Nicholas Bean, Undergraduate – Poster Presentation (Dr. Allan T. Scholz, Mentor)

Diet and Growth of Northern Pike (Esox lucius) in Box Canyon Reservoir, Pend Oreille River, Washington

The objectives of this study were to determine the food habits and growth of northern pike, discovered in Box Canyon Reservoir in 2004. Their diet consisted entirely of fish. Northern pike preyed opportunistically on pumpkinseed (27% of the relative abundance of all fish in the reservoir and 27% of the pike diets). Pike selected peamouth (7% reservoir, 24% pike diet) and mountain whitefish (< 1% reservoir, 7% pike diet). Pike avoided tench (7% reservoir, < 1% pike diet), and consumed fewer yellow perch than available in the reservoir (26% reservoir, 11% pike). Lengths at age were: age 1 (194 mm), 2 (312 mm), 3 (401 mm), 4 (530 mm), 5 (656 mm), 6 (764 mm), 7 (883 mm) and 8 (1,012 mm), which were longer than North American populations. Northern pike are apex predators that exert top-down structuring of fish communities and cause cascading trophic interactions in a food web. Their presence in the reservoir has potential to undermine efforts to protect a federally listed endangered species (bull trout) and other native fishes (e.g. peamouth and mountain whitefish).

Mike Clawson; Co Authors: Matthew Berg, Sasha Greenspan, Markael Luterra, Michael Panza & Alex Rigney, Undergraduates – Oral Presentation (Dr. Margaret O’Connell, Mentor)

Diversity and Abundance of Multiple Taxa in Ecuadorian Cloud Forest: Trees, Epiphytic Ferns, Invertebrates, and Birds

The tropical montane cloud forest of the Ecuadorian Andes is one of the earth’s most diverse ecoregions and is being deforested at an alarming rate. While lowland forests in Ecuador have been extensively studied, montane cloud forests, which have many more endemic species, have been virtually ignored by researchers. This study compared two elevational zones within upper montane cloud forest, in terms of species richness and abundance, using a multi-taxa approach. The taxa used were trees and understory vegetation, epiphytic ferns, invertebrates located within canopy bromeliads, and birds. All of the studies were done along the same four transects, two transects per elevational zone. The results of the vegetation study showed no difference in forest structure between the two elevations, but a strong difference in species composition. Ferns were found not only to be different in species richness between the transects, but also stratified into different height zones within the trees. Within the invertebrate taxa, the most abundant species were found on all four transects, but the majority of species was limited to a single transect. Bird species were not found to be different between the transects, although many new species were recorded in the area. Throughout all taxa in this study, the sample sizes were insufficient to yield representative samples of the populations. Our results, however, may serve as a basis for future research in the practically unstudied montane cloud forest.
Aj DeBee, Undergraduate – Oral Presentation (Dr. Judd Case, Mentor)

Phylogenetic Relationships of Fossil Penguins from Seymour Island, Antarctica

*Sphenisciformes* (penguins) are a highly derived order of birds which have evolved specialized morphology that allows for a primarily aquatic lifestyle. The order is represented by 17 extant species, and at least several dozen extinct species. Although their fossil record is extensive the phylogenetic relationships of these extinct penguins remains largely unknown. Understanding these relationships will shed light on the phylogenetic relationships of extant forms. I compiled and analyzed over 100 morphological characters from both the humeri and the tarsometatarsi of 15 fossil taxa from Seymour Island, Antarctica. Using a MacClade data matrix and Phylogenetic Analysis Using Parsimony, I found several potential evolutionary relationships between these Seymour Island taxa. Preliminary analyses include findings that taxa of smaller penguins, such as Delphinornis and Mesetaornis, are more primitive than taxa of larger penguins such as Anthropornis.

Isaura J. Gallegos, Undergraduate – Oral Presentation (Janani Varadarajan & Dr. Ronald Coolbaugh, Mentors)

Interacting Partners for Putative (*Arabidopsis thaliana*) Cytochrome P450 Reductase III

Cytochrome P450 reductases are essential for the function of another family of enzymes called cytochrome P450s. Cytochrome P450 reductases interact and transfer electrons to P450 enzymes to make them catalytically active. There are two known Arabidopsis cytochrome P450 reductases, ATR1 and ATR2. Analysis of the Arabidopsis genome revealed a third putative ATR, ATR3, which has been partially characterized. This project tested the interaction between ATR3 and three P450 enzymes, CYP701A3, CYP76C2, and CYP72A7 using the SRS yeast two-hybrid assay. ATR3 was cloned into the "bait" vector, the candidate protein partners were each cloned into the "prey" vectors individually. Yeast cells were transformed with the "bait" and the "prey" constructs. Interaction between the "bait" and "prey" was tested by checking for growth at 37°C in glucose or galactose medium. None of the three proteins tested displayed growth at 37°C.

Jennifer Gunnarson & Nick Bean, Undergraduates – Poster Presentation (Dr. Allan Scholz, Biology & Bill Baker, Washington Department of Fish and Wildlife, Mentors)

Identification and Reconstruction of Ingested Prey Fish Lengths Using Diagnostic Bones

"Diagnosis" bones are useful for identifying prey fishes in the diets of piscivorous fish and the types of fish bones found in archeological sites. Between genera and species of fishes there are distinct characteristic differences in the shapes of diagnostic bones that identify them. Our objective was to develop a dichotomous key to identify the diagnostic bones of Eastern Washington fishes. Fish were measured (total length in mm) and either fed to dermestid beetles or boiled to remove flesh from bone. Diagnostic bones (cleithrum, dentary, opercle, preopercle and pharyngeal arches) were extracted and measured to the nearest 0.01 mm using a dial caliper. Measurements of original fish length (dependent variable) and its diagnostic bones (independent variable) were related in a linear regression equation. A fisheries biologist or archeologist can use this regression equation to estimate fish length from a diagnostic bone measurement.
Identification of the Bacterial Community of Medical Lake, Washington, Using Molecular Methods

Bacteria play an important role in processes such as fermentation, infection and recycling of organic and inorganic compounds. In aquatic systems, bacteria decompose detritus to release terrestrial carbon and act as a food source for other organisms. Historically, the bacterial contribution to aquatic systems has been largely ignored by ecologists. This is principally due to difficulty in studying and identifying bacteria; it is estimated that only 10% of bacteria from any environmental sample can be cultivated under laboratory conditions. For example, the limnology, fish population and water quality of Medical Lake, Washington, have been studied, but no research has examined the bacterial constituents of the lake. Recent advances in molecular technology now provide an avenue to address this question.

Therefore, in this study of Medical Lake, molecular methods are used which do not require growth of bacteria in the laboratory. Bacterial 16s rRNA genes will be amplified, cloned and sequenced, then compared against international databases for identification. Using molecular techniques, I will develop a more complete picture of the bacterial composition of Medical Lake, adding to the existing biological profile of the lake.

Frog Egg Fertilization Using Sperm or Sperm Nuclei

We will compare fertilization rates achieved by injecting single intact spermatozoon or a sperm nucleus into frog eggs of species *Xenopus laevis*. It is routine in humans to use sperm injection with those whose sperm motility is impaired. The success rates are low (1-5%), but high enough for some to opt for the procedure. During natural fertilization, the spermatozoon does not penetrate the egg; instead, the sperm membrane fuses with the egg's membrane. The outer sperm membrane never contacts the interior of the egg. Stripping the plasma membrane from a sperm and injection of the nucleus into the egg would seem to more closely resemble the natural process.

Sperm will be spun in a centrifuge and resuspended in nuclear preparation buffer (NPB). Half will be treated with lysolecithin to remove the cell membrane. The remaining half will be in NPB until injection into eggs. All injections will be done with a glass pipette pulled to an interior diameter of 7 µm. The injection needle, fitted to a micromanipulator, is attached to tubing connected to a three-way valve so that it can be switched, to provide positive pressure, or vacuum.

Factors Affecting Growth and Success of Stocked Rainbow Trout in Eastern Washington Lakes

Rainbow trout (*Oncorhynchus mykiss*) are a popular trophy fish commonly stocked into many Eastern Washington lakes in order to provide profitable fisheries which benefit local economies. In Eastern Washington, the number of trout stocked into lakes is solely determined by the size of the lake expressed in surface acres. Currently, other than the size of the lake, no criteria exists for determining an appropriate number of trout to stock within each lake. Although physical and biological factors have been shown to affect the growth rate of trout, these factors are not considered at the time of stocking. Twenty lakes in Eastern Washington were sampled for abiotic variables such
as temperature, dissolved oxygen, chlorophyll, nitrate and phosphate, as well as biotic variables such as density and biomass of all prey, and fish composition in each lake. The relationships between rainbow trout growth and other variables are presented.

**Rachel Lassman, Undergraduate – Oral Presentation (Dr. Margaret O'Connell, Mentor)**

**A Field Trip to Turnbull National Wildlife Refuge: An Introductory Video**

As part of the National Wildlife Refuge System, the mission of Turnbull National Wildlife Refuge (TNWR) is to conserve, protect, and enhance fish and wildlife and their habitat for continuing benefit of people. Educating children about the importance of, and threats to, wildlife habitat, is one way the refuge addresses its mission. Given its proximity to Spokane, TNWR provides environmental programs to over 8,000 students annually. This video is designed to both educate children about the importance of refuges and to provide teachers and classes with necessary information to help them prepare for field trips to TNWR.

**Cole Lindsey, Undergraduate – Oral Presentation (Dr. Margaret O'Connell, Mentor)**

**Effects of Human Development on the Home Range Size of Moose and Elk in Spokane County**

Human development in wildlife habitat can have both negative and positive effects on wildlife and these effects vary by species. Elk (*Cervus elaphus*) and moose (*Alces alces*) are both large hoofed mammals belonging to the family Cervidae, but elk are herding animals whereas moose are solitary. My research examines whether human development has different effects on the home range sizes of elk and moose. I radio-tracked 7 female elk and 7 female moose during winter-spring 2007 in the Peone Prairie region of northern Spokane County and combined my data with Washington Department of Fish and Wildlife data. I established home ranges for each animal. Elk had larger home ranges than moose regardless of zoning category. County zoning maps were used to categorize levels of human development. Home ranges and the frequency of sightings of both species were compared between categories.

**Kevin Mahoney, Graduate – Oral Presentation (Dr. Ross Black, Mentor)**

**Stable Isotope Assessment of Aquatic Communities in Eastern Washington Lakes, with Impacts on Rainbow Trout**

Rainbow trout (*Oncorhynchus mykiss*) is a sought after sport fish and is therefore stocked annually in many lakes across eastern Washington. However, little is known about how the lakes’ animal constituency impact rainbow trout ecology. Stable isotope analysis is a technique used to determine the organization of aquatic communities. This project will examine the stable isotope signatures of rainbow trout and their respective prey/food and competitor species. More specifically, analysis of stable isotope signatures will establish each sampled species’ use of different energy sources and feeding location in the lake (open-water vs. near shore), what trophic position they occupy, and whether this community structure changes in different age classes of fish species. Twenty eastern Washington lakes, all of which are managed for trout fishing and are stocked annually with rainbow trout, will be established as research sites. This study will provide a unique opportunity to collect stable isotope data from each of the lakes. Ultimately, such knowledge may be used by Washington Department of Fish and Wildlife to permit targeted management of individual lakes and thereby improve sport fishing of this important resource.
Amanda Matthiessen, Graduate – Oral Presentation (Dr. Charles Herr, Mentor)

Further Development of a Novel Buffer for PCR

Polymerase Chain Reaction is a powerful molecular technique used for forensic science, disease detection, as well as other important applications. PCR replicates specific DNA from a complex mixture of DNA. A problem currently exists with the technology. Typically, PCR generates more than the desired target DNA sequences. One problem sequence generated is called primer-dimer. Other unwanted sequences are generated when primers interact with non-target DNA. This noise degrades assay reliability. We have developed a novel PCR buffer system that reduces pH fluctuations during PCR thermocycling. One benefit of the buffer, containing 5-5, diethylbarbituric acid (BART buffer), is that it almost eliminates this noise, and increases the replication of the desired sequences. This has been observed in two substantially different PCR systems. One allows bovine sexing, (annealing temperature is 65Â°C), and the other, bird sexing (annealing temperature is 48Â°C and uses degenerate primers). This study will address a remaining problem with BART: frozen storage. Also, the development of a complete reaction mix for use in either bovine sexing or bird sexing will be attempted. Thaw; simply add cells, and the reaction will be ready to run.

Andy Miller, Graduate – Poster Presentation (Dr. Allan T. Scholz, Mentor)

Diet, Age, and Growth of Tiger Trout (Salmo trutta x Salvelinus fontinalis) from Ten Eastern Washington Lakes.

Tiger trout are a sterile hybrid between a male brown trout and a female brook trout. These fish are currently being stocked into eastern Washington lakes, even though their diet and interactions within the lakes are unknown. The purpose of this investigation was to determine the feeding habits of tiger trout in ten eastern Washington lakes. Study lakes ranged from small desert lakes, to large alpine lakes. There were two main objectives for this investigation: (1) determine if tiger trout feeding habits were uniform between these lakes, or if there were regional differences; and (2) to determine the age and growth of tiger trout in these lakes. Tiger trout were collected by angling, creel surveys, electrofishing, and gill nets. After their stomachs were pumped via gastric lavage, they were released back into the lake. Tiger trout were generalist feeders, preying upon the most abundant prey in their environment. Prey organisms included zooplankton, midges, mollusks, and small forage fish. Those over 300 mm in total length changed their diet and became piscivorous.

Corey Peone, Undergraduate – Poster Presentation (Dr. Allan T. Scholz, Mentor)

Ungulate Survey on the Colville Indian Reservation

The objective of this project was to estimate the populations of the following species of ungulates on the Colville Indian Reservation: mule deer (Odocoileus hemionus), whitetail deer (Odocoileus virginianus), Elk (Cervus canadensis), and Moose (Alces Alces). A helicopter with a pilot and two observers was used to make the counts. Animals on randomly selected management units were counted. These numbers were expanded using a computer model called "Aerial survey," created by the Idaho Department of Fish and Game to account for animals that were present in the management area but not seen by the observers. The model also calculated 90% confidence intervals for the estimates. Helicopter counts were 2,693 mule deer, 1,936 white-tailed deer, 68 moose, and 555 elk. These data were inputted into the aerial survey model, which gave estimated numbers for the both species of deer combined at 11,066 with a 90% confidence interval + 1,800, elk at 1,115 with a 90% confidence interval + 383. Moose were not put into the model. [This work was conducted during the Winter break of 2006-2007 as part of an internship with the Colville Confederated Tribes Fish and Wildlife Department.]
Caitlin Valentin, Graduate – Poster Presentation (Dr. Charles Herr, Mentor)

DNA from Bone and Feathers

Our lab is developing methods for extracting DNA from bone and feathers. It is difficult to extract DNA from bone and feathers due to the small amounts present and the tight association of DNA to calcium phosphate in bone and/or keratin and collagen present in bone and feather. Increasing the yield of DNA would be valuable for the forensic, anthropologic, and archeologic fields. We have discovered that the use of an extraction buffer containing 600mM ethylene glycol tetraacetic acid (EGTA) will de-mineralize bone, and 2-Mercaptoethanol and heating in a microwave will dissolve the collagen fibers that are present. To separate the EGTA and 2-Mercaptoethanol from the DNA after use, we utilize a procedure we have termed electrophoretic dialysis. This procedure uses electrophoresis to drive ions out of a dialysis bag. In the extraction of DNA from feathers, we have discovered that a 50/50 mixture of EGTA and 2-Mercaptoethanol paired with heating in a microwave solubilizes the majority of the feather, potentially releasing DNA. The feathers also undergo a modified version of electrophoretic dialysis to separate the DNA from the chemicals in preparation for additional experiments.

Lacie Westbrook, Undergraduate – Oral Presentation (Dr. James G. Hallett, Mentor)

Patterns of Genetic Variation Among Geographic Subpopulations of the Meadow Vole

Geographic barriers can influence patterns of genetic substructure by reducing migration. I asked how differences in the size of geographic barriers affected genetic variability in the meadow vole (Microtus pennsylvanicus). The Pend Oreille River, sloughs, and streams were predicted to have declining effects on genetic subdivision. DNA was extracted from 307 individuals collected from six habitat restoration areas along the Pend Oreille River. An additional 58 samples were obtained from two areas in Idaho as outgroups. Genetic variation was characterized using six microsatellite markers. Microsatellites are segments of DNA consisting of variable numbers of repeated sequences that are nonfunctional. Allele frequencies were used to calculate Nei’s genetic distance between all pairs of subpopulations. These distances were compared to geographic distances with a Mantel test. I will discuss the observed patterns of genetic substructure in relation to the additional habitat changes that will result from habitat restoration.