been touted as a new means to address complex ecological issues that involve a variety of individuals and jurisdictions. Our research explored the process of collaboration among private landowners in north-central Indiana, who built upon existing community programs to bring information about natural resources to fellow community members.

We focused on creating “community forums” to bring these landowners together to discuss private land management and natural resource issues. This research, which was Kenli’s doctoral project, aimed to explore how private landowner collaboration could be established and sustained. A dedicated group of landowners, which included farmers, private forest owners, and new rural residents, met monthly to discuss local natural resource issues and potential solutions. Area natural resource professionals from agencies such as Natural Resources Conservation Service, Cooperative Extension, and Indiana Department of Natural Resources also participated in these meetings. After about a year of meetings, landowner participants had identified several common interests, developed new contacts within the community, agreed upon a name for the group – CommuniTree - and decided to move forward and take action. Now, two years after the initial meeting, the group is working to implement their goals and make a difference in their community.

During these monthly meetings, our role as researchers was to identify how private landowner collaboration could be accomplished. For example, we were interested in knowing what elements were required for landowners to come together around a common issue and through what stages such collaborations would proceed. Two qualitative case studies conducted in two watersheds of north-central Indiana were used to collect individual and group level data through participant observation and focus groups, respectively.
In addition to using a framework of collaboration, we relied on theories of sense of place, sense of community, community attachment, and social capital to study the phenomenon of collaboration on private lands. Using a combination of inductive and deductive approaches to analyzing the data, we asked questions that sought to understand the role of this new and potentially effective and meaningful avenue for private landowner engagement. In summary, we wanted to know how collaborative natural resource management arrangements among private landowners could be initiated, sustained, and evaluated.

Most participants reported to have had a positive experience with the community forums, yet some felt frustrated by the lack of a more tangible outcome at the time of the focus group interview. It was clear to us that although we wanted to create an open atmosphere within which individual interests could be expressed and common interests could potentially emerge, more structure and direction was necessary to help the group identify and move toward a common goal.

Due to the exceptional dedication and commitment of members of CommuniTree, many continued to meet well after the focus group interview as they planned the materials for Pine Village Elementary School’s Outdoor Laboratory. Pine Village Elementary School has a small patch of forest at the northern edge of the school property that is bisected by a small stream and a meandering trail. Once used as a dump for school waste, the area has been restored over the past ten years by school staff, students, and community volunteers who recognized the value of the resource. Now, the Outdoor Laboratory boasts several species of flora and fauna, including shagbark hickory, American elm, and white oak. School children regularly visit the Outdoor Laboratory to learn about nature as they engage in physical education and develop writing and mathematics skills.

CommuniTree partnered with teachers from Pine Village Elementary School to help develop interpretive material for the school’s Outdoor Laboratory. To help teachers incorporate the Outdoor Laboratory into their curriculum, CommuniTree has developed a series of signs, posters, brochures, and maps for the Outdoor Laboratory. At Pine Village School’s 2006 Spring Program, which is attended by many members of the community, signs were unveiled which highlight a number of tree species as well as topics such as exotic species invasion and forest management. It is hoped that the outcomes of this partnership, which began as one graduate student’s research project, will bring landowners, community members, and elementary school children together to explore and appreciate the natural world that surrounds them, as well as to consider new means to manage private lands and collaborate with neighboring landowners. Furthermore, this research makes a contribution to a burgeoning field of research studying processes of collaboration and how such processes can contribute to new means of natural resources management.

Amy and Kenli received their Ph.D. in human dimensions at Purdue under the direction of Dr. Shorna Broussard. Kenli is a John A. Knauss Marine Policy Fellow with the U.S. Navy. Amy is a post-doctoral researcher at Purdue working with Dr. Keith Woeste and Dr. Doug Jacobs. Amy can be reached at arossdav@purdue.edu.
I am a member of Dr. Andrew DeWoody’s lab where we conduct research in ecology and evolution. We work on a variety of taxonomic groups in the fields of immunology, conservation genetics, genomics, evolutionary biology and wildlife science. Many of the projects in our lab address questions in basic research without clearly defined practical applications. Basic research need not be directed by potential practical implications and in this way, unexpected results can refocus research objectives or introduce entirely new questions. Additionally, while the results of basic research may not always seem to have an obvious application, they form an important foundation of scientific knowledge and theory upon which to build further research.

My dissertation research addresses the rapid rate of evolution in a diverse group of voles. By using their two genomes - one located within the mitochondria and the other in the nucleus - I am able to compare rates of evolution among different taxa and examine gene transfers that have occurred between the two genomes. This research has allowed me to gain insight into the evolutionary history of these rodents and the general dynamics of genome evolution in mammals.

Arvicoline rodents - The subfamily Arvicolinae, comprising the voles, lemmings and muskrats, is one of the most diverse rodent subfamilies. Most of the diversity within the Arvicolinae is found within the 65 species of voles in the genus Microtus. The genus underwent a rapid diversification approximately two million years ago, making its rate of speciation 60–100 times higher than other vertebrates. Despite high levels of chromosomal variation, voles exhibit little morphological diversity; even the most experienced mammalogists can have difficulty telling species apart in the field. Could it be that the chromosomal diversity exhibited within the genus is somehow driving its speciation rate? Is there evidence for this rapid evolutionary rate within its DNA sequence? We addressed these questions by studying different elements within arvicoline genomes.

Since morphological differences between voles are subtle, understanding their rate of evolutionary divergence requires DNA sequences. Each panel of colored DNA bases is from a different individual.
Mitochondrial genomes—The field of vertebrate genomics is familiar to most people because of the recent sequencing of the human genome. This genome, consisting of three billion nucleotides, is enclosed within the nucleus of each cell (i.e., the nuclear genome). However, most people may not be familiar with the second genome contained by all eukaryotic cells – the mitochondrial genome. In mammals, the mitochondrial genome is 16,000-17,000 nucleotides in length. Although the mitochondrial genome is physically separated from the nucleus, DNA fragments can transfer from the mitochondria to the nucleus and be integrated into the nuclear genome sequence. This type of transfer between genomes is widespread among animals and plants but how and why it occurs is not yet known. We used mitochondrial genome sequences of voles to test whether their rapid evolution is reflected in their mitochondrial DNA and looked for fragments that have been transferred to the nuclear genome.

Laboratory work—I obtained tissues from natural history museums and completed all of the DNA work here in the Genetics Lab in FNR. We have a large facility with most of the resources that the graduate students in our group need. I sequenced the entire mitochondrial genome of a Eurasian species of vole and compared it to another vole mitochondrial genome from GenBank, a public repository of DNA and protein sequences. I also compared these vole sequences to the mitochondrial genome sequences of other mammalian taxa and found that the rate of evolution in Microtus far exceeds that of other mammals. We still do not know the cause of this rapid rate of evolution in voles, nor do we know if there is a connection between the rate of evolution within their mitochondrial genome and that found within their nuclear genome.

Another question that intrigued me was whether pieces of vole mitochondrial genome have been transferred to the nuclear genome. I found multiple fragments that ranged in size, evolutionary age and number. The largest fragment that I have found is over 4,000 nucleotides - at least 25% of the mitochondrial genome. It appears that pieces of the mitochondrial genome have been transferred repeatedly throughout the course of arvicoline evolution. Because this group of rodents diversified so quickly, it has been difficult for researchers to reconstruct evolutionary relationships. These displaced fragments can provide insight into vole phylogenetics and resolve taxonomic relationships that previously may have been confusing.

Results from this study may also have applications within the field of human disease. Little is known about the mechanisms of mitochondrial DNR transfers. By studying them, we can understand how interactions between two genomes might influence genetic diseases and shape our own evolution.
Most people would not let their children swim near a sewage treatment outflow in a river. In Indiana, where almost a third of the 1,800 miles of waterways are considered polluted, this significantly reduces the number of areas available for water recreation. However, unlike humans, aquatic organisms are sometimes unable to distinguish between contaminated areas and more pristine aquatic systems. Many of the waterways inhabited by fish are subject to sewage outflows from water treatment facilities, and the effluents that flow into the water can contain contaminants that have not been treated to reduce their toxicity.

Contaminants known as endocrine disrupting chemicals (EDCs) are often found in sewage but are not usually treated at the facilities. These compounds act by changing hormone levels in the body, thereby causing physiological or reproductive changes that either mimic or are inconsistent with natural activity. One example of a common EDC is ethinyl estradiol (EE₂), the main active ingredient found in birth control pills. Over 1,690 kg of EE₂ is manufactured in the United States each year. Twenty percent of all women in the United States take these products, which are then eluted into sewer systems and eventually waterways. The presence of high levels of EE₂ can negatively affect fish by causing changes in their spawning behavior, which may in turn affect the number of offspring they produce. Previous studies have determined that concentrations of the compound above .57 ng/L are of ‘high risk,’ meaning that exposure to these waterways could cause negative health effects. EDC concentrations well above the high-risk level for fish and humans have been noted in waterways throughout the developed world. As more women begin to take birth control, these levels will continue to rise. Other EDCs found in waterways include zearalanone, a natural phytoestrogen, and trenbolone, a potent androgen used to increase muscle growth in cattle. Two other compounds of interest include ZM 189, 154, an anti-estrogen used to treat breast cancer patients, and flutamide, an anti-androgen used to treat prostate cancer. In addition to being exposed to various EDCs, fish are particularly susceptible to these chemicals because they can be readily absorbed through their gills and skin and ingested.

Endocrine disrupting chemicals are found in concentrations well above the high-risk level for fish and humans in waterways throughout the developed world.

Photo by Andrew Muir
Sonia Mae Johns, an M.S. student majoring in Fisheries and Aquatic Sciences, uses a small net to collect fathead minnows from their holding tank to use in her research.
through their food. Fish are also susceptible to the effects of EDCs because these hormones affect their reproductive systems and therefore affect sex determination, which can occur up to two months after the fish are born.

Studies have shown that chemicals causing changes in hormone levels in fish are widespread across the United States. Feminization of male fish, masculinization of female fish, changes in the ratios of males to females in a given population, and changes in reproductive success have all been found in natural aquatic systems where EDCs occur. Recent studies have identified effects of these chemicals on the fecundity, fertility, frequency of spawning, and hatch rate of numerous species. Other studies have focused on the effects of these chemicals on biomolecular activities in these species. However, no studies have been done to link the changes in gene and protein expression to these reproductive and physiological endpoints.

Although many species are affected by these compounds, the United States Environmental Protection Agency (US EPA) has chosen the fathead minnow (Pimephales promelas) as a model species for these investigations. Fathead minnows are relatively small, have a short generation time, and are easy to raise and reproduce in a laboratory setting. Their reproductive cycle can be controlled by regulating the temperatures and amount of light they are exposed to, making it easier to perform reproductive experiments throughout the year. There are also databases that contain the information for over 10,000 genes available that make it easier to compare expression of these genes for fish exposed to different chemicals.

My master’s research is part of a multi-investigator project that involves linking gene and protein expression with reproductive and physiological endpoints (the actual changes that occur) using the approach outlined by the EPA using the fathead minnow. Research efforts will focus on the aforementioned EDCs. Our research group has attained preliminary results for the exposure of the fathead minnow to EE₂. An increase in the frequency of spawning events was observed in the study when the fish were exposed to the compound, and an increase in expression of certain genes typically seen in reproductively-mature females was also noted in mature males.

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