

# Intermountain Journal of Sciences

#### The Intermountain Journal of

*Sciences (IJS)* is a regional peer-reviewed journal that encourages scientists, educators and students to submit their research, management applications, or viewpoints concerning the sciences applicable to the intermountain region. Original manuscripts dealing with biological, environmental, health and human development, mathematics, molecular-cellular, pharmaceutical, physical and social sciences are welcome.

Co-sponsors/publishers include the Montana Academy of Sciences, the Montana Chapters of The Wildlife Society and The American Fisheries Society. It is the intent of the governing bodies of the co-sponsoring organizations that this journal replace and standardize printed proceedings from the respective annual meetings. Format and style should follow the Guidelines for Meeting Abstracts Submitted to the Intermountain Journal of Sciences, 1st revision 2016.\* It is the policy of the editorial board that abstracts from presentations at annual meetings be published in the last issue of IJS for that year of the annual meeting. Submission of manuscripts for review and publication without regard to membership is encouraged.

Baseline funding is provided by the co-sponsoring organizations. Long-term funding will be derived from page charges assessed manuscript authors at \$60/page, sponsoring organizations at \$40/page for annual meeting abstracts and annual subscriptions: student - \$6, regular member - \$15, patron member - \$25, international member - \$25 and library - \$25. One time subscriptions are: life member - \$150 and sustaining subscriber - \$2,500. The intent of the co-sponsors and editorial board is that *IJS* be expanded to a quarterly journal. Achieving that objective depends upon numbers of acceptable manuscripts received and available funding. The editorial board's policy is that contributing authors be assured of publication within 12 months of acceptance of their manuscript. It is also intended that *IJS* be converted to an eJournal.

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Associate editors and referees judge submitted manuscripts on originality, technical accuracy, interpretation and contribution to the scientific literature. Format and style should follow the *Guidelines for Manuscripts Submitted to the Intermountain Journal of Sciences, Dusek 1995, 2<sup>nd</sup> revision 2016.*\* Organization may vary to accommodate the content of the article, although the text is expected to elucidate application of results.

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Balance 01/01/18		\$4,134.87
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	Subscriptions Total	\$310.00
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		8,600.00
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Total Income		\$9,810.00
Expenses:		
Design and Printing		\$6,817.99
Postage		\$166.75
P. O. Box Rental		\$140.00
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Total Expenses		\$9,812.02
Balance 12/31/18		\$4,132.85

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The *Intermountain Journal of Sciences* (IJS) is a fully refereed journal.

Manuscripts are submitted to the Editorin-Chief (EIC) for initial consideration for publication in the IJS. This review shall include, but not be limited to, appropriateness for publication in IJS, correct formatting and inclusion of a letter of submittal by the author with information about the manuscript as stated in the "Guidelines for manuscripts submitted to the Intermountain Journal of Sciences" (Dusek 1995, 2007) available on the IJS website, www.intermountainjournal.org under the Publish tab. This cover letter must also include a statement by the author that this paper has not been submitted for publication or published elsewhere. The EIC notes the date of receipt of the manuscript and assigns it a reference number, IJS-xxxx. The EIC forwards a letter of manuscript receipt and the reference number to the corresponding author. The corresponding author is the author who signed the submittal letter.

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For accepted manuscripts, each copy of the manuscript containing comments thereon and other comments are returned to the corresponding author. Revised manuscripts are to be returned to the EIC in hard copy and four copies if further review is required. These copies can be submitted in digital form by email. The revised manuscript shall be returned to the EIC within 14 days of notification. Review of the revised manuscript by the Associate Editor and reviewers shall be completed and returned to the EIC within 14 days. An accepted manuscript will then be forwarded to the Managing Editor (ME) for final processing.

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The identity of all reviewers shall remain anonymous to the authors, called a blind review process. All criticisms or comments by authors shall be directed to the EIC; they may be referred to the ME or the Editorial Board by the EIC for resolution.

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Submissions concerning management applications or viewpoints concerning current scientific or social issues of interest to the Intermountain region will be considered for publication in the "Commentary" Section. This section will feature concise, well-written manuscripts limited to 1,500 words. Commentaries will be limited to one per issue.

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## LITERATURE CITED

Dusek, Gary L. 1995, revised 2007. Guidelines for manuscripts submitted to the *Intermountain Journal of Sciences*.Int. J. Sci. 1(1):61-70. Revised guidelines are available on the Intermountain Journal of Sciences web site: (www.intermountainjournal.org)

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# EVALUATION OF THE ARTICLE "ESTIMATING HERBACEOUS BIOMASS OF GRASSLAND VEGETATION USING THE REFERENCE UNIT METHOD" BY BOYDA, ET AL. 2015

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## Abstract

An evaluation of the scientific article "Estimating Herbaceous Biomass of Grassland Vegetation Using the Reference Unit Method" by Boyda, et al. 2015 (Prairie Naturalist) is relevant because authors state that herbaceous biomass can be accurately and precisely estimated throughout the entire Buffalo Gap National Grasslands (BGNG) and beyond with the Reference Unit Method. The authors failed to provide easy to follow methods, a complete data set with all results, a study site map while only providing partial data and analyses for prairie dog colonies or areas adjacent to prairie dog colonies. The authors did not provide an improvement in protocol and methodology of the weight estimated method (double sampling-estimating and clipping) described by Pechanec and Pickford (1937) and its application on rangelands to estimate above ground biomass for many ecological types. Evaluations were very limited to few plant species: western wheatgrass (Pascopyrum smithii), purple three-awn (Aristida purpurea), needle-and-thread (Hesperostipa comata), and green needle grass (Nassella viridula). Other comparisons were groups of plants with unidentified species and may produce questionable results with different species mixes when applied to other grasslands or locations on the BGNG. The protocols developed with double sampling (clipping with oven dry weights and corrected to visual estimates) is still the standard with no improvement by Boyda et al. (2015). The article by Boyda et al (2015) may provide erroneous results with application of the Reference Unit Method and is not recommended for estimating herbaceous biomass.

Key words: estimating biomass, double sampling, grasslands, plant biomass.

## DISCUSSION

Authors of the article by Boyda, E. D., J. L. Butler and L. Xu. 2015 Prairie Naturalist suggests that by applying the Reference Unit Method to estimate herbaceous biomass of grassland vegetation can provide more accurate and precise estimates of plant biomass with plant species and functional groups that involve combining species with similar origin (native or introduced), life form and duration. The protocol and procedures presented are not an improvement in methodology to estimate herbaceous biomass, especially not the standard double sampling method. The weight estimated

method (double sampling-estimating and clipping) described by Pechanec and Pickford (1937) and its application on rangelands to estimate above ground biomass for many ecological types is still the standard with no improvement. Since the inception of the double sampling method, it has been the standard to estimate plant biomass on the grasslands for years (Wilm et al. 1944, NAS-NRC 1962, Francis et al. 1979, Cook and Stubbendieck 1986, Robin et al. 1983, Bonham 2013). The article by Boyda et al. 2015 did not provide an improvement of the double sampling procedure by using the Reference Unit Method.

The authors failed to present the double sampling equation used for analyses in this publication which made methodology more difficult to follow. Equations listed in Table 1 are not provided in the methods such as;

 $\tilde{Y}_e = y_r + b(x_e - \bar{x}_r)$ , as given in Cook and Stubbendieck, 1986, Society for Range Management, p 246; Equations 42 and 43.

 $\tilde{Y}_{e}$  = estimated corrected yield.

 $y_r = ratio estimate (actual/estimated).$ 

b = regression coefficient.

 $x_e = ocular estimated weight of a plot.$ 

 $\bar{\mathbf{x}}$  = average of ocularly estimated weights of plots in regression sample.

According to the field methods which are not clear; it was a ratio estimated as defined on page 75 of the publication (The biomass of each individual species and functional group was estimated within each plot as a ratio of the reference unit: estimated unit in increments of 0.1). That would be " $y_r$ " in the equation above. However, the authors then weighted the "y<sub>r</sub>" with the "b", a regression coefficient. The authors did not provide any numerical results of statistical analyses for these equations for testing the intercepts and coefficients in Table 1. The "b" coefficient did not add anything to the estimated weight and could have been deleted. All equations in Table 1 were apparently solved with the ratio of reference/actual combined as stated on page 75. Their equation as given in Table 1 contains "x" which is the ratio as stated. It is difficult to believe that a ratio of two different plot areas (references in sample areas) can be estimated at 0.1 of unit (gram?) increments.

The authors applied equations in Table 1 with the "b" coefficient (slope) to convince the reader that they estimated the constant, and the mean clipped value was a fraction of the estimate to the nearest one tenth of  $g/0.25 \text{ m}^2$ , which only adds confusion to the analyses. Therefore, all results and conclusions are questionable.

The first objective to evaluate the Reference Unit Method on a broad spatial scale was not accomplished because of their limited homogenous site selection procedures within two ecological soil sites, Clayey and Loam. The authors describe the experimental design and selection of nine prairie dog colonies to provide sampling and placement of four transects on each colony, colony edge and off colony. Off colony sites were located adjacent to the prairie dog colonies, resulting in very similar vegetation.

The authors state that this allowed them to evaluate along a "considerable gradient of plant community composition and abundance". However, only nine carefully selected prairie dog colonies with conditions that the prairie dog colony had to be greater than 12 ha in size, near roads, restricted to two soil types (Clayey and Loamy), with consistent soil texture characteristics were evaluated. Sites selected for sampling were in relatively flat terrain and excluded the nearby undulating topography. This failure is observed with results of a single plant species (western wheatgrass) and a combination of buffalograss (Bouteloua dactyloides) and blue grama (Bouteloua gracilis) defined as (SHORT) for their claims of multiple species assessment with the Reference Unit Method. Other evaluations were groups with unidentified plant species. The article lacked a map of nine prairie dog town locations to demonstrate broad spatial scale, the edge sites were still within the colony, and off colony sites were within 200 m, providing variability of prairie dog activity, but little or no variability of the Buffalo Gap National Grasslands (BGNG) (Boyda et al. 2013).

The second objective was to examine multi-species reference calibrations. Unfortunately, western wheatgrass (PASSMI, *Pascopyrum smithii*) was the only plant species to be identified individually and included in their results (Table 4). The other "visually dominant" species like purple three-awn (ARIPUR, *Aristida purpurea*), needle-and-thread (HESCOM, *Hesperostipa comata*), and green needlegrass (NASVIR, *Nassella viridula*), which represent a significant part of the vegetation net primary production depending upon range condition on the BGNG, were so infrequent on or near these prairie dog colonies that creation of regression equations with the Reference Unit Method failed to address these plant species. The "SHORT" functional group which is a combination of two species (buffalo grass and blue grama) had results presented, so at best they can claim multi-species (3 plant species) were evaluated. Plant species that were combined into the other groups were never identified from the list of 481 plant species on the (BGNG) (Kostel 2006). Therefore, their objective to examine the multi-species reference calibrations and abundance was not achieved.

Objective three was to evaluate seasonlong calibration equations. This study claims that Reference Unit Method can be used over the growing season which was at most a 12 -week sample period. Season long grazing on the BGNG is approximately 24 weeks. Evaluation of the Reference Unit Method did not assess early vegetative plant growth, mid-growth or mature (dry vegetation) to determine the feasibility of the method.

The analyses as presented in Methods have a total sample size of 108 transects across three treatment effects related to prairie dogs (interior, edge, and adjacent offcolony) over two ecological sites. Only 70 transects were used as the main data set and 38 transects for validation with statistical procedures. However, sample sizes in tables and figures are confusing and do not add up to either 70 or 38 transects used for analyses. Nevertheless, 19 transects are considered as outliers in Table 1, but some outlier transects were apparently reused for analyses of functional groups. Sample size for various analyses and precise methods were not clearly defined nor presented.

The INFLUENCE option used in the regression analysis is based on statistics developed by Belsley et al. (1980) and measures the influence that each observation has on the parameter estimates (SAS 1988). Influential data identified in the study may not always indicate true outliers, or variation within the sample procedures, biological variation, or observer variation. Removing all data identified as influential increases the

regression model precision and accuracy. It also fails to provide a robust procedure for estimating herbaceous biomass and fails to evaluate the reference unit method by limiting the range of data (See Figure 1, majority of data near origin).

The INFLUENCE option provided authors a method to carefully remove all data that reduced best model results with no explanation. There appears to be more than 19 transects deleted as outliers throughout the analyses. In addition, the validation data set was calibrated using previously created regression equations and while doing this calibration of the validation data set, the observation weights are changed, or set to a value of zero, which cause select observations to be excluded from the analysis (SAS 1988). Therefore, by both calibrating the validation dataset and weighting the data greatly improves linear regression performance, however no true comparison of estimated weight is made with actual weights.

The fourth objective (Validation) includes comparisons among different observers and are displayed partially in the tables and figures. Regression plots displayed in Figure 1 clearly demonstrate why differences among observers were low, giving the perceived appearance of great accuracy and precision. A sample size of 38 transects for validation was defined in the methods but ranged from 13 to 15 transects (Table 2). Validation of functional groups for each observer between calibrated biomass estimations and actual biomass showed that a total of 13 t-tests were different from estimated vs actual among observers at P<0.15 (Table 2). Based on 9 functional groups and TOTAL in Table 2, (three observers (n=29)), the results showed a 45% error rate or 14% error rate for each observer after estimates had been corrected and estimates were made to a tenth of a gram. On an individual bases, observer 1, with experience with this method was correct 90% while observer 2 and 3 failed 60% and 67% of the time respectively, using functional groups (*P*<0.15). Other inconsistencies with no explanations were

Observers 1 and 2 are included in analyses for Table 2 with functional group PFI, while Observer 3 is absent from Table 2, but is then used for the same PFI functional group in Table 3, then in Figure 1 all three observers are used for the regression lines with only n=16 or less and all values were less than 1.5 grams. Results are not positive when comparing calibrated biomass with actual biomass with 60% of the functional groups being different (p=0.10) (Table 4).

The reference unit method evaluated by Boyda et al. 2015 cannot be repeated as presented and explained within the publication. Field sampling and statistical analysis sections are very confusing as to repeatability, in addition the statistical gyrations required are too cumbersome for efficient fieldwork. Improved accuracy and precision by the Reference Unit Method with an improvement of the double sampling procedure would be welcomed in science and rangeland management. However, this study falls short of achieving and improving the double sampling protocol defined as the Reference Unit Method for both science and management.

## AKNOWLEDGEMENTS

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# Long Term Study of an Undisturbed Cormorant Colony

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## Abstract

Arboreal nesting by double-crested cormorants (*Phalacrocorax auritus*) has not been studied as intensively as ground-nesting populations, and there are little published data on tree colony initiation and development, as well as long-term use of trees for nesting. Cormorants established nesting in an existing great blue heron rookery at Ninepipe National Wildlife Refuge (NNWR) in Western Montana in 1974. The primary goal of this study was to record the initiation and growth of an arboreal cormorant colony under undisturbed natural circumstances. The colony has been intensively studied since its inception in 1974, and data on nest success, population dynamics, foraging sites and behavior were collected over the next 18 years, until 1992. Foraging sites and resource partitioning developed with the growing colony. Nest stratification changed over time in response to substrate condition and nest re-use. Areas where nests were concentrated moved within the colony, and nest success was correlated with weather patterns and natural events. Anti-predator response varied with the avian predator involved. Display behavior and fledging of young varied in some aspects from these behaviors in ground nesting populations.

Keywords: double-crested cormorant, long-term study, arboreal nesting, undisturbed

## INTRODUCTION

Much has been written on coloniality in cormorants and other waterbirds (Lewis 1929, Mendall 1936a, Jenni 1969, Burger et al. 1977, McCrimmon 1978), but little on the colonization process itself (Baille 1947, Siegel-Causey, 1986) or on longterm use of a site from its initiation over an extended number of seasons. I examined the colonization and long-term use of an arboreal double-crested cormorant (*Phalacrocorax auritus*) colony.

The double-crested cormorant is known to abandon nesting colonies and establish elsewhere in response to disturbance, population pressures and loss of habitat (Anderson and Hamerstrom 1967, D.

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Thompson 1977, Ellison and Cleary 1978, Markham and Brechtel 1978, Kushlan and McEwan 1982). After a period of decline due to these factors as well as pesticide pollution, reduced water quality, and in some cases direct persecution (Houston 1971, Anderson and Hamerstrom 1967, Vermeer 1970, D Thompson 1977, Crehore 1979, Des Granges and Reed 1981, Pierson 1984), double-crested cormorants experienced a population increase and range expansion (Weseloh and Brechtel 1977, Markham and Brechtel 1978, Skaar 1975 and 1980, Scharf and Shugart 1981, Milton 1983, Buckley and Buckley 1984, Roney and Blady 1984, Vermeer and Rankin 1984, Craven and Lev 1987, Findholt 1988 and Bergeron et al. 1992).

Many new colonies have been established, especially in the west (Markham and Brechtel 1978, Pierson 1984, Findholt 1988), where they have followed the development of irrigation reservoirs (Markham and Brechtel 1978, L. Thompson unpub. man.). Double-crested cormorants established an arboreal colony in an existing great blue heron (*Ardea herodias*) rookery at Ninepipe National Wildlife Refuge (NNWR) in western Montana in 1974. Prior to 1974 double-crested cormorants did not nest in Montana west of the Continental Divide.

Very little has been published on the initiation and development process in these new colonies or on their rate of increase or nest success (Baille 1947, Siegel-Causey 1986), and I could find no published data on the long term use of an arboreal colony, its population dynamics, use of foraging sites and or behaviors specific to arboreal nesting. The primary goals of this study were to record the natural growth of an undisturbed colony of tree nesting cormorants and the subsequent development and expansion of foraging sites from the colony's inception in 1974 and monitor the effects of this cormorant colonization on the existing great blue rookery and on the tree substrate. The colony was intensively studied from 1974 to 1992

## **Study Area**

Great blue herons first established a rookery at the NNWR near Charlo, Montana in 1969 (Haderlie, USFWS per. comm., Klingsporn, unpub.man). The double-crested cormorant study colony was established within this existing rookery. NNWR lies between two mountain ranges at approximately 920 m. in elevation in the broad valley of the Rocky Mountain Trench. The refuge consists of a man-made reservoir and its surrounding meadows and marshes. This refuge is managed for waterfowl production and the nesting area is closed to the public during the nesting season. The study colony nested in trees that were spread over three groups of islands extending approximately 1.5 km along the southwest side of the reservoir (Fig. 1). Nest trees were black cottonwood (Populus trichocarpa). Ample foraging areas in addition to the colony site were found throughout the valley at Pablo National Wildlife Refuge (PNWR), a similar reservoir 24 km to the north, as well as the lower Flathead River, Flathead Lake and numerous small reservoirs, glacial pothole ponds and mountain lakes.

## **Methods**

Study methods employed emphasized maintaining the colony in an undisturbed state. To avoid abandonment and prevent data bias I took special precautions to maintain the study colony in an undisturbed natural state. Refuge management cooperated by preventing public access to the site and by timing refuge activities to avoid critical times in the nesting cycle.

Recommendations of Fyfe and Olendorf (1976) on handling of sensitive species were followed, and in keeping with the goal of "zero" human disturbance, research methods avoided examining nests during the laying and incubation periods. No egg counts or measurements were taken. Clutch sizes and egg weights are well established for this species, and I felt that the intrusions necessary to obtain these data for the present colony would seriously bias results.

The only nest entry made was for the banding of nestlings in order to assess site fidelity, origins of the colony and wintering sites. Every effort was made to reduce disturbance and handling mortality. No young were lost due to handling. Banding was done at night to prevent gull predation and because nestlings were more docile (Mendall 1936a, Ainley per. comm.) and less apt to fledge early due to our activities. Banding operations were initiated when the young no longer required brooding and most adults spent the night away from the colony.

During the banding process, researchers wore head-lamps which were only used when necessary and a safety helmet with face guard to protect from branches and beaks in the darkness. We worked quietly, with as little light as possible using moonlight nights when available. Cottonwoods are notoriously unstable, so ropes and regular mountain climbing tackle with a back-up anchor to the tree trunk were used for safety.

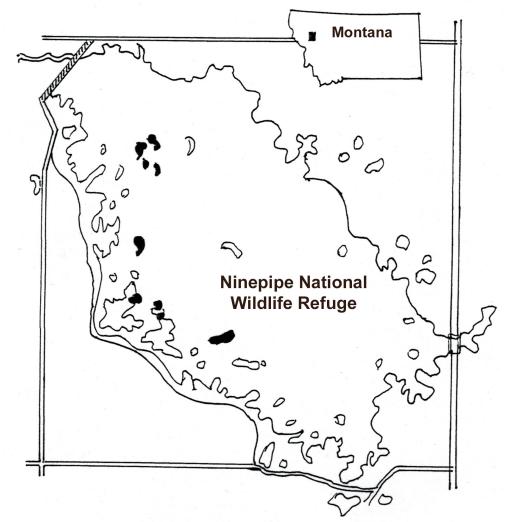


Figure 1. Ninepipe National Wildlife Refuge in Montana with colony nesting sites shown in black.

Nests being worked on and any nearby, regardless of species, were covered with a square of dark corduroy cloth on approach using methods described by Thomas (1977). This quieted the young so they could be removed one at a time for banding with minimal trauma. Birds were transferred to a ground banding site in a wicker case. A cloth was laid over each bird's head to quiet it during banding. Tools were kept on a white cloth for easy location in the darkness, and actual banding and measurements were done under one shaded lamp.

The banding and color marking system consisted of a colored Herculite leg streamer

bearing a single letter code and a standard USFWS aluminum leg band. Color and leg placement of streamers indicated hatch year and island group while the letter code isolated to natal nest. Letters were sent to refuge managers throughout possible wintering areas to make them aware of this marking program and to enhance the possibility of sightings of the unavoidably small number of birds that could be reached for banding.

Even though the islands of the colony were spread over approximately 1.5 km., there was continuous use of the available tree substrate. For this reason and for the purposes of this study, I considered the three island groups to be a single biological colony (Buckley and Buckley, 1979). Nest trees were diagramed, numbered and grouped by north, central and south islands (Fig. 2). These diagrams were duplicated for field use and all nests and young of all species were plotted on these diagrams annually (Fig. 2). Similarly, preference between peripheral branches or core locations near the trunk were plotted. This provided data on nest locations, nest

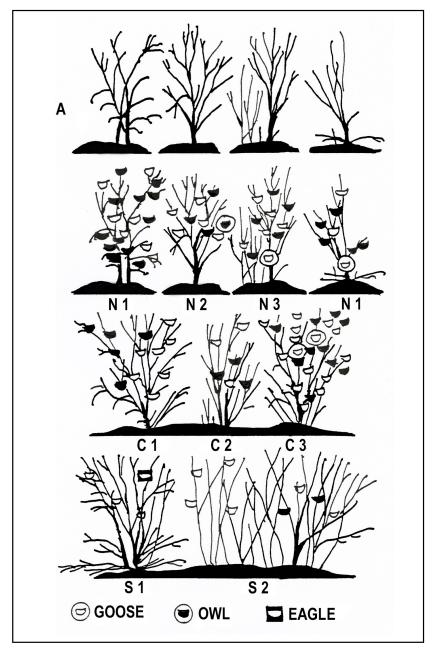


Figure 2. Trees were diagramed as shown in "A" above and duplicated for field use. All nests and young of all species were plotted on diagrams. Trees or tree groups were numbered and designated by island group north (N), central (C) and south (S). Diagram shows a sample year with cormorant nests shown in black and herons in white and geese, eagles and owls as shown it the key.

success, nests reused by other species in subsequent seasons as well as nests that were abandoned or usurped. To check for stratification, nest locations were plotted at three levels within the trees: upper, middle and lower.

Weekly observations were made from shore with a 15 x 60 mm. zoom telescope or from a camouflage covered canoe anchored in reeds at a discreet distance. Necessary photographs were taken with a 600-mm. mirror telephoto lens. All nest trees were photographed in June 1976, when trees were in full leaf to provide baseline data for the effects of cormorants on the tree substrate. Photos were repeated in August 1981 to establish the tree mortality and decline in relation to the charted cormorant use over six breeding seasons.

For the purposes of this study an occupied nest was defined as any nest on which a bird actively displayed, an active nest as any nest on which a bonded pair began incubation activities, and a successful nest was any nest which produced at least one chick of fledging age. Nest success calculations were based on active nests. Young per nest averages were computed annually by island, tree group and for the whole colony.

Castings and regurgitated meals were analyzed for fish otoliths and other undigested bone material to determine species of fish taken by adults and fed to nestlings. Fishermen were questioned and data were obtained from Bureau of Indian Affairs and Confederated Salish-Kootenai Tribal Fisheries studies in progress to determine dominant fish species present at feeding sites. Owl pellets were collected and examined for evidence of cormorant depredation.

Probable foraging sites were surveyed weekly to determine feeding areas used and, if possible, the prey items taken. Expansion and use of foraging sites were recorded for each season. The number of adult and juvenile cormorants were counted in feeding areas at midday when birds had exited the water and were congregated on islands and snags. During this time, cormorants extended their water-logged wings to facilitate drying and re-oiled their feathers in preparation of continued foraging. Locations of color marked birds were also determined at this time.

Museum skins for two subspecies of double-crested cormorants (Phalacrocorax *auritus auritus* {*P. a. auritus*}) and (Phalacrocorax auritus albociliatus {P. a. *albociliatus*}) were viewed and measured to obtain data on plumage and size variations in order to determine possible origins of this colony. In addition to skins at the University of Montana, forty-one skins were measured at the Museum of Vertebrate Zoology at the University of California-Berkeley and thirtynine skins were measured at Field Museum in Chicago. These were checked against measurements of 20 birds confiscated by wardens during fall waterfowl hunting seasons.

Young from two nests blown down in wind storms, ranging in age from 2 to 4 weeks of age, were hand-reared and observed for feeding preference and quantity requirements. Behavioral and growth data collected are to be reported elsewhere.

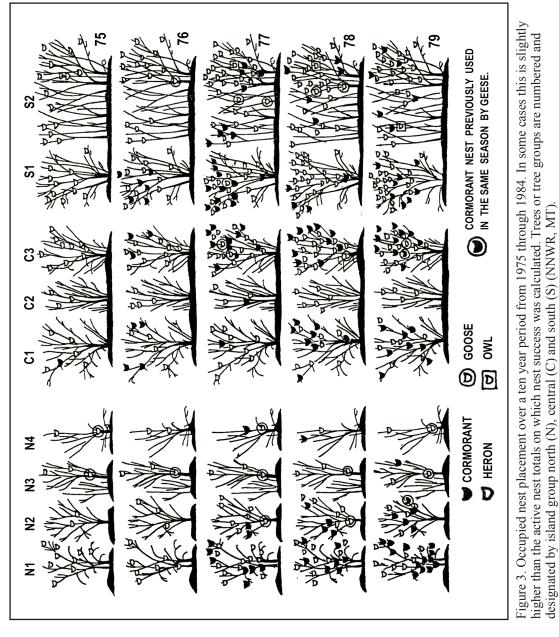
## RESULTS

## **Colonization by Cormorants**

In 1974, five birds spent the summer at NNWR on the western slope where they had previously been recorded only as occasional transients (Hand 1969). These five birds spent a great deal of moving about from island to island, flying in low lines over the water. Most of the cormorants displayed from a tree on the central islands through early August and that tree served as their roost site for that season.

Of the five original birds present in 1974, three were in immature plumage. One pair including a mature plumaged male and immature plumaged female (determined from size differential and positioning) established a pair bond and initiated a nesting attempt in a vacant great blue heron's nest in the roost tree. Although incubation appeared to be in progress no young were produced and the nest was later abandoned. No evidence of eggs were found in the nest. Whether or not this pair laid eggs or if the nest was depredated by gulls is unknown.

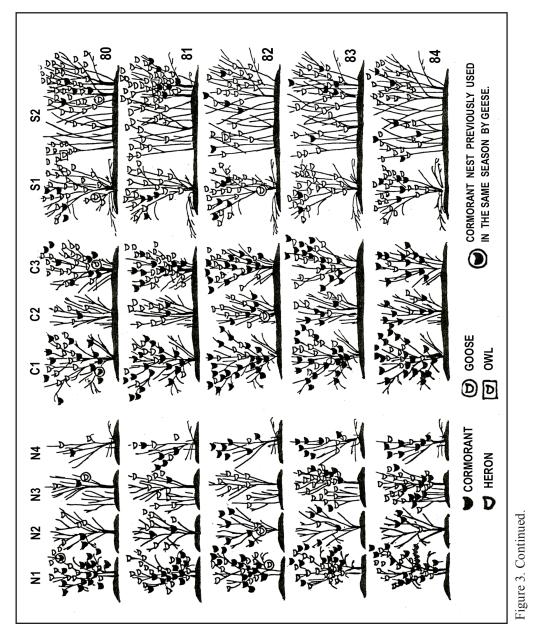
In 1975 a single pair of cormorants nested and fledged two young from the nest built in 1974 (Fig. 3). At this time both birds were in mature plumage and, since they were not marked, it is unknown if this successful pair included one or both members of the bonded pair of the previous year. In 1976, there were 22 young reared in 13 nests and 1977 saw a rapid expansion to 36 active nests. In subsequent years additional immigration supported natural colony growth and in 1990 the colony produced a peak number of 339 cormorant young in 158 nests (Table 1). Birds banded as nestlings did return to nest within the colony. Stabilization at 50 to 60 nests with a slower growth thereafter started to occur in 1980.



#### **Great Blue Herons**

Nesting within heterospecific colonies is usual for double-crested cormorants and, as with the Ninepipe colony, it is most commonly found in nesting associations with the great blue heron (Lewis 1929, Mendall 1936a, Gerbacher 1939, Mitchell 1977, D. Thompson 1977, L. Thompson 1981) (Fig. 3). The Ninepipe heron rookery originated in 1969 (Haderlie USFWS, pers. com.) with approximately 60 nests when the herons moved there "en masse" from their original colony site on the Flathead River after human disturbance during the pairing and nest initiation stage. Klingsporn (unpub. man.) found 77 nests occupied in 1972 and 74 nests in 1973. Heron nest success varied with the year (Table 2).

As in other colonies reported in the literature (Lewis 1929, Gersbacher 1939, L. Thompson 1981) there was



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Table 1. Cormorant nests (N) and young (Y) are shown by tree group, with totals and young per nest (YPN), averages by north (N), central (C), and south (S) islands. Nests, young and young per nest averages are also shown for the entire colony for each year from 1975 through 1992. (Ninepipes NWR) Grand totals for all columns per vear (GRTTL).

sou	Ν		
9.	4	Bishop	

							CORMOF	RANT NE	CORMORANT NEST SUCCESS	SESS					
Year		N	N2	N3	N4	TTL	C	C2	C3	C4	Π	SI	S2	TTL	GRTTL
75	z						-				-				
	≻						2				2				2
	ΥΡΝ										(2.00)				(2.00)
76	z						4		ю		7	9		9	13
	≻						7		Ŋ		12	10		10	22
	ΥNΡ										1.71			1.66	1.69
77	z	9	2			8	-		∞		10	6	6	18	36
	٨	17	2			19	2	2	19		23	25	23	48	06
	ΥΡΝ					(2.30)					(2.30)			(2.66)	(2.50)
78	z	7	4		-	12	80		11		19	4	5	6	40
	λ	17	7		0	24	12		26		38	6	1	20	82
	ΥΡΝ					(2.00)					(2.00)			(2.22)	(2.05)
79	z	13	5	-		19	7		11		18	7	9	13	50
	λ	32	15	ო		50	18		29		47	13	17	30	127
	ΥΡΝ					(2.63)					(2.61)			(2.30)	(2.54)
80	z	16	2	-	-	20	13	-	8		22	4	4	80	50
	λ	42	ო	0	2	47	27	ო	19		49	7	10	17	113
	ΥΡΝ					(2.35)					(2.23)			(2.13)	(2.26)
81	z	12	2	-	5	20	18	2	10		30	4	9	10	60
	λ	28	9	ო	15	52	59	9	23		83	1	14	25	160
	ΥΡΝ					(2.65)					(2.77)			(2.50)	(2.67)
82	z	16	5		7	28	21	4	11		36	5	5	10	74
	λ	52	10		17	62	51	10	28		89	1	13	24	192
	ΥΡΝ					(2.82)					(2.47)			(2.40)	(2.60)
83	z	14	4	5	7	39	19	5	14	ę	41	2	9	œ	62
	У	36	10	12	17	75	43	14	33	9	96	4	13	17	188

Table	Table 1. Continued.	tinued.													
Year		N	N2	N3	N4	TTL	c	C2	C3	C4	TTL	SI	S2	TT	GRTTL
	ΥΡΝ					(2.50)					(2.34)			(2.13)	(2.38)
84	z	18	с	6	7	37	21	7	12	5	45	5	9	11	93
	λ	41	1	18	18	88	40	12	31	œ	91	10	15	25	204
	ΥΡΝ					(2.38)					(2.02)			(2.30)	(2.19)
85	z	32	9	œ	17	63	2	10	1	2	25	m	4	7	95
	У	87	13	18	37	155	5	21	29	5	60	5	ω	13	228
	ΥΡΝ					(2.46)					(2.40)			(1.85)	(2.40)
86	z	26	5	ę	14	48		7	12	7	26				74
	У	67	13	9	37	123		16	23	15	44				177
	ΥΡΝ					(2.56)					(1.69)				(2.39)
87	z	37	7	7	15	99	~	16	13	∞	38				104
	>	91	10	13	37	151	2	40	31	18	91				242
	ΥΡΝ					(2.28)					(2.45)				(2.33)
88	z	30	2	22	12	99		2	5	13	20				86
	У	99	5	53	26	150		5	13	31	49				199
	ΥΡΝ					(2.27)					(2.45)				(2.31)
89	z	32	9	10	17	65		12	6	12	33	7	12	19	117
	У	69	12	12	42	135		29	7	28	64	19	27	46	245
	ΥΡΝ					(2.10)					(1.90)			(2.40)	(2.09)
06	z	33	4	15	12	64		11		27	38	14	32	56	158
	У	93	12	31	34	170		28		65	93	33	43	76	339
	ΥΡΝ					(2.65)					(2.40)			(1.30)	(2.15)
91	z	30		8	12	50	٢	9		9	13	15	17	32	95
	λ	54		16	23	93	2	14		16	32	34	37	71	196
	ΥΡΝ					(1.86)					(2.46)			(2.21)	(2.06)
92	z	26		15	14	55		2		23	25	19	10	29	109
_	У	59		34	35	128		ო		52	55	36	20	56	239
	ΥΡΝ					(2.33)					(2.20)			(1.93)	(2.19)

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Table 2. Heron nests (N) and young (Y) are shown by tree group, with totals and young per nest (YPN), averages by north (N), central (C), and south (S) islands. Nests, young and young per nest averages are also shown for the entire colony for each year from 1975 through 1992. (Ninepipes NWR). Grand totals for all columns per year (GRTTL).

							HERC	<b>DN NEST</b>	HERON NEST SUCCESS	Ņ					
Year		N	N2	N3	N4	TTL	с	C2	C3	C4	Ę	SI	S2	TT	GRTTL
73	z	15	-	13		29			4		13	~	6	17	59
	٨	15	0	20		35	17		4		21	ω	22	30	94
	ΥΡΝ					(1.21)					(1.62)			(1.76)	(1.59)
27	z	1	9	2	~	20		с	13		24	21	22	43	87
	y	28	12	5	2	47	22	6	32		63	50	53	103	213
	ΥΡΝ					(2.35)					(2.63)			(2.39)	(2.45)
78	z	10	5	2	-	18	7	9	14		27	23	24	47	92
	λ	21	5	5	2	33	17	6	30		56	65	57	122	211
	ΥΡΝ					(1.83)					(2.07)			(2.59)	(2.29)
62	z	œ	4	-	-	14	∞	4	13		25	19	21	40	62
	λ	19	13	സ	ო	38	22	10	37		69	33	37	20	177
	ΥΡΝ					(2.71)					(2.76)			(1.75)	(2.24)
80	z	1	4	-	2	18	12	4	20		36	25	41	66	120
	λ	25	ω	2	4	39	25	6	45		29	31	76	107	225
	ΥΡΝ					(2.17)					(2.19)			(1.62)	(1.88)
81	z	20	7	10	4	41	ω	ω	22		38	18	51	69	148
	y	47	17	24	10	98	21	22	58		101	39	121	160	359
	ΥΡΝ					(2.39)					(2.66)			(2.32)	(2.43)
82	z	12	1	6	-	23	9	8	15		29	22	23	45	97
	λ	26	4	17	2	49	14	20	29		63	50	51	101	213
	ΥΡΝ					(2.13)					(2.17)			(2.24)	(2.20)
83	z	16	-	19	с	39	6	5	13	9	33	23	23	46	118
	У	36	2	40	6	87	17	15	·34	12	78	45	44	89	254
	ΥΡΝ					(2.23)					(2.36)			(1.93)	(2.15)

Year	N	N2	N3	N4	TTL	c	C2	C3	C4	TTL	SI	S2	TT	GRTTL
84 N	17	-	19	2	39	9	ю	2	ω	19	20	29	49	107
λ	44	2	35	4	85	14	9	5	17	42	49	61	110	237
ΥPI	7				(2.18)					(2.21)			(2.24)	(2.21)
85 N	9		23	-	30		2	2	13	17	12	25	37	84
λ	15		52	2	69		Ŋ	ო	28	36	30	56	86	191
ΥPI					(2.30)					(2.11)			(2.32)	(2.27)
86 N		Ļ	18	2	23			2	18	20	2	11	13	56
λ		2	44	4	54			4	35	39	4	26	30	123
ΥP	7				(2.34)					(1.95)			(2.30)	(2.19)
87 N			22		24		З	2	38	43				67
λ			42		47		7	4	83	94				141
ΥP	7				(1.95)					(2.18)				(2.10)
88 N	2		10		12			4	35	39				51
λ	14		22		26			1	80	91				117
ΥPI	7				(2.16)					(2.35)				(2.29)
89 N		-	9	-	8				40	40		6	11	59
λ		ო	4	2	19				68	64	2	18	23	110
ΥΡΙ	7				(2.38)					(1.70)			(2.09)	(1.86)
N 06			2		2				56	56	7	9	13	71
У			2		2				125	125		15	33	160
γPI	7				(1.00)					(2.23)			(2.53)	(2.25)
91 N	-		5	<del>.                                    </del>	7				42	42	2	З	5	54
λ	33		7	2	12				80	80		7	13	105
ΥPI	_				(1.71)					(1.90)			(2.60)	(1.95)
92 N			S		5				24	24		4	4	33
Y			11		1				47	47		6	6	67
ΥPΙ	-				(2.20)					(1.96)			(2.25)	(2.03)

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little interspecific aggression between herons and cormorants in this colony, although their nests were frequently in close proximity. Altercations occurred between conspecifics, but rarely between the two species. Some degree of interspecific dominance by herons was apparent. Any typical heron-cormorant interaction was usually a brief encounter which was quickly brought to an end when the heron stood up to its full height and presented an aggressive arched-neck display (Mock 1976), whereupon, the cormorant responded with a head-averted appeasement display and the incident was over. Interspecific competition for food resources was minimal due to the spatial partitioning inherent in their different foraging methods.

# Other Species Nesting in the Colony

Five to eight pairs of Canada geese (Branta canadensis) usually nested in the colony trees each year where they occupied old heron nests or nest baskets provided in lower levels of some colony trees (Fig. 3). Great horned owls (*Bubo virginianus*) nested within the colony nine of the 18 years of the study. They raised three young there each year (Fig. 3). House sparrows (Passer domesticus) nested within the lower portions of the heron and cormorant nests. They primarily used nests built by herons. Cormorant nests, unless built on top of old heron nests, were usually too compact to admit them. Several of these nest associations occurred annually throughout the colony. California gulls (Larus californicus) and ring-billed gulls (L. delawarensis), nested on the ground on most of the islands with populations increasing from about 200 gulls in the early years of the colony to approximately 3000 nests by 1989.

## Nests of Great Blue Heron and Double-Crested Cormorants

Nests constructed by herons and cormorants were readily distinguishable, at least in their first season, with the herons laying a loose platform of sticks, while the cormorants constructed a securely

woven nest with a more compact bowllike appearance (Fig. 4). Primary nesting material for both species were twigs from the black cottonwood nest trees, and while the herons added little else, the cormorants incorporated a variety of items such as greenery, gull feathers, bird wings and other oddities including the remains of one flattened and desiccated gull chick into their nests. Lewis (1929) also describes similar inclusions in cormorant nests. Nests of both species varied greatly in size with those nests which survived from season to season becoming much larger. Newly constructed cormorant nests also had some size variation with young birds building noticeably smaller nests than more mature birds. Most nests of the year were approximately 40 cm. in diameter. Dimensions of a representative nest of the year were: outside diameter, 40.5 cm; inside diameter of the shallow bowl, 27.9 cm; height of the nest mass, 40.6 cm; depth of the bowl, 7.6 cm. In 1978 one pair



Figure 4. Nests of the two species were readily distinguished. Herons laid a loose platform of sticks while cormorants constructed a securely woven compact bowl.

with the female in immature plumage fledged one chick from a nest only 17 cm across with little bowl indentation.

Both species reused old nests in subsequent seasons and nest platforms were utilized by each without regard for the species which originally constructed them. Additional new nests were constructed by both species and nests were abandoned and usurped by both species. Nest ownership changes between conspecifics were not detectable unless the actual interaction was observed.

Herons arrived on the nest site earlier in spring than cormorants and usually did not take over cormorant nests because they were often paired and well established before the cormorants arrived. Cormorants occasionally displaced male herons who had not secured a mate and were still displaying into late April. In these cases, the cormorants occupied the nest while the herons fed and then drove him off when he returned. No nests of either species were observed to change ownership after pairing except in the case of immature plumaged cormorants which often lacked nest attentiveness. On three occasions occurring in 1979 and 1980 cormorants took over nests previously occupied earlier in the same season by Canada geese, after their broods had fledged.

Cormorants habitually demolished unattended nests, both those of herons and of conspecifics by pillaging them for nest materials. They were able to carry away an entire nest in a few hours. This behavior has also been reported by Lewis (1929) and Mitchell (1977). Previous nest occupants usually rebuilt at the same location within a few days.

Nest abandonments appeared to be due to failure to secure a mate, loss of the clutch, or to reduced nesting readiness of young birds. In 1978, four of the five nests abandoned had been occupied by pairs with at least one bird in immature plumage.

#### Stratification

Mitchell (1977) and L. Thompson (1981) reported marked stratification in tree-nesting associations between herons and cormorants. Herons have been reported to favor and compete for nests in the tops of trees (Owen 1960, Henny and Bethers 1971, Ives 1972, Wilburn 1972, Burger et al, 1977 and Parker, MS Thesis 1977). Some stratification of nests seemed apparent in the first few seasons of this mixed colony with the cormorants nesting throughout the rookery but also building many nests in the smaller peripheral branches. After continuous monitoring for several seasons, however, this stratification was less apparent. Cormorants were able to construct their more securely woven nests in smaller forks and branches but in subsequent seasons these platforms were frequently taken over by herons (Fig. 3). When tree diagrams for the entire 18 years were lined out, selected two or three-year periods showed some apparent stratification but this did not hold up over the long term (Fig. 5).

Similarly, preference between peripheral branches or core locations near the trunk were plotted. There were no noticeable differences between the two species in the selection of these locations (Fig. 4). One major factor breaking down stratification was the widespread re-use of both species nests by both species in subsequent seasons.

Although cormorants sometimes clustered near conspecifics they also nested randomly throughout the colony and in close proximity to herons. Nest concentrations were not always situated in the same locations in subsequent seasons. Species composition shifted throughout the colony with nesting in any given tree or on any given island varying greatly from season to season (Figs. 3 & 6). In addition to this mobility in nest site selection there was a great deal of interaction between the three island groups, especially during the courtship period and with regard to foraging flights to other feeding sites.

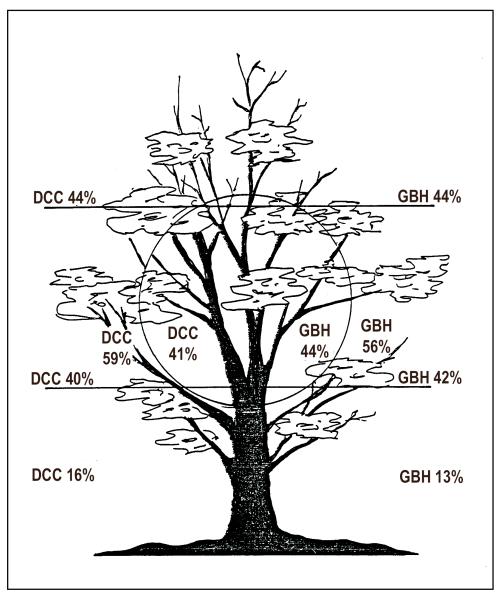


Figure 5. Nest stratification was plotted for high, middle and low levels and for core versus peripheral branches (NNWR). Percentages of nesting in each of these areas are shown for cormorants (DCC) and herons (GBH).

## **Nesting Chronology**

Great blue herons arrived at NNWR, usually in March, and before the arrival of the cormorants. Males took up old nests or started new ones and displayed for mates. Pairing was usually complete by the middle of April when the cormorants arrived. Cormorant arrival depended greatly on the weather, and if it was a very cold spring they straggled in slowly. The earliest arrival recorded was a single bird on 19 March, with no other birds present for over two more weeks. In some seasons they all seemed to arrive at once.

Upon arrival, cormorant males selected from the remaining old nests of either species or initiated new nests by laying a few sticks in a tree fork and displayed there for a mate. As more birds arrived, displays became more active. Wing-waving, the male

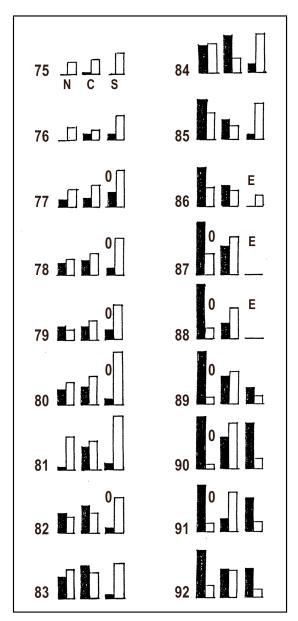


Figure 6. Nest distribution for cormorants (black) and herons (white) are shown by island group, north, central and south, showing how nesting concentrations varied and moved from year to year (NNWR). Owl (O) and eagle (E) nests are noted on island groups for years nesting occurred by these species.

advertising display, was directed at any cormorant passing overhead as described by Van Tets (1965) and also to birds beside or below the branch. Males continued to point their beaks at the sky in the standard display but sometimes turned the body orientation to face the direction of an interested female. Females thus attracted were at first repelled, but were gradually accepted.

The final establishment of a pair bond took from a few hours to several days. Some males displayed on bare branches at more visible sites and then led the female down the branch to an existing nest or a more secure nest site. Cormorant pairing was usually complete by late May with incubation of approximately 28 days taking place during May and June. Incubation was reported to begin with the third egg of a clutch for this species by Mendall (1936a) and Palmer (1962). In the Ninepipe colony, there was a noticeable size differential between the young after the first two chicks hatched which as of the end of this study (1992) has not been reported in the literature.

The onset of nesting varied within the colony by as much as a month or more with no apparent nest synchrony. Nest initiation at this site appears to be primarily correlated to the arrival times of individual birds and their pair bonding time. This also varied greatly between pairs, with one pair in 1980 establishing a pair bond within hours and beginning nesting activities within two days of their April arrival. It is possible these birds were pair bonded before their arrival at NNWR.

Nestlings fledged on their own initiative at approximately seven to eight weeks of age. The larger voung of a given nest left first and smaller nest mates often waited several days to as much as a week before departing. Fledging ages in this study do not differ noticeably from the projections of Lewis (1929) for cormorants fledging from tree nests but present a sizable variance from the nest departure age of four weeks reported by Mitchell (1977). Premature nest exits may have resulted from the frequent nest entry essential to Mitchell's research.

Young reared in terrestrial colonies leave the nest at about three to four weeks of age and move about on their natal island, returning to the nest to be fed (Lewis 1928, Mendall 1936a, and Palmer 1962). It was previously thought that once fledged from tree or cliff nests, young did not return to the nest (Lewis 1929, Mendall 1936a, and Mitchell 1977). The undisturbed young of the Ninepipe colony did not leave the nest until physically capable of flight. They then sailed or fluttered down to the water. After perfecting their flying abilities, usually in about two to four days, they returned to their tree nests where they were fed supplementally in or near the nest until about nine weeks old. This corresponds to the supplemental feeding chronology in ground colonies (Lewis 1929, Mendall 1936a and Palmer 1962).

I never observed parental feeding at any location other than in or near the nest. In one instance parents totally ignored the food cries of their young from a dislodged nest on the ground situated directly below its former position. These parents returned repeatedly to the former nest site searching and vocalizing for their young which called in vain from no more than four meters below.

Young forced from the nest at early ages remained beneath the trees, avoided the water and usually starved. Hand-reared birds swam well at three weeks of age but left the water at the first opportunity. They did not dive, except to escape, before they were five and one-half to six weeks old and well after their external nares were closed. After this age, they dove readily and pursued fish, swimming under water with the aid of a slight cupping action of the wings. It is unlikely that birds under this age, or with open nares could forage efficiently and survive.

Fledglings spent a large portion of their day swimming and developing foraging skills, within groups of birds of similar age, in waters near the nest site. Age-related foraging efficiency and learning has been addressed in the literature (Morrison, et al. 1978, Bildstein 1983, Erwin 1983). Young often foraged as a group and while adult cormorants of this colony have never been observed to engage in communal fishing activities, this method of foraging has been reported for the species (Taverner 1926, Bartholomew 1942, Palmer 1962).

By nine weeks of age, young flew well enough to follow adult birds to feeding sites up to 24 km. distant. After this dispersal only a few birds fed at Ninepipe, though a number of them continued to roost for the night on flat islands far out in the reservoir. Birds in juvenile plumage often foraged near each other and ranged out to other areas in the valley and were probably responsible for the initiation of new feeding areas. Color-marked siblings did not maintain any feeding associations with each other and often foraged at totally different sites.

Fall migration depended greatly on the weather with the birds remaining only until heavy frosts occurred at night. Migration dates ranged from mid-October to early December. The last birds remaining in the area were usually in juvenile plumage. Combined heron and cormorant phenology for the period of the study is shown in Figure 7.

#### **Nest Success**

Herons displayed huge fluctuations in nest numbers while cormorants had a rather steady increase in nests until later years (Fig. 8). Overall, nest success could be directly correlated to weather conditions, such as a cold rainy spring, severe wind storms or natural disasters (Fig. 9). The two species were not necessarily impacted by the same events. Heron young were most affected by late cold, wet spring conditions. The cormorant nesting cycle was usually late enough to preclude any impact by these early storms. Nest destruction in strong wind storms caused the highest cormorant chick mortality, with young literally being tossed from the nest on occasion. Storm loss was also observed by Burger (1982), and by Meier (Pierson 1984). The eruption of Mt. St. Helens in 1980 affected both species. Heron young which were just old enough to not require brooding suffered greatly as the ash fall covered their faces and plastered their eyes shut. Also herons, which forage

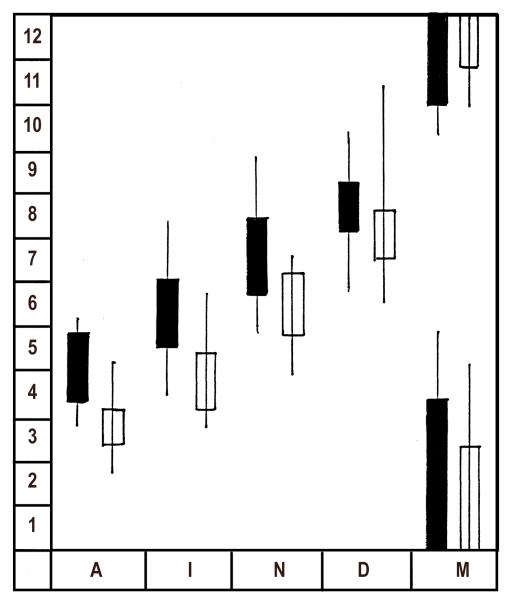


Figure 7. Phenology graph showing average time spans and extremes for arrival (A); incubation (I); nest life (N); dispersal (D), and migration and wintering periods (M) for cormorants (black) and herons (white) (NNWR).

visually, had a hard time locating enough food for the young along ash-clogged shores. Cormorants were in the incubation stage during the May eruption and fared better but suffered in foraging efficiency in the clouded waters.

After young were visible in the nest, usually about two to three weeks of age, they were subject to very little mortality other than accidents. After nest destruction, renesting did occur in some cases with birds incubating into late August on two occasions.

When banding first started in 1978, both ectoparasites and endoparasites were minimal on nestlings. However, juvenile birds recovered in the fall of 1980 contained large quantities of nematodes. Effects of these endoparasites on nestlings have not been pursued.

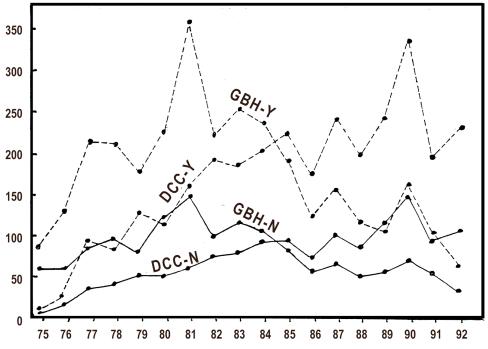


Figure 8. Population trends for herons and cormorants showing nests for cormorants (DCC-N) and herons (GBH-N) and young (DCC-Y and GBH-Y).

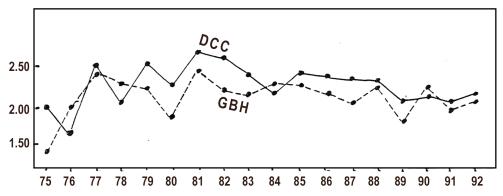


Figure 9. Success per nest fluctuations were greatly affected by natural disasters during nest life (NNWR). Eruption of Mt. St. Helens in 1980 affected both species. Herons were most affected by wet, cold spring weather and cormorant low seasons were the result of strong wind storms.

Eggshell scraps collected showed no excessive thinning from pesticides in this colony, but the state of pollution in waters at all foraging sites was not explored. Studies have indicated high chlorinated insecticide levels in fish from at least one of the major foraging sites (Plummer 1984).

#### **Avian Predators**

Cormorant response to avian predators varied with different predators. Gull predation was the chief cause of mortality in young nestlings during the early stages of the colony. A contributing factor to this heavy predation was the reduced attentiveness among the first-time nesters in immature plumage. Ellison and Cleary (1978) also found heavier gull predation at nests of inexperienced cormorants. Predation by gulls was greater in nests on peripheral branches and near the tops of the nest trees. Siegel-Causey and Hunt (1981) also found that the more accessible nests in a colony were the least successfully defended while McNeil and Leger (1987) found similar differences with success of nests positioned centrally or peripherally.

Cormorants responded to the increasing gull populations with more attentiveness but they did not abandon islands with large gull populations in spite of a constant threat of predation. They protected young until they were large enough to defend themselves, about three or four weeks of age, when the chicks open mouthed threat display was sufficient to repel most gulls. Mendall (1936a) also reports nest defense behavior in young cormorants. Parents with young defended nests more aggressively than did cormorants with only eggs in the nest. Ellison and Cleary (1978) noted this pre and post-hatching defense relationship as well.

Cormorants abandoned a number of nests on one island when great horned owls established in the colony with a late season nest. However, in the subsequent eight seasons, the owls nested in the colony and were ignored. Herons and cormorants made no effort to avoid them, frequently nesting on the same branch less than a meter away (Fig. 5). One known instance of owl predation was observed, and that was by young owls in the act of fledging. Two owlets, in their efforts to leave the nest, accidentally landed in a heron nest in a nearby tree where they promptly consumed the three small occupants. By the next day they had made their way to the mainland and no other nestlings were missing to indicate any further predation.

Owl pellets contained only the remains of *Peromyscus, Microtus* and *Phasianus* and provided no evidence that owl predation had involved any avian species of the colony. Suspected owl predation on egrets has been reported (Callahan and Carey 1979) and Meier found owl predation on cormorants in Wisconsin colonies (Pierson 1984). Owl predation on cormorant chicks was not a factor in the Ninepipe colony; however, young owls fledged and left the area before cormorant young had hatched during most seasons.

Eagles nesting in the colony were avoided and their presence caused the abandonment of an entire island by both species, even though no predation was ever observed by eagles or their young. When the eagles began nesting on the south islands in 1986 no new nests of either herons or cormorants were established on that whole island complex. Heron nests already occupied were not abandoned and raised a normal number of young. However, cormorants abandoned the island that season and no heron or cormorant nests were established on this island until after 1988 (Figs. 3 & 6). Both species returned to the island only after the eagles no longer nested there. Such desertion due to eagles has also been reported in a Wisconsin colony (Pierson 1984)

#### **Foraging Sites**

The expansion of cormorant foraging sites paralleled the increases in colony size, with some unexpected partitioning of use by age groups developing as the colony grew. In the initial stages of colony development, from 1974 through 1976, the birds fed exclusively in the vicinity of the heronry islands, and in the shallows on the north and south margins of NNWR. By 1977, they began ranging out to Kicking Horse and Mission Reservoirs and other small lakes in the vicinity even though the colony size, as yet, did not put any apparent pressure on the local food supply. In 1978, birds began feeding at PNWR and Crow Reservoir as well as on some additional small lakes and ponds and on the Flathead River and Crow Creek. The expansion continued each year to more areas of the lower Flathead River and north as far as Polson Bay on Flathead Lake. Birds now range up to 35 km. or more from the colony site (Fig. 10).

During the colony's formative years few non-breeders stayed near the colony

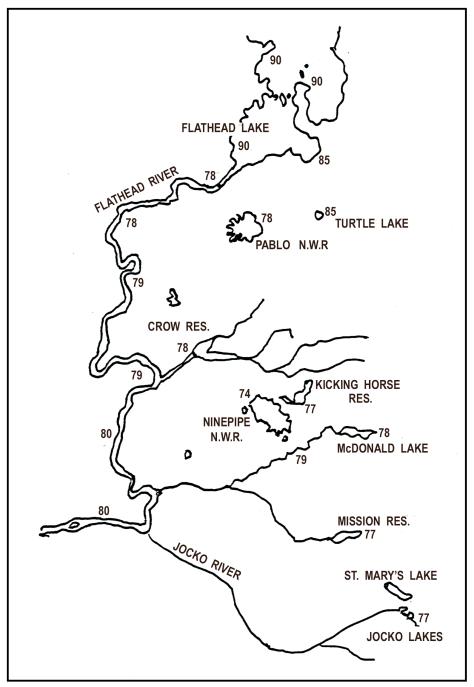


Figure 10. Foraging sites expanded with the expanding colony. This map shows the major feeding sites and the years cormorants began using them in western Montana.

islands and roosted together in one of the trees at night. By 1979 the number of nonbreeders increased and they began to stay at PNWR all night, which also became the primary feeding site for the breeding birds as well. After 1979, the adult birds foraged at Ninepipe only during the courtship and pairing period. Even after destruction of their dominant prey fish at Pablo through reservoir draw-down the adult cormorants did not return to fish Ninepipe heavily but moved to the river as a primary feeding site until the fish population recovered at PNWR.

#### **Prey Species**

Analysis of cormorant castings did not provide quantitative data on food consumption due to the irregularity with which castings are regurgitated (Williams 1977) as well as their loss in the water or to gulls (Jordan 1959, Ainley et al. 1981). However, castings did provide fairly accurate information on fish species taken (Williams 1977, Ainley et al 1981). Young birds do not eject castings until they fledge (Lewis 1929, Williams 1977). Remains of prey species fed to the young were readily obtained from regurgitated meals. These also were not effective for quantitative analysis since regurgitation occurs only when they are disturbed.

Analysis of castings of adult cormorants, regurgitated meals of the young, stomach contents of salvage birds, and field observations at foraging sites have determined that yellow perch (Perca flavicens), black bullhead (Ictalurus melas) and mountain whitefish (Prosoplum williamsoni) were the primary prey species for this colony. Some other fish species such as suckers (Catostomus spp.) and northern pike minnow (Ptychocheilus spp.) were taken in small quantities but no indication of any trout or salmon species (Oncorhynchus spp., or Salmo spp.) were found in their diets. This may relate to fishing methods with the species selected being associated primarily with bottom feeding as is employed by cormorants (Taverner 1915, Ainley et al. 1981), or to the ease of capture of these slower moving fish.

Hand-reared young displayed a distinct preference for firm-bodied rough fish over trout to the point of spitting out the trout unless they were very hungry. Similar prey preference was also reported by Lewis (1929) and selection for prey fish without commercial value has been indicated in other research (Taverner 1915, Gabrielson and Jewett 1940, Vermeer 1970, Crehore 1979, Roney 1979). Primary commercial fish and the preferred fish of anglers in this area are trout species. A survey of area trout hatcheries revealed active predation by herons, but none by cormorants. In fact, most hatcheries were not even aware that cormorants nested in the valley. One of the reintroduced hand-reared birds did feed at a late fall salmon run in the valley as a two year-old. However, I attribute this to behavior modification due to the food studies performed and to a developed tendency toward opportunistic feeding and the use of easily obtained prey.

Perch were the dominant prey species fed to young in the Ninepipe Colony. These were brought to small young as a regurgitated partially predigested mass and later in small sized fish remarkably suitable to the size of the nestling. This selective prey size feeding of young has also been reported for two tern species (Baltz et al. 1979), and prey selectivity in foraging has been addressed for several waterbird species (Ogden et al. 1976, Erwin 1983). By the time young fledged, they were consuming individual fish weighing up to 170 to 220 grams. Based on nestling intake studies by Dunn (1975) which show nestling intake at 1.14 kcal/g per day and on my own food studies with hand-reared young, which consumed approximately 450 to 475 gr. per day, the Ninepipe colony at the peak of nesting demand would require from 140 to 170 kg of fish biomass per day.

#### **Effects on Vegetation**

Cormorants are reported to have adverse effects on the trees in which they nest (Lewis 1929). This was explored in the Ninepipe colony with comparison photographs made before and after a period of six nesting seasons. Herons had occupied the colony trees for six seasons before the first photographs were made and before any heavy cormorant use had occurred. At this time colony trees were all in good condition except for four trees which showed some slight deterioration due to standing in water. These were excluded from the analysis.

Photographs made at the end of the six-year period showed remarkable correlation between tree use by cormorants and tree mortality. Trees most heavily used by cormorants (NI, CI, C3 and SI) showed the greatest degree of deterioration and some were totally dead, while control trees in group S3 on islands not yet used by cormorants continued to be lush and green. The most dramatic indication of the cormorant impact was evidenced in tree group N3 in which only one cormorant nest occurred during the test period. This tree group also had several heron nests but showed no change during the six-year period other than normal growth except in the area directly below the single cormorant nest. This tree group showed marked cormorant impact in the area of the single nest after just three years of occupancy. Some cumulative effects of both herons and cormorants over the 18 year life of the heron rookery must be acknowledged. However, trees with primarily herons and few cormorants showed far less deterioration than did trees used exclusively by cormorants.

## **Possible Origins**

Two plumage types were present in the Ninepipe colony as evidenced by the nuptial plumes which are worn during the breeding season. Birds here displayed both the short, uniform length, black, curled plumes described for (P. a. auritus) as well as the long, flowing, white streaked plumes of the (P. a. albociliatus) subspecies (Taverner 1926, Palmer 1962) (Fig. 11). Some tarsal measurements in the Ninepipe colony exceeded those given for (P. a auritus) but fell within the range of (*P. a. albociliatus*) (Palmer 1962). The Ninepipe Colony lies midway between the plotted ranges of these two subspecies and initial band returns seem to indicate the possible use of the winter ranges of both subspecies. (Fig. 12) (Palmer, 1962).

## DISCUSSION

The first cormorant activity in the development of the Ninepipe Colony was the initial scouting of the area. These birds foraged at the site for two seasons before

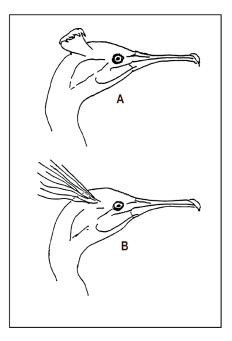


Figure 11. Nuptial plumes represented are of the two types present in the NNWR colony. Plumes shown are common to *Phalacrocorax auritus auritus* (A) and *Phalacrocorax auritus albociliatus* (B). Tarsus measurements in the colony also fall within the ranges of both subspecies.

establishing serious nesting, giving them ample opportunity to determine the quantity and quality of the fishery. Most of these first colonizing birds and several of those nesting in subsequent early seasons wore immature plumage. This follows the assertions of Lack (1954, 1966) that experienced breeders tend to breed where they did before, and dispersion is probably brought about mainly by individuals breeding for the first time. Since the Ninepipe colony developed gradually from a single originating nest it is apparent that this colonization was not the result of a mass desertion of an entire colony from some other location (Fig. 3).. So it is logical to assume that it was the avoidance by young birds of crowded areas and the inevitable competition there with experienced birds. The lack of parasites which was noted in nestlings early in the colony life could also denote a possible reason for the beginning of this and other nesting colonies or for the movement of

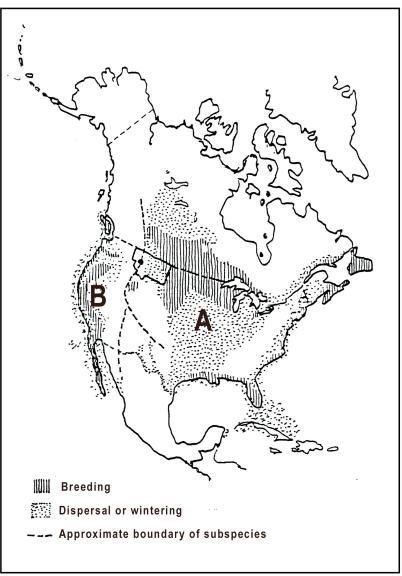


Figure 12. Ranges of two subspecies prior to the Ninepipe colonization for *P. a. auritus* (A) and *P. a. albociliatus* (B) are shown. The Ninepipe colony lies on this median between ranges.

nesting sites within the colony. King and his associates (1978) found ticks to be a factor in nest desertion in pelicans. The use of green plant material in birds nests has been indicated as an ectoparasite avoidance measure (Wimberger 1984).

In the initial stages there was rapid growth in the Ninepipe colony (Fig. 14) which required an influx of immigrant birds of breeding age and could not have derived solely from natural growth via birds reared within the colony for two reasons. First, the double-crested cormorant first breeds at two to three years of age (Lewis 1929, Mendall 1936b, Palmer 1962) and second, the number of young reared in the colony was insufficient to support the initial growth rate. In 1980, the colony's growth slowed which could have resulted from the return of maturing birds raised in the colony. The fact that birds banded as nestlings did return to nest within their natal colony implies some degree of site fidelity. Further, the short amount of time needed for establishing a pair bond between some early arrivals may indicate that these birds were together in previous years. Both of these concepts need further study.

The forces in operation in the initiation and development of the colony indicate an ability for rapid recruitment in this species following a decline or in the formation of a new colony. The successful nesting of young birds, the ability of most birds in the colony to nest, and the immigration of additional birds to support natural colony growth, all signify such rapid recruitment potential. Larger clutch sizes, which is the other common requisite for rapid population growth (Lack 1966), can only be hypothesized in the Ninepipe colony since clutch size data were not taken to avoid disturbance. However, nest successes of 2.5 to 2.6 young per nest in several of the early years are considerably higher than the 1.1 to 2.2 reported in the literature (Hickey 1952, Ellison and Cleary 1978). Some nests in protected sites produced as many as four or five young. This higher success could also be attributed to other factors including the survival of smaller sibling nestlings of a clutch due to undepleted food resources in the new colony and to the undisturbed nature of the colony.

Nest successes greater than 2.2 young per nest have been the trend in the colony with poor seasons usually having some adverse climatic condition or other natural occurrence during the nestling period, such as the eruption of Mt. St. Helens which layered the entire area with ash fall. Waterfowl production on the refuge was also down 40% that year. In the absence of egg data one must also consider some negative forces on success in operation in early stages of the colony such as the higher degree of gull predation and that young birds might lay comparatively smaller clutches.

The origins of the colonizing birds at NNWR was not been fully determined. Banding was terminated due to the deteriorating condition of the nesting trees which precluded putting out enough banded birds to assure this determination. Initial banding results have indicated, and plumage characteristics and measurements imply that colonizing birds most probably were derived from populations of two different subspecies. Banding returns and the location of high mountain ranges suggest that the migration routes from this colony probably follow water courses through southeastern Idaho. Birds nesting or passing through that area have wintered on both the Pacific and Gulf coasts.

Recoveries of cormorants banded in nearby Alberta and Saskatchewan have all been from the Gulf of Mexico (Houston 1971, Brechtel, pers. comm.). These colonies, however, are situated on the eastern side of the Continental Divide and appear to be primarily (P. auritus) plumage (Brechtel pers. comm.). Birds banded in the present study have been recovered at Rockport, Texas on the Gulf Coast and at American Falls Reservoir in S. E. Idaho. while birds banded at Minidoka Reservoir near American Falls have been recovered on the Pacific Coast (Balph 1981, Trost pers. comm.) (Fig.13). One bird banded in Idaho was also recovered in Arizona (Finholt, pers. comm.). Birds nesting or passing through S. E. Idaho have wintered in the territories of both subspecies. The nuptial plumage of these two subspecies and a gradation between has also been reported for another intermountain colony at Great Salt Lake (Behle 1936).

Other double-crested cormorant colonies in the northern Rocky Mountain region prior to 1975 from which the Ninepine colonization could have occurred are shown in Figure 14. Both tree and ground nesting colonies are represented. Data on colony sites, in addition to personal observations, are from the following: for Montana: Skaar (1975 and 1980), and L. Thompson (unpub. survey); Idaho: Gabrielson and Jewett (1940), Trost (pers. comm.) and Findholt (pers. comm.), Wyoming: Skinner (1929), Findholt (1988); Utah: Behle (1936) and Knopf (1981). It is

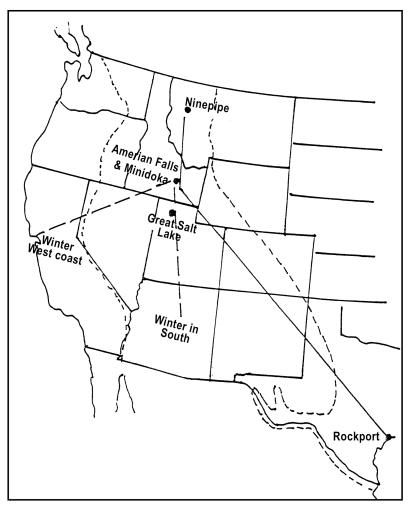


Figure 13. Band recoveries from the Ninepipe colony were from Rockport, Texas on the Gulf coast and from American Falls and Minidoka reservoirs in southeastern Idaho. Some birds from southeastern Idaho are known to winter on the Pacific Coast.

of note, that Figure 14 includes a number of nesting sites which have developed in this intermountain region during the 1970's in areas shown by Palmer in 1962 as dispersal areas (Fig. 12).

The site selected for this colony appears to fulfill all colony requirements for food resources, security and nest sites. There was an ample fish supply at the colony site to provide for fledglings and for older birds during the nest initiation period. Other food resources were available within a flying distance of up to 35 km. In addition, there were secure sites with adequate nesting substrate on islands that were virtually undisturbed during the nesting period. Nest site selection within the colony favored the more open and in some cases dying trees over those with full heavy foliage. This has been true of both herons and cormorants. It is my hypothesis that this derived from both the greater ease with which these more open trees were accessible to birds with large wing spans as well as the need of both species to be visible during their courtship displays.

Stratification as reported by other researchers appears to be offset in the

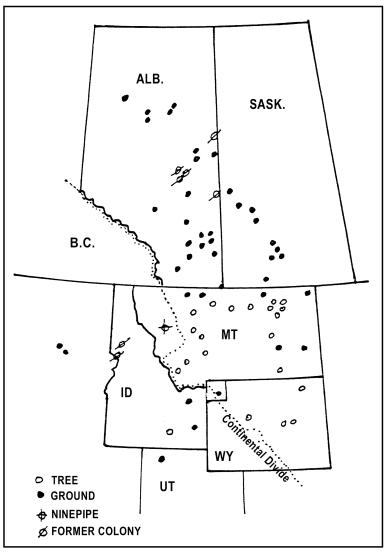


Figure 14. Cormorant colonies in the Intermountain West prior to 1974 from which colonization could have occurred (NNWR, MT). Tree colonies are shown in white, ground colonies in black.

Ninepipe colony by the re-use of both species nests by both species. The cormorants have the ability to construct their well-woven nests in smaller peripheral branches, but herons frequently nest in these the following season. Viewed in any given set of years, some degree of stratification might be visualized. But when considering the overall nest distribution over the 18 years of the study, this stratification does not hold up (Fig. 3). Nest concentrations not only varied within trees but shifted from island to island. Lundquist (1932) also found these cormorants to shift nesting from one island to another.

In 1978, the majority of cormorant nests were high in the trees and on peripheral branches, an obvious stratification which was the direct opposite of that found by Thompson (1981). However, in subsequent years cormorant nests were predominantly low in trees, clustered near the trunks, or scattered about at random. Viewed in any one or two-year period this colony could have presented an appearance of well defined stratification. I feel that the appearance of stratification was more a consequence of the tendency of cormorants to cluster near conspecifics. Islands occupied first in the beginning of any given season tended to be the ones containing the most cormorant nests that year.

The foraging site partitioning practiced in the Ninepipe colony which reserved the feeding areas immediately adjacent to the nesting site for the newly fledged young has obvious advantages (Rodgers, 1979). It provided the young with a food source available to them before they were mobile enough to travel any distance. It assured them of a sizeable mass of fish that increased their chances of prey capture and compensated for their undeveloped fishing skills. It eliminated their need to compete with experienced adults while foraging and young birds often continued to forage separately from adults after dispersal.

This reservation of food supply in the vicinity of the nesting area for the inexperienced newly fledged birds has not previously been described for doublecrested cormorants, although it has been indicated that they often feed at substantial distances away from the colony (Mendall 1936a). Distant foraging by adults to prevent depletion of resources essential to fledglings and to avoid competition with them, has been reported for ibises and herons (Rodgers 1979).

At NNWR two other factors increased the level of successful prey capture by young cormorants. This reservoir is used for irrigation and experiences a sizable draw down by mid-summer leaving fish more concentrated in smaller, shallower pools and consequently more available for young birds who are communally foraging. Several authors have shown that ecological density of prey increases as water levels decline (Kushlan et al. 1975), and that this food availability even triggers the initiation of reproduction in some species (Clark 1989, Powell 1983). The nesting asynchrony in the study colony may also have created greater foraging distances and reduced pressure in the natal area at fledging time.

The reservation of resources for fledglings does not totally explain the long distances traveled by some adults to forage even while they were feeding nestlings, especially since other apparently adequate fishing grounds were closer. One possible reason for this could be their requirements for prey size selection appropriate to nestling size. Knopf and Kennedy (1981) state that cormorants cannot fly efficiently to outlying wetlands to forage. The energetics of these long flights in the study colony may have been improved when conditions permitted, by the use of soaring. I have observed two instances of cormorants soaring on thermals near cumulus clouds. In one case eleven birds rose to such a height that they could no longer be observed with the naked eye. Such soaring has not previously been reported for this species and was surprising considering their heavy wing loading.

The existing heron population has grown fairly steadily along with the cormorant colony without being adversely affected. However, the effects of cormorant excrement on the nesting trees have been far more deleterious than one would have anticipated in 1974, when the cormorant colony first began. This eventually will affect both herons and cormorants as the nesting substrate is depleted. The initiation of a satellite colony by both species at PNWR in 1985 may be a response to such loss of substrate. Lewis (1929) stated that cormorant fecal material was very caustic and damaged trees. There is also the possibility that as this very liquid fecal material thoroughly coated the leaves that transpiration and photosynthesis were impaired or prevented.

While Lewis (1929) reported both tree and ground-nesting on the same island, there is no evidence in the literature to indicate that, once the trees are gone, cormorants will nest on the ground. Lewis (1929) does report one colony on St. Genevieve Island which moved into trees as a result of severe persecution. Colonies in the central plains have been deserted after the trees were gone (Crehore 1979, Robbins pers. comm.) and the Wisconsin DNR has built artificial trees and platforms at several sites to retain the cormorant population after tree destruction (Crehore 1979, Pierson 1984). There have been no studies to date to determine if this species is imprinted to nest type and whether a bird reared in a ground nest will ever nest in a tree or vice versa. This requires a great deal more study and a large number of banded and permanently identified individuals.

There is some discussion in the literature on which type of nidification, terrestrial or arboreal, is the original form. Lewis (1929) felt the ground or cliff nesting was primary, while Van Tets (1965) considered that all of the *Pelecaniformes* derived from tree nesting ancestors. It is my hypothesis that since this cormorant constructs such a fine, compactly woven and quite securely anchored tree nest that it is unlikely to have very recently evolved from ground nesting stock.

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# FECAL VS. RUMEN CONTENTS TO DETERMINE WHITE-TAILED DEER DIETS

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### Abstract

The purpose of this study was to evaluate and determine the feasibility of white-tailed deer (*Odocoileus virginianus*) diet composition of rumen versus fecal contents during winter months in the Black Hills of South Dakota. Sixty-two deer were shot over five winters (December – May). Major plant composition was approximately the same; however, juniper, pine, lichen, and *Astragalus* species showed significant differences between fecal and rumen comparisons. Spearman rank order correlation indicated ranks were in good agreement (r = 0.82, P = 0.01). Average Kulczynki's similarity was 75 percent overall and is considered very good for major forage species. Significant differences were observed for shrubs and lichen when considering all forage species. Overall, fecal analyses for major forage species will provide excellent information and help managers make more informed decisions with regard to habitat resources for deer.

Key words: diet composition, methods, microhistological, forage, plants

### INTRODUCTION

Examination of dietary forage species of herbivores has been accomplished primarily by using the micro-histological technique for many years with procedures developed by Sparks and Malechek (1968). However, few studies have made direct comparisons to better understand herbivore diets by using fecal and rumen contents from the same animal (Smith and Shandruk 1979). Comparisons between esophageal fistula (method to collect forage directly after consumed from esophagus) and fecal have been examined with good results (Vavra et al. 1978, Johnson and Pearson 1981). Smith and Shandruk (1979) reported fewer plant species were identified in fecal samples when compared to rumen contents for pronghorn (Antilocapara americana). These studies reported some differences between esophageal fistula and fecal botanical composition to determine diets of herbivores.

Knowledge of dietary food habitats of herbivores is important for resource managers to improve or sustain habitat conditions for the deer. Comparing whitetailed deer (*Odocoileus virginianus*) diets between fecal and rumen content from the same animals within a ponderosa pine forest in the Black Hills of South Dakota is important to improve our understanding of these methods. Micro-histological analyses of fecal samples is the most common method to determine diets of deer (Uresk and Dietz 2017). However, agreement (or not) of botanical composition between fecal and rumen samples has not been documented for the ponderosa pine (*Pinus ponderosa*) forest.

The purpose of this study was to evaluate white-tailed deer diets within a ponderosa pine forest. Specific objectives were to compare fecal with ruman samples from the same individual deer and determine the feasibility of fecal analyses for dietary composition for future and past diet studies.

### **STUDY AREA**

The Black Hills of South Dakota is approximately 5000 km<sup>2</sup> and ecompasses portions of Wyoming and South Dakota (USDA-Forest Service 1983). Elevation of the study areas ranged from 1600 m to 1800m. Precipitation during the study period was 51cm and 80% occurs from April to September (Dietz et al. 1980, Uresk and Dietz 2017). Annual precipition since the study is 53 cm (Uresk and Dietz 2017). The average growing season is 89 days and temperatures ranges from -6.2° C to 35° C.

This study was conducted in two areas, McVey Burn and Experimental Forest dominated by ponderosa pine and a detailed description of the study area is presented by Dietz et al. (1980) and Uresk and Dietz (2017). Common understory shrubs include kinnikinnick (Arctostaphylos uva-ursi), chokecherry (Prunus virginiana), creeping barberry (Mahonia repens), Saskatoon serviceberry (Amelanchier alnifolia) and common snowberry (Symphoricarpos albus). Graminoids include roughleaf ricegrass (Oryzopsis asperfolia), timber oatgrass (Danthonia intermedia), sedges (Carex spp), Kentucky bluegrass (Poa pratensis) and needle-and-thread (Stipa *comata*). Common forbs include cream pea (Lathyrus ochroleucous) and bluebell bellflower (Campanula rotundifolia). Habitat types and plant species throughout the Black Hills are described by Hoffman and Alexander (1987).

### **Methods**

A total of 62 white-tailed deer were shot in 1967, 1969, 1970, 1971 and 1972 (Uresk and Dietz 2017) during 5 winters (December-May). At the time of collection, both fecal and rumen samples were collected from each individual deer. Samples from each deer were kept frozen prior to examination. Individual samples were then thawed, dried, and ground through a Wiley mill fitted with a 1 mm screen. All material was washed over a 0.1-mm screen (Sparks and Malechek 1968). Fecal and rumen material was cleared of chlorophyll and other composites with Hertwig's solution. Microhistological examinations of the samples were examined with 5 slides per rumen and fecal samples. Twenty fields per slide were observed under a binocular

microscope at 100 power for identifiable plant fragments (Sparks and Malechek 1968, Rogers and Uresk 1974, Johnson et al. 1981). Hand compounded test mixtures of plants were used periodically to check accuracy of reading slides to determine plant identification and maintain quality control. A 90 percent similarity was maintained between actual test mixtures of plants and estimated values (Rogers and Uresk 1974).

Data from microhistological examination of fecal and rumen contents were reported as percentages of dietary density (Sparks and Malechek 1968). Comparisons between fecal and rumen contents were analyzed using a paired t-test and Spearman's rank order correlation  $(P \le 0.10)$  for all food items (SPSS 2003). Kulczynski's similarity index was determined for comparisons of dietary foods (Oosting 1956).

### RESULTS

Grasses were dominated by Poa spp and Stipa comata (Table 1). Poa spp were 4 percent and 6 percent in fecal and rumen, respectively, but were not different ( $P \leq$ 0.10). There were no differences among forage items between fecal (10%) and rumen contents (12%) for grasses and sedges (Table 2). Forbs were similar for the fecal and rumen category. Astragalus succulentus, a forb, in fecal and rumen contents was 5 percent and 2 percent, respectively (Table 1). All forbs in the fecal and rument forage contents were similar (Table 2). Shrubs were comparable in both fecal vs rumen contents except for Juniperus communis, that was greater ( $P \le 0.10$ ) in the rumen (1.6%) compared to the fecal (0.5%) (Table 1, Table 2). Shrubs were higher in fecal (69%) versus the rumen (60%) (Table 2,  $P \le 0.10$ ). Trees were similar in both fecal and rumen contents (Table 2). Pinus ponderosa, a tree, was estimated at 10 percent in the rumen compared to 4 percent in the fecal contents (Table 1,  $P \le 0.10$ )). Lichen was present in greater amounts within the rumen.

Similarity indices for major forage species ranged from 10 percent to 99 percent

Category and Species	Fecal Mean ± SE	Rumen Mean ± SE	Similarity <sup>1</sup> Mean
Carex spp.	1.3±0.5	1.8±0.6	84
Danthonia intermedia	0.4±0.2	1.1±0.4	53
Poa spp.	3.7±2.0	5.7±2.0	79
Stipa comata	3.7±1.9	3.6±2.4	99
Forbs			
Antenneria spp.	2.7±1.4	7.1±2.7	55
Astragalus succulentus	5.0±1.6	2.0±0.7*	57
Potentilla geum	2.8±0.7	2.7±0.6	98
Solidago spp.	3.2±1.4	2.4±0.8	86
Trifolium pretense	2.9±2.9	2.3±2.3	89
Shrubs			
Arctpstaphylos uva-ursi	12.2±2.7	11.9±3.8	99
Artemisia frigida	22.8±4.5	16.1±3.7	83
Berberis repens	1.9±1.6	1.3±1.0	81
Juniperus communis	0.5±0.4	1.6±1.2*	48
Rosa woodsii	1.4±0.7	1.9±1.3	85
Rubus pubescens	3.5±1.7	2.6±0.8	85
Salix spp.	18.1±2.6	15.6±2.5	93
Shepherdia canadensis	5.3±1.6	6.3±2.0	91
Trees			
Pinus ponderosa	3.9±0.8	9.6±2.6*	58
Lichen			
Lichen sp.	0.1±0.1	2.0±0.8*	10

Table 1. White-tailed deer diets (%± SE) comparing fecal vs rumen contents for 62 deer for major plant species or variable that were estimated at >1% of the dietary composition.

\* Significant different at  $p \le 0.10$ 

<sup>1</sup> Kulczynski's similarity index (%).

with an overall average of 75 percent (Table 1). Spearman's rank order correlation was r = 0.82 (P < 0.01) indicating that the rankings were alike between fecal and rumen contents.

### DISCUSSION

Comparisons between fecal and rumen contents from the same animals have received mixed results (Smith and Shandruk 1979). However, their pooled results for rumen and fecal contents with pronghorns

Category	Fecal	Rumen	Similarity <sup>1</sup>
	Mean ± SE	Mean ± SE	Mean
Grasses and Sedges	10.3 ± 1.3	12.4 ± 1.4	91
Forbs	16.8 ± 1.4	16.5 ± 1.5	99
Shrubs	68.7 ± 2.7	$59.6 \pm 2.5^*$	93
Trees	4.1 ± 0.8	10.0 ± 2.6	58
Lichen	0.1 ± 0.1	$2.0 \pm 0.8^{*}$	10

Table 2. White-tailed deer diets (%± SE) comparing fecal vs rumen contents for 62 deer by category for 49 food items.

\*Significantly different fecal vs rumen at  $P \leq 0.10$ .

<sup>1</sup>Kulczynski's similarity index (%).

were 85 percent similar. An evaluation between esophageal fistula and fecal material with cattle was 90 percent similar and highly correlated, r = 0.99 (Johnson and Pearson 1981). Wydeven and Dahlgren (1982) reported that fecal forage species from prairie dogs provide a reasonable estimate of dietary composition when compared to stomach contents.

In our study, *Juniperus communis* and *Pinus ponderosa* were differnet between fecal and rumen content with greater amounts in the rumen. Minson (1990) reported that coarse textured plant fragments spend more time in rumen than fine textured plant fragments. Although, Anthony and Smith (1974) obtained higher values for *Juniperus* spp in fecal than in rumen estimates. Similarly, Johnson and Pearson (1981) obtained greater values for *P. ponderosa* in fecal than in the rumen.

# CONCLUSIONS

Overall, white-tailed deer fecal analyses for major forage species items provided accurate information when compared to rumen contents to determine dietary composition. Relative densities of major species were approximately the same, although relative density estimates for Juniper and pine, were different ( $P \le 0.10$ ) between fecal versus rumen comparisons. Spearman's rank order correlation was very good between fecal and rumen contents, r= 0.82, indicating the ranks were in good agreement for food items. The average similarity index was 75 percent and is considered very good for the major forage species. Fecal analyses will provide resource managers the information required to improve or sustain adequate forage resources for deer.

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# MONTANA CHAPTER OF THE WILDLIFE SOCIETY

### 56<sup>ND</sup> ANNUAL CONFERENCE 2018

#### Telling Our Story - Lesson's Learned

February 19-23, 2018 Copper King Hotel & Conference Center Butte, Montana

Kelvin Johnson, President Elect 2017-18 Montana Chapter of The Wildlife Society

### Introduction

Our conference theme this year revolved around telling our story, as well as sharing our lessons learned. There are many success stories that have come to fruition large in part due to wildlife professionals effectively communicating our management experience and research knowledge with others. Conversely there are situations where our wildlife messages have been lost due to one reason or another. And in almost all cases, if we were to do it over, we'd choose to communicate something differently. Given the issues surrounding wildlife and wildlife conservation, it is becoming more important that we, collectively, are effective at communicating our wildlife knowledge and experiences with others. Whether we are bringing our information from the field to our managers or administrators, from agency to agency and other partners, to stakeholder groups, members of our public, or to our policy and law makers, we need to effectively tell our story.

Before the main conference began we had three workshops. One explored how to effectively promote and utilize conservation programs when working with private landowners; another provided a hands-on and in the field experience learning about one of the most powerful means to tell our story to the public through wildlife photography; and finally, an in-depth workshop was designed to help wildlife professionals find their voices when it comes to talking policy and conservation.

After the workshops, the conference began with a plenary session that focused on how wildlife conservation perspectives can be effectively communicated with others. To do this, we had 5 panelists that shared their experiences and ideas from their various perspectives, which included government agency administration, partnership coordination, popular journalism, stakeholder groups and law-making processes. By gaining additional insight into their perspectives, we aimed to better understand how effective communication can influence wildlife conservation within and beyond the boundaries of Montana.

We had 48 papers that were presented during concurrent sessions. There were 15 students presenting this year and we were fortunate enough to schedule their presentations so that no two students presented at the same time. There were 20 posters presented, with 10 of them presented by students.

At this year's banquet, our keynote speaker was Andrew Mckean, former editor-in-chief of Outdoor Life. He shared, from his perspective, how social science trends will change our jobs. The evening also included a tribute by John Weigand to one of the founding charter members of Montana TWS, Dr. Richard Mackie, who passed away January 6, 2018.

### Welcome to Butte – 1984 Redux

Terry N. Lonner - A "Butte Rat" and Wildlife Research Biologist - Retired, MFWP

The last time the annual meeting of the Montana Chapter of the Wildlife Society's annual meeting was held in Butte was in 1984. At this meeting a "Welcome to Butte" presentation began by T. Lonner assisted by his wife, Martha, using two slide projectors, a dissolve unit and a "Boom Box" for audio. At this 2018 meeting a very similar introduction was presented, but this time a state-of-the-art digital audio/video system was used to present his introduction with a short movie celebrating the immigration of 12 million people to America through Ellis Island. For many of them Butte was their major destination. Butte developed from a silver and gold mining camp of a few hundred people in the 1860s and by 1919 became the largest city between Minneapolis and Seattle with a population of 100,000 including 38 nationalities and 20,000 mine workers. During Butte's mining heydays there were about 10,000 miles of shafts, tunnels, drifts and stopes underneath its surface and some of them were a mile deep. In 1917 Butte was producing almost 50% of the nation's copper and about 25% of the world's copper. Because of poor working conditions the miners eventually formed the Butte Miner's Union, one of the richest and strongest unions in the United States. The Berkeley Pit was started in 1955 and operated until 1982. After the underground mines were shut down in 1983 and the water pumps were turned off, the Berkeley Pit began filling with water until it reached a current depth of about 900 feet. It has become one of the largest bodies of toxic water in the world and is now the focus of our Nation's largest Superfund site with white geese using it to their detriment. Montana Resources resumed open pit mining in 1985 with the Continental Pit. In spite of all this mining activity and environmental degradation, the citizens of Butte have been and still are very involved with helping to conserve Montana's wildlife. The Butte Gun Club began in 1885 and in the early 1900s Butte sportsmen's clubs were organized like the Butte Anglers Club and the Rocky Mountain Sportsmen's club. They were instrumental in helping fund the first elk transplant in 1910 to the Mt Fleecer area southwest of Butte. Jim McLucas, a Butte native and WWII Marine Veteran, became Montana Fish and Game Department's first trapper in 1946. He supervised the trapping and transplanting of over 16,000 big game animals throughout Montana and the west including Hawaii. The sportsmen of Butte reorganized in 1957 and formed the Skyline Sportsmen's Association. They continue to remain vigilant in monitoring Montana's wildlife management and conservation issues and activities.

Tap 'er Light!

#### AGENCY PERSPECTIVES

Martha Williams, Director, Montana Fish, Wildlife & Parks, Helena

Abstract not available at press time.

## DECREASING THE NOISE TO SIGNAL RATIO THROUGH BETTER COMMUNICATION AND PARTNERSHIPS

Carolyn Sime, Sage Grouse Habitat Conservation Program Manager, Montana DNRC, Helena, MT

Science is embedded throughout our lives. We fight over how science is used or misused in public policy decision making. In particular, group decision-making on natural resource issues is hard wired for conflict. Individuals have different values and desired outcomes, yet a decision has to be made by some public policy making body on behalf of the whole. Science informs the decisions, but narratives and facts compete. Conflicts are often perpetuated when narratives and facts are contradictory. This dynamic creates a very high noise to signal ratio for decision makers. Wildlife professionals can take a leadership role and increase their effectiveness by taking a more narrative approach to communicating scientific information to non-scientists rather than the way we are traditionally taught and communicate with each other. As natural resource challenges have become more complicated, collaboration and partnerships have become more integral to the finding durable solutions. Keys to successful partnerships include: a shared vision of desired outcomes; ownership in the outcomes; a common understanding of applicable science and facts; honest dialogue; and sustained commitments.

### CATCH AND RELEASE: HOW TO WORK WITH JOURNALISTS TO TELL YOUR WILDLIFE STORY

Brett French, Outdoor Editor, Billings Gazette, Billings, MT

Those odd subspecies of humans known as journalist can help wildlife biologists and agencies tell their unique story in a "hey Martha" way. But how do you deal with them? What do they want to know? And is it OK to dart one if they get out of hand? Veteran newspaper journalist Brett French will offer suggestions to help wildlife professionals catch a journalist's attention and release them to get your story across to the public or associates in a clear, concise and helpful manner. Montana residents care about what's happening with wildlife and wildlands. Communicating to that constituency in an interesting and visual way can help build trust, establish a rapport with the public and lead to support for initiatives or complex problem solving.

#### **BUILDING RELATIONSHIPS FROM THE GROUND UP**

Jay Bodner, Natural Resources Director, Montana Stockgrowers Association, Helena, MT

Montana, like many states, has taken an active role to be more effective in managing wildlife. With private land comprising 67 percent of the state and 80 percent of the habitat needed for species on the endangered species list, landowners play a critical role in wildlife management. Private landowners recognize they can be a valuable asset to wildlife professionals. Landowners have the unique ability to share the knowledge gained from living and working on the land; they have a unique understanding of wildlife and the conditions that affect them. The key to an increase in management effectiveness is developing a working relationship with landowners and communication. When positive interpersonal relationships are developed, trust is built. This trust results in an increased exchange of quality information and successful outcomes.

### Condensing the Science of Wildlife Management into Everyday Language

Nick Gevock, Conservation Director, Montana Wildlife Federation, Helena, MT

How do professional biologists effectively communicate to the public about wildlife? What do we as biologists do well, do poorly, and how can we improve? And what can we provide to wildlife conservation organizations to help them implement good wildlife policy? Wildlife biologists spend a lot of time talking to each other. But in order to convey wildlife science and management to the public, they need to learn how to condense the science of wildlife management into everyday language that hunters, anglers and wildlife enthusiasts understand. Those questions will be explored in this plenary session by Nick Gevock, conservation director for the Montana Wildlife Federation in Helena. MWF works during the Legislature and throughout the year to promote abundant wildlife and healthy habitat. Please join in this session to learn better ways to communicate our professional work.

# **PRESENTATION ABSTRACTS**

In Order of Presenting Author \* Denotes Presenter \*\*indicates student presentation

### SURVEILLANCE STRATEGY FOR DETECTING PSEUDOGYMNOASCUS DESTRUCTANS (PD) AND WHITE-NOSE SYNDROME IN MONTANA 2016-2017

Emily Almberg\*, Montana Fish Wildlife and Parks, Bozeman Dan Bachen, Montana Natural Heritage Program, Helena Lauri Hanauska-Brown, Montana Fish Wildlife and Parks, Helena

The devastating bat disease, White-Nose Syndrome (WNS), caused by the fungus Pseudogymnoascus destructans (Pd), was detected in western Washington state in March of 2016. This detection was 1,300 miles from the previous westernmost detection and highlighted the urgency for surveillance in other western states like Montana. Early detection of the disease may provide valuable insights into the statewide status of WNS, research opportunities, mitigation options and cave management. The goals of Montana's surveillance plan include 1) surveying for WNS/Pd in new geographic areas outside the WNS-affected zone and/or biologically important sites and 2) surveying for WNS infection in bat species that are not currently known to be susceptible. In the absence of information or a risk assessment to help Montana focus on priority surveillance areas other than winter hibernacula, the 2017 strategy focuses on sampling at six hibernacula representing all regions where aggregations of bats overwinter. Both active and passive sampling of bats and hibernacula environments will be conducted. Active sampling can detect Pd from swabs of bats or in hibernacula soils. Passive sampling will be conducted into the early summer specifically targeting bats found dead outside of hibernacula, bats showing clear signs of WNS infection, and bats found dead as part of a large mortality event. Bats submitted for rabies testing may also be sampled when circumstances or characteristics of the carcass indicate WNS may be the cause of mortality. While surveillance efforts can be costly it may provide information with enough time to better inform decision making.

### **CHRONIC WASTING DISEASE IN MONTANA**

Emily Almberg\*, Montana Fish, Wildlife, and Parks, Bozeman John Vore, Montana Fish, Wildlife, and Parks, Helena Jennifer Ramsey, Montana Fish, Wildlife, and Parks, Bozeman Zach Mills, Montana Fish, Wildlife, and Parks, Bozeman Keri Carson, Montana Fish, Wildlife, and Parks, Bozeman Justin Gude, Montana Fish, Wildlife, and Parks, Helena

In 2017, Montana Fish, Wildlife, and Parks (FWP) convened an internal working group and a Citizen's Advisory Panel to update the state's chronic wasting disease (CWD) surveillance and management response plans. As part of the plan, FWP initiated intensive CWD surveillance in deer and elk in southcentral Montana in the fall of 2017. Through this effort, we detected our first cases of CWD in wild mule deer and white-tailed deer south of Billings, Montana. We also happened to sample a hunter-harvested mule deer that was part of a mule deer movement study north of Chester, MT, near the Alberta border; this animal

turned out to be CWD-positive as well. Although disappointing, neither of these detections were surprising. We've known that CWD is likely to spread to these areas from infected populations in our neighboring states and provinces. With commission approval, FWP called special hunts in both areas to determine prevalence and distribution of CWD within the affected deer populations. Here we will report on the results from our general surveillance and special hunts and will discuss the options for future management of the disease.

### \*\*Analysis of Spiny Softshell Turtle Population Structures in Five River Systems in Eastern Montana

Gabriel H. Aponte\*, Rocky Mountain College, Billings, MT Kayhan Ostovar, Rocky Mountain College, Billings, MT Andrhea Massey, Rocky Mountain College, Billings, MT Ulrich Hoensch, Rocky Mountain College, Billings, MT

The spiny softshell turtle (Apalone spinifera) is designated as a species of concern in the state of Montana due to a lack of knowledge regarding their conservation status, loss of habitat connectivity and anthropogenic changes in hydrology. Information on population abundance and basic population structure for this species is necessary to understand how altered hydrological regimes and catastrophic events may affect this highly aquatic species. Spiny softshell turtles were studied in five river and creek systems in southeastern Montana. Over three years a total of 553 spiny softshell turtles were captured. The proportion of females to males captured across all five systems was not significantly different between creeks and rivers, with a total of 89.69% females and 10.31% males. We developed four length / age classes (juvenile, sub-adult, reproductive adult, mature adult) based on reported age cohorts from other studies. Numbers of spiny softshell turtles in each cohort were found to be significantly different between the five systems p < 0.00001. The Musselshell and Yellowstone Rivers and Pryor Creek had evidence of juvenile age classes and a more even distribution of age classes than the other systems. The Bighorn River had mostly larger adults and the Musselshell River lacked significant numbers of mature adults. Average seasonal water temperatures, timing and magnitude of spring pulse flows, ice cover and scour in winter, and abundance of open gravel bars, should be examined as possible factors which may explain the observed differences in spiny softshell turtle demographic structures on these five systems.

### \*\*Factors Influencing Migratory Behavior of Elk Across Montana

Kristin J. Barker\*, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula Michael S. Mitchell, USGS Montana Cooperative Wildlife Research Unit, University of Montana, Missoula

Kelly M. Proffitt, Montana Fish, Wildlife and Parks, Bozeman

Increasing numbers of ungulates that remain resident on winter range year-round rather than migrating to higher-elevation summer range pose a common management challenge in western North America due to issues of property damage and reduced public hunting opportunities. Although the effect of forage on migratory behavior of ungulates is fairly well-understood, relative influences of other factors that may drive individual variation in migratory behaviors remain less clear. We are investigating how forage variability, conspecific density, animal age, and human land use affect the likelihood that an adult female elk (*Cervus canadensis*) will migrate or exhibit intermediate behavior rather than remain resident on winter range. We assessed behavior of 312 elk in 16 herds across southwestern Montana

using radiocollar data collected during 2006-2016. Approximately 64% (n = 200) of elk migrated, travelling up to 110 km from their winter locations. Preliminary results suggest migrants tended to be older than residents or intermediates (19% of migrants  $\geq$ 10 yrs old vs. 6% and 4%, respectively; p = 0.003), to experience higher conspecific density during winter (F2,309 = 12.19, P < 0.001; p-adj. < 0.01 in both cases), and to be located in areas where forage varied more predictably across space and time (F2,309 = 28.94, P < 0.001; p-adj. < 0.001 in both cases). Identifying the factors that most strongly influence migratory behavior will help managers understand current patterns of behavior, predict how patterns may change in the future, and identify potential means of manipulating behaviors to meet management objectives.

### THE SEARCH FOR BLACK SWIFTS: A SPECIES OF CONCERN, INVENTORY NEED AND LEGEND

Lisa Bate\*, Glacier National Park, West Glacier Chris Hammond\*, Montana Fish, Wildlife and Parks, Kalispell Amy Seaman\*, Montana Audubon, Helena

Since 2004, when only three known nesting colonies had been identified in Montana, Black Swifts (*Cypseloides niger*) have been a state priority species for inventory and monitoring. They are an unusual neotropical migrant bird that initiates nesting in Montana when most other birds are completing their breeding seasons, with young not fledging until August or September. Black Swifts are reliant upon perennial streams, waterfalls, and insect prey for survival and are considered at high risk of extirpation, due to climate change. In 2015, Canadian biologists listed Black Swifts as endangered due to significant population declines. Since 2012, partnerships have coalesced around the search for this illusive and hard-to-detect bird, striving for standardized surveys that can shed light on the bird's presence and distribution in Montana. Over time, we have refined our protocol, training, and survey methods, and with these lessons learned, turned 2017 into one of our most successful survey seasons yet, more than doubling the known number of swift colonies in Montana. At the same time, however, we documented possible losses of colonies. As we continue with swift inventories, we are developing long-term monitoring plans, and exploring the use of new tools and models to help us better assess waterfalls for presence of Black Swifts.

### \*\*Lewis's Woodpecker Nest Success and Nest Site Selection in The Bitterroot Valley

William M. Blake\*, MPG Ranch, Missoula, MT Kate R. Stone, MPG Ranch, Missoula, MT Tom E. Martin, USGS Montana Cooperative Wildlife Research Unit, Univ. of Montana, Missoula

Identifying factors that drive habitat selection and how these factors influence reproductive success is important to species conservation, especially for promoting growth of populations in danger of extirpation. We studied Lewis's Woodpecker (*Melanerpes lewis*), a declining species in which information regarding reproductive success is limited. Lewis's Woodpeckers are found in high concentrations along cottonwood forests, yet prior research suggests they have low reproductive success in this habitat. We investigated the relationship between nest site selection and nest success by monitoring over 200 nests across two preferred habitat types: cottonwood floodplain forests, and burned coniferous forests. We also compared vegetation and local habitat characteristics to determine the drivers of nest site selection and their influence on nest success. Our results demonstrate that Lewis's Woodpeckers primarily selected tall nest trees, and high density of large mature trees. They particularly avoided dense canopy cover in the floodplain, and high density of small snags in the burned forest habitat. Despite this, we found limited evidence that vegetation characteristics influenced nest success. Overall, nest success was best explained by habitat type and hatch date, with nest success being highest in burned forest habitat and in individuals that initiated nests earlier in the season. Our data improves our understanding of the relationship between habitat selection and reproductive success in a species of conservation concern, and will provide land managers with information on the drivers of nest success in this species, as well as other species that share similar ecological niches.

### FROM KISHINENA TO POM: MONTANA'S WOLF RECOVERY STORY

Diane K. Boyd\*, Montana Fish, Wildlife & Parks, Kalispell

Wolves were extirpated from 95% of their historical range in the lower 48 states by the 1930's. Montana's wolf recovery began in the 1970's through natural dispersal from Canada, monitored by students/staff from the University of Montana. The USFWS coordinated western recovery efforts, culminating in the reintroduction of wolves to central Idaho and Yellowstone National Park in 1995-97. In 2004 FWP's Wolf Management Plan was approved by USFWS and federal funding awarded to FWP to staff and monitor recovery efforts. Wolves were delisted in Montana in 2011. Reintroduced and naturally recolonizing wolf populations increased and expanded rapidly and merged into one metapopulation by 2007, connected to Canada's 10,000 wolves. Most importantly, monitoring and research efforts, technology, public attitudes toward wolves, and agency approaches have radically changed over time. The author was involved with wolf recovery from the first wolf, Kishinena, to the present tri-state (MT, ID, WY) population of 2000 wolves, and current development of a Patch Occupancy Model (POM) to estimate Montana's wolf population. Many misperceptions are circulating in the public and private sectors about wolf origins, behavior, impacts on big game populations, and management strategies. Discussion will incorporate forty years of sociopolitical, ecological, and technological changes that can be applied to enhance wolf management decisions.

### **BEST PRACTICES FOR WATERFOWL MITIGATION AT THE BERKELEY PIT, BUTTE, MONTANA**

Stella Capoccia\*, Biological Sciences, Montana Tech, Butte Gary Swant, Go Bird Montana, Deer Lodge, MT Matt Vincent, Rampart Solutions, Butte, MT Mark Mariano, Rampart Solutions, Butte, MT Janet Ellis, Montana Audubon, Helena, MT Jay Selmer, Biological Science, Montana Tech

November 28, 2016, marked an unprecedented fall migration event: an estimated 60,000 Snow Geese (*Anser caerulescens*) and Ross's Geese (*Anser rossii*), often termed light geese as a combined flock, landed in the Berkeley Pit. Mine personnel successfully hazed most of these birds off the Pit; however, about 3000 geese died despite round-the-clock hazing efforts that continued for days. Until that time, the Berkeley Pit's records on preventing avian mortality showed a 99.8% success rate, including light geese. This success draws questions of why mortality rates were so high in 2016 and what could be done to improve practices. This research reports on the first year of an on-going investigation effort geared towards those questions. Findings address climate trends, biodiversity, and avian biology as they relate to the area. We examined historic climate trends and compared them to regional weather events that led up to the November 2016 light goose event. We estimate 42 bird species may use the Berkeley Pit for seasonal/regional activity and are tracking site-specific preferences for this unique environment. Specifically, we surveyed waterfowl trends, and determined that identification of and information on the different species, combined with weather patterns, may enhance or detract from deterrent and hazing efforts. Collectively, our work provides a biological overview of the Berkeley Pit, the role it plays for waterfowl specifically during migration, and how this knowledge can be used to minimize the use of the Pit in the future use of and loss of waterfowl that land on Pit water.

### CONFESSIONS FROM SAGEBRUSH REHAB: EXPERIENCES RESTORING SAGEBRUSH IN CENTRAL MONTANA

Matthew J. Comer\*, BLM, Lewistown, Montana

Recent wildfires and the desire to reestablish Wyoming big sagebrush (*Artemisia tridentata* ssp. *Wyomingensis*) from local seed sources has had varying degrees of success in the Lewistown Field Office. Hand stripping sagebrush seeds adjacent to a 2010 wildfire and immediately broadcasting them in areas with high tree mortality exceeded expectations for sagebrush recruitment and growth. Early successes led to refining techniques to efficiently collect native seeds used to speed sagebrush reestablishment and store for future rehabilitation efforts in the event of a large scale disturbance. Sagebrush seeds were cleaned and grown out in partnership with Special K Ranch and Montana prisons, then planted in the fall using tree planters, gas powered augers and dibble bars. Depending on site conditions, the three planting methods could be considered and scaled to larger areas. Planting seedlings was most efficient in areas with low vegetative regrowth.

#### **GRIZZLY BEAR RECOVERY AND DELISTING: PROGRESS REPORT**

Hilary S Cooley\*, USFWS Grizzly Bear Recovery Program, Missoula

The grizzly bear was listed as a threatened species in the lower 48 states in 1975. Accordingly, The U.S. Fish and Wildlife Service (Service) developed a Grizzly Bear Recovery Plan and updated that plan as necessary. The Greater Yellowstone Ecosystem was the first population to achieve recovery. On June 30, 2017, the Service issued a final rule delisting the Greater Yellowstone Ecosystem Distinct Population Segment. The Service is now reviewing recovery of grizzly bears in the Northern Continental Divide Ecosystem. We review progress toward grizzly bear recovery and delisting, including litigation challenges and future management once delisting has been achieved.

### POTENTIAL PATHS FOR MALE-MEDIATED GENE FLOW BETWEEN THE NCDE AND GYE GRIZZLY BEAR POPULATIONS

Christopher P. Peck, USGS Interagency Grizzly Bear Study Team, Bozeman, MT Frank T. van Manen, USGS Interagency Grizzly Bear Study Team, Bozeman, MT Cecily M. Costello\*, Montana Department of Fish, Wildlife and Parks, Kalispell, Mark A. Haroldson, USGS Interagency Grizzly Bear Study Team, Bozeman, MT Lisa A. Landenburger, USGS, Interagency Grizzly Bear Study Team, Bozeman, MT Lori L. Roberts, Montana Department of Fish, Wildlife and Parks, Kalispell Daniel D. Bjornlie, Wyoming Game and Fish, Cheyenne Richard D. Mace, Montana Department of Fish, Wildlife and Parks, Kalispell

For decades, grizzly bear populations in the Greater Yellowstone Ecosystem (GYE) and the Northern Continental Divide Ecosystem (NCDE) have increased in numbers and range extent.

The GYE population remains isolated and although effective population size has increased since the early 1980s, genetic connectivity between these populations remains a long-term management goal. We delineated potential paths for male-mediated gene flow between the populations. We first developed step-selection functions to generate conductance layers using landscape features associated with non-stationary GPS locations of 124 male grizzly bears (199 bear-years). We then used a randomized shortest path (RSP) algorithm to estimate the average number of net passages for all grid cells in the study region, when moving from an origin to a destination node. Repeating this process for 100 pairs of random origin and destination nodes, we identified paths for three levels of random deviation from the leastcost path. We observed broad-scale concordance between model predictions for paths based on NCDE individual versus GYE individuals for all three levels of movement exploration. Models indicated that male grizzly bear movements could involve a variety of routes, and verified observations of grizzly bears outside occupied range supported this finding. Where landscape features concentrated paths into corridors, they typically followed neighboring mountain ranges, of which several could serve as pivotal stepping stones. The RSP layers provide detailed, spatially-explicit information for agencies and organizations to identify and prioritize conservation measures that maintain or enhance the integrity of areas conducive to male grizzly bear dispersal.

# MOOSE POPULATION DYNAMICS IN MONTANA: RESULTS FROM THE HALFWAY POINT OF A 10-YEAR STUDY

Nicholas J. DeCesare\*, Montana Fish, Wildlife and Parks, Missoula Jesse R. Newby, Montana Fish, Wildlife and Parks, Kalispell

Facing indication of moose (*Alces alces*) population declines in Montana and elsewhere in recent decades, Montana Fish, Wildlife & Parks began a 10-year study in 2013. We aimed to estimate population growth rates and vital rates, and to assess the relative influence of a suite of factors potentially limiting moose populations in 3 study areas. During 2013–2017 we captured and radio-collared 122 adult female moose, and annually monitored vital rates of adult survival, pregnancy, parturition, litter size, and calf survival associated with each collared female. Preliminary results from the first 4 biological years of monitoring are indicative of 3 different scenarios playing out across the 3 study areas. In the Cabinet-Fisher area of northwest Montana, relatively high adult female annual survival (0.93, SE=0.026) coupled with lower rates of fecundity (0.63 calves/female) and calf survival (0.31, SE=0.065) have yielded a stable to slightly increasing population growth rate estimate of  $\lambda = 1.02$ . In the upper Big Hole Valley of southwest Montana, somewhat lower adult survival (0.837, SE=0.036) coupled with higher rates of fecundity (0.71 calves/female) and calf survival (0.437, SE=0.063) translate to stable to slightly decreasing population growth rate estimate of  $\lambda = 0.98$ . Lastly, on the Rocky Mountain Front, high rates of all vital rates, including adult female survival (0.927, SE=0.027), fecundity (0.81 calves/female), and calf survival (0.507, SE=0.063) indicate an increasing population, with an estimated growth rate of  $\lambda = 1.12$ . We have not yet completed formal sensitivity analyses of vital rates or limiting factors, but results to date suggest meaningful roles being played by parasites, predators, and nutrition, each to varying degrees depending upon the study area.

### ELK FORAGE AND RISK TRADEOFFS DURING THE FALL Archery Season

Jesse D. DeVoe\*, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula Kelly M. Proffitt, Montana Fish, Wildlife and Parks, Bozeman Michael S. Mitchell, U.S. Geological Survey, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula Craig Jourdonnais, MPG Ranch, Missoula, MT Kristin J. Barker, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula Justin Gude, Montana Fish, Wildlife and Parks, Helena

Elk (Cervus canadensis) need access to adequate nutrition during late summer and fall to support physiological requirements for reproduction and overwinter survival. Montana's fall archery hunting season can affect distributions of elk that may require animals to choose between accessing adequate forage and minimizing risk of harvest. We used radiocollar data from female elk in the Sapphire Mountains during late summer and fall to evaluate the extent of this potential tradeoff across a gradient of hunting risk. Our results indicated that during the archery season elk with higher-risk summer-fall ranges (i.e., those with higher proportions of hunter accessibility) selected more strongly for areas away from motorized routes than elk with lower-risk ranges. Elk distribution was most influenced by forage quality, however, and elk selected strongly for areas of high forage quality regardless of the level of risk, suggesting that elk in the sapphires do not compromise access to nutritional resources during the archery season. Elk with lower-risk summer-fall ranges had access to higher mean forage quality than elk with higher-risk ranges, possibly due to the availability of haystacks and pivot-irrigated areas on private lands. Managers interested in encouraging elk to remain on public lands during the hunting seasons may consider balancing hunting risk and forage resources across the landscape during the archery season. Strategies for public lands might include closures to motorized travel, limiting hunter pressure, and improving forage quality, and strategies for private lands might include working to enhance hunter accessibility and restrict elk access to high-quality forage resources.

### MOVEMENTS OF RED-TAILED HAWKS CAPTURED DURING FALL MIGRATION IN WESTERN MONTANA

Robert Domenech\*, Raptor View Research Institute, Missoula, MT Adam Shreading, Raptor View Research Institute, Missoula, MT

We have studied the fall migration of raptors at the MPG Ranch, located in the northern Bitterroot Valley of western Montana since 2011. Our fall migration count annually records more Red-tailed Hawks (*Buteo jamaicenis*) than any of the five other raptor migration count sites in Montana. Banding efforts at our site have yielded modest totals, but impressive species diversity, with a relatively high proportion of Red-tailed Hawks. In 2015, we began a satellite telemetry study to specifically target fall migrating Red-tailed Hawk. We hope to identify migration routes, wintering and summering ranges and discover if our birds use stopover areas while on migration. We are also interested in learning about the seasonal movements of non-breeding aged Red-tailed Hawks. For this pilot project, we outfitted 12 Red-tailed Hawks (4 adults, 4 hatch-year), with 22g solar Argos/GPS transmitters. We will share basic movement demographics and anecdotal observations collected thus far.

# WHAT WE KNOW ABOUT WEATHER, CLIMATE, PLANTS, WILDLIFE AND FISH

Phil Farnes\*, Snowcap Hydrology, Bozeman, MT

Using data from NRCS SNOTEL (SNOw TELemetry) sites and NWS climate stations can help define climatic conditions at locations occupied by concerned species. Relationships between climate and different animal species, aquatic life, trees and forage that have been observed will be presented. Some of the variables used are snow water equivalent (SWE), temperature, precipitation, growing degree-days winter severity, fall green-up, soil moisture and temperature. Daily data is generally more useful than monthly or seasonal averages. Climate variability that has occurred over long time periods is also useful when observing changes in survival, migration, reproduction or other physical changes. There is generally a poor correlation between climatic variables at lower elevations and higher elevations. It is imperative that this relationship be understood when evaluating climatic effects on species that move from lower to higher elevations or from higher to lower elevations during different seasons. It is also important that valley climatic conditions are not used to define relationship of species that occupy higher elevations.

### \*\*Evaluating Sample Size to Estimate Genomic Relatedness in Bighorn Sheep Populations

Elizabeth P. Flesch\*, Animal and Range Sciences Department, Montana State University, Bozeman Jay J. Rotella, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman

Jennifer M. Thomson, Animal and Range Sciences Department, Montana State University, Bozeman

Tabitha A. Graves, Northern Rocky Mountain Science Center, USGS, West Glacier, MT Robert A. Garrott, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman

Genetic research may be a useful approach for understanding factors that could impact productivity and restoration of bighorn sheep herds. For example, genetic consequences of inbreeding in small populations can impact recruitment, and relative relatedness among herds can help inform translocation decisions. This empirical simulation study quantified genetic attributes of bighorn sheep populations with a range of different herd histories in Montana and Wyoming to investigate genomic relatedness within and between herds and to estimate an optimal sample size per population for evaluating genetic diversity and distance. Employing an Ovine array containing ~700,000 single nucleotide polymorphisms (SNPs) with approximately 24,000 markers that are informative for Rocky Mountain bighorn sheep, we conducted whole genome genotyping. We analyzed genetic material from 30 individuals from each of four different populations that we predicted would differ in genetic characteristics due to population dissimilarities that included origin (native/reintroduced), population size, bottleneck history, degree of connectivity, and augmentation history. The four populations provided samples across a spectrum of these herd attributes and included Fergus, Taylor-Hilgard, and Glacier National Park in Montana and the Beartooth Absaroka in Wyoming. We examine relatedness estimates within herds using two different metrics to evaluate the potential for links between genomics and herd demography. By evaluating our simulation results, we conclude that a sample size of 25 is adequate for assessing intra- and interpopulation relatedness. We discuss the utility of genetic analyses for improving knowledge of bighorn sheep populations and potential implications for bighorn sheep management.

### GRIZZLY BEAR RECOVERY PLANNING: REQUIREMENTS UNDER THE ENDANGERED SPECIES ACT

Jennifer K. Fortin-Noreus\*, US Fish and Wildlife Service, Missoula, MT

There are many common misconceptions about Recovery Plan requirements under the Endangered Species Act (Act). The Act requires the U.S. Fish and Wildlife Service to develop and implement recovery plans for endangered and threatened species, unless it would not promote the conservation of the species. Recovery Plans are guidance documents that must include site-specific management actions; objective and measurable criteria; and an estimate of the time and cost required to implement the recovery plan. The Service frequently receives questions and comments from partner agencies and the public about the Grizzly Bear Recovery Plan. The first recovery plan for grizzly bears was published in 1982 and subsequently revised in 1993. There have been multiple ecosystem specific revisions and supplements to the 1993. One of these supplements was the development of habitat-based recovery criteria for the Greater Yellowstone Ecosystem (GYE), as required by a 1997 court settlement agreement. In addition, the demographic recovery criteria were updated for the GYE concurrently with the delisting of the GYE grizzly bear population in both 2007 and 2017. This presentation will address frequently asked recovery plan questions, such as: When does the Service have to update recovery plans? Are recovery plans litigable? Do all recovery criteria have to be met to remove a species from the list of endangered and threatened wildlife?

# \*\*Elk Calf Survival in the Upper Bitterroot Valley, Montana

Michael J. Forzley\*, Ecology Department, Montana State University, Bozeman Jay J. Rotella, Ecology Department, Montana State University, Bozeman Kelly M. Proffitt, Montana Fish, Wildlife and Parks, Bozeman Robert A. Garrott, Ecology Department, Montana State University, Bozeman

In 2011, researchers from Montana Fish, Wildlife and Parks and the University of Montana initiated the Bitterroot Elk Ecology Project to evaluate the factors driving changes in elk populations in the southern Bitterroot Valley of Montana. After three years of evaluating the bottom-up and top-down effects on survival and recruitment of area elk populations, the research team determined that increasing elk calf survival in the southern Bitterroot may have positive effects on area elk abundance and low recruitment. Furthermore, the research team identified mountain lions as a major source of elk calf mortality. Thus, in 2012, area wildlife managers increased the harvest of mountain lions in the study area to increase elk calf survival. To evaluate possible changes in calf survival several years after the mountain lion harvest effort, we are formally repeating the original calf survival study. We have been monitoring survival of elk calves via radio telemetry from 2016 to present (n = 248 calves over the 2 seasons) in the East Fork and West Fork watersheds of the Bitterroot River. We will present comparisons of current calf survival in the upper Bitterroot study area with previous estimates. We will also present the results of an evaluation of associations between calf survival and factors such as forage availability, predation risk, and individual characteristics of elk calves.

## Montana Statewide Bighorn Sheep Research Project: A Progress Report

Robert A. Garrott\*, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman
Jay J. Rotella, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman
Kelly Proffitt, Montana Fish Wildlife and Parks, Bozeman
Carson Butler, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman
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Blake Lowrey, Fish and Wildlife Ecology and Management Program, Ecology Department, Montana State University, Bozeman

The fourth year of the Montana Statewide Bighorn Research Project has been completed and we have met all of our objectives to date. We have completed two capture events in each of the eight study herds, sampling a total of 495 animals and radio collaring 222 adult ewes. Analysis of the pathogen data has provided minimum sampling recommendations for determining what pathogens are present in a herd and have demonstrated that the pathogens associated with pneumonia are resident in most herds sampled. The first deployment of GPS store-on-board collars have been recovered from 7 of the 8 herds and have revealed divergent seasonal movement strategies among study herds with habitat modeling analyses currently underway. Pilot genomics studies have been completed providing guidance on the number of genetic samples required to adequately assess genetic attributes of herds, with preliminary results suggesting notable differences among herds. We have employed new analytical tools to economically identify and quantify a suite of 81 metabolites from a small serum sample from each bighorn captured. We are currently in the process of evaluating the resulting data in an attempt to develop a 'health panel' that can inform managers of the physiological and nutritional condition of animals that should reflect habitat conditions and resources available on each herd range. Adequate data have been collected to estimate adult ewe pregnancy and survival rates and, with management monitoring data on annual recruitment, will provide the data required to develop integrated population models.

# Understanding Montana Landowners' Values, Attitudes & Behaviors towards Wildlife & Wildlife Habitat

Larry M. Gigliotti\*, USGS South Dakota Cooperative Research Unit, South Dakota State University, Brookings

Lily A. Sweikert, Natural Resource Management Department, South Dakota State University, Brookings

The Plains & Prairie Potholes Landscape Conservation Cooperative (PPP-LCC) identified habitat loss as a key research need in 2012. This grassland-wetland ecosystem provides essential habitat for an array of wildlife, especially waterfowl. Many factors contribute to loss of wildlife habitat, but ultimately it comes down to decisions made by the private landowner. Montana was part of a five state study (IA, MN, SD, ND & MT) of landowners in the PPP-LCC. Each state wildlife agency developed their questionnaire based on their agency's needs for information about landowners. Montana's specific objectives were to: (a) measure characteristics, attitudes, values and behaviors towards participating in a variety of conservation programs, and (b) identify meaningful segments of Montana landowners

for understanding conservation behaviors. The Montana landowner survey received 438 usable returns for a 32% response rate. We analyzed landowners based on four segmentation models: (1) Landowner Occupation Model (farmer, rancher, farmer/rancher, and non-ag), (2) Owning Lands Model, which was based on reasons for owning land in Montana, has four types based on how each group scored on three scales (personal use, conserve/protect habitat, and economics/income), (3) Wildlife Habitat Model has three types (low, medium, and high) based on the level of positive support for wildlife and wildlife habitat on private land and (4) Wildlife Value Orientations Model, based on how people feel about wildlife, has four types (Utilitarian, Mutualist, Pluralist, and Distanced). Each model is evaluated based on predicting behavior and other characteristics of the various sub-groups.

### Common Loon Conservation and Management: A Success Story That Began 35 Years Ago

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The first comprehensive work on the common loon (*Gavia immer*) in Montana began in 1982 when Don Skaar initiated surveys across the breeding distribution in the northwestern part of the state. Eight years later, he completed the first management plan that identified 62 lakes with territorial pairs. Based on recommendations in the plan, research evaluating the effects of increased recreational use and development on common loon nesting was initiated. This group of individuals would go on to create the nonprofit organization that would become the seed for future growth in common loon conservation, the Montana Loon Society. The Common Loon Working Group was formed some years later to guide professionals in the conservation, management, and research on common loons in the state. I will discuss the history of common loon conservation in Montana and summarize research from the Montana Loon Study in the early 1990s and the Loon Ecology Project (2002-2008). I will share our successes and what I believe to be potential failures, as well as, what we may expect to see in the future.

### WOLVERINES IN WINTER: INDIRECT HABITAT LOSS AND FUNCTIONAL RESPONSES TO BACKCOUNTRY WINTER RECREATION

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Outdoor recreation is increasingly recognized to impact wildlife yet few studies have looked at recreation within large natural landscapes that are critical habitat to some of our most rare and potentially disturbance-sensitive species. Over 6 winters (2010 - 2015) and four study areas (>1.1 million ha) in Idaho, western Wyoming and southwestern Montana, we radio-collared 24 individual wolverines and acquired >54,000 Global Position System (GPS) locations over 39 animal-years. Simultaneously, we monitored winter recreation, collecting ~6,000 GPS tracks from backcountry winter recreationists representing ~200,000km of recreation activity. We combined the recreation GPS tracks with trail use counts and aerial-

based recreation surveys to map the extent and relative intensity of motorized and nonmotorized recreation. We modeled habitat selection of male and female wolverines within their home ranges using resource selection models and functional responses. We found some differences in habitat selection between the sexes, including the females selecting colder, snowier areas possibly associated with denning. We then estimated the habitat selection responses of wolverines to backcountry winter recreation, assessed the potential for indirect habitat loss from winter recreation and tested for functional responses of wolverines to differing levels and types of recreation. Motorized recreation occurred at higher intensity across a larger footprint than non-motorized recreation in most wolverine home ranges. Wolverines avoided areas of off-road motorized and non-motorized recreation and females experienced higher indirect habitat loss than males. Wolverines showed negative functional responses to the level of recreation exposure, with increasing avoidance as levels of the recreation increase within the home range.

### \*\*Assessment of Bridges in Eastern Montana to Identify Active Season Bat Roosts

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Bats fulfill important niches in the ecosystems they occupy and control insect populations that are agricultural pests. Bat species across North America face several significant threats including habitat degradation/roost disturbance, wind turbine-related deaths, and White-Nose Syndrome (WNS). In Montana, bat roost information has been lacking in the nine eastern counties where Northern Myotis (Myotis septentrionalis) is listed as Threatened, as well as a large number of additional central and eastern Montana counties. To identify active season roosts across these regions, we surveyed bridges for bat use. From the surveys, we established baseline knowledge about bat roosting preferences and the locations of active roost sites to monitor, which can be used to better inform conservation efforts. Survey data were collected from bridges in nine counties in Eastern Montana, as well as from bridges in eight counties in central Montana. Bridges surveyed were classified by roost type (day, night, maternity, none). Structural materials, surrounding habitat characteristics, and other attributes were recorded. Night roosts were the most common roost type, concrete bridges were the most used bridge type, and maternity roosts were only found in wooden bridges with ideal crevices. Results which show widespread use of concrete bridges as roosts, and the importance of ideal crevices for day and maternity roosting bats, are consistent with previous studies in western and central Montana. Ideal crevices are an important structural characteristic for bridge roosting bats, which, when implemented in bridge design, provide suitable habitat in the face of increasing urbanization and human disturbance.

## ESTABLISHING A CONTEMPORARY BASELINE OF WOLVERINE DISTRIBUTION AND GENETICS ACROSS FOUR WESTERN STATES

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Wolverines (Gulo gulo) were extirpated from their historical range in the conterminous U.S. by about 1920. Since that time, some recovery has occurred, but the extent of their distribution has remained undefined due to their naturally low densities and use of rugged, remote terrain. Conservation of this metapopulation would benefit from initiation of a population monitoring program along with an understanding of the extent of current distribution, gaps in distribution, genetics and connectivity. We established a camera/DNA survey across current range states to obtain baseline information on occupancy and genetics of wolverines. During winters 2015–16 and 2016–17, we sampled 185 of 633 grid cells (15x15 km) that were >50% wolverine habitat. We detected wolverines at 59 of 185 cells. Citizen volunteers detected wolverines in an additional 35 cells. Probability of a wolverine being detected at least once at a site that was occupied was high (p=0.92) and did not differ between sites that were visited a single time vs. multiple times. Average estimated probability of wolverine occupancy during our study was 0.42 (95% CI 0.29-0.55). We detected more females than males. Spatial models indicated wolverine occupancy varied across the region. with highest probability in the Northern Continental Divide Ecosystem and lower on the southern and eastern periphery of the study area. Our regional occupancy estimates provide a baseline for future evaluations of change in wolverine distribution and occupancy through time, including the possibility of detecting influences on distribution due to climate, human influences, or continued range expansion.

### **\*\*Estimating Recruitment of Wolves with Limited Data**

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Successfully managing a species can require information on demographics. Recruitment is particularly important for harvested species because it determines the level of harvest that can be sustained by the population; thus, this information can be used to improve harvest decisions. When management occurs across large spatial scales, however, these data can be costly to collect and are often unavailable. Management of gray wolves (*Canis lupus*) in Montana could benefit from information about variation in recruitment. Existing data are insufficient for traditional methods to estimate recruitment, therefore a new approach is needed. Our objectives were to develop a method to estimate recruitment using available data and determine the amount of data needed to produce reliable estimates. Integrated population models can be a useful tool for demographic analyses from limited data sets. We developed an integrated population model to use collar, hunter survey, and group count data

to estimate recruitment of wolves. This method did not require collecting additional data and used available data from ongoing monitoring. We tested the model on a simulated data set to determine model sensitivity to the amount of data used. We found that the model estimated the true parameter values from the simulated data accurately with at least 12-25 group counts and approximately 10 collars per year. Ultimately, we will use this method to evaluate the spatial and temporal variation in recruitment across the state. This method can help reduce costs associated with monitoring wolf populations while providing valuable information needed to help inform management decisions.

### ACOUSTIC MONITORING OF AVIAN NOCTURNAL MIGRATION IN THE BITTERROOT VALLEY, MONTANA

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Millions of birds migrate at night that emit sounds we can record and identify to species. In 2012, we began to record nocturnal flight calls of migratory birds at three monitoring sites. By the summer of 2017, we had expanded to over 25 sites throughout the Bitterroot Valley, ranging from valley-bottom high schools to ridge-top fire lookouts. We have collected and processed terabytes of recordings from spring and fall migration using customized, opensource software. Our archive now includes thousands of detections of passerines, the majority of which are calls from sparrows, warblers, and thrushes. In this presentation, we will share some of the migration patterns we have documented, the influence weather and other variables can have on migration, and how these data can contribute to our knowledge and conservation of bird populations. A longer-term goal of this project is to develop a network of acoustic monitoring stations across Montana, building a collaboration between private individuals, non-profits, and managing agencies. We will provide information on how you can participate in this project and attend a training workshop taking place this spring.

### Better Understanding Montanans Thoughts Regarding Wolves and Wolf Management in Montana

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Following the 2016-17 wolf hunting/trapping seasons, Montana Fish, Wildlife & Parks (FWP) conducted four separate surveys of resident Montanans to better understand their views regarding wolves and wolf management in Montana. This research builds upon previous work of the agency to survey state residents in 2012. Survey finding revealed that tolerance for wolves on the Montana landscape remains relatively low. However, comparing the 2017 survey data to identical data collected in 2012 shows a slight shift in the direction of more tolerance for wolves over time, particularly among Montana households. Results also showed continued tolerance for wolf hunting in Montana across all four survey groups. In contrast, tolerance of wolf trapping varied substantially. While hunters and landowners were very tolerant of wolf trapping, nearly half of the respondents to the Montana household survey

were not tolerant of wolf trapping in the state. Lastly, for each of the four survey groups there was little agreement among respondents regarding whether the regulations for the Montana wolf hunting and trapping seasons were satisfactory or not. These survey results speak to the contentious nature of wolf management in Montana, and the importance of continue efforts on the part of FWP to involve the public in wolf management decisions and season setting processes.

### Using the Scavenging Rate of Native Birds and Bats to Adjust Wind Farm Fatality Estimates

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Wind Farms have the potential to negatively affect bird and bat populations due to collisions with turbines. To estimate bird and bat fatality, the number of carcasses found beneath turbines is commonly corrected for removal by scavengers, which is quantified by measuring persistence of experimental carcasses. Often these studies use domestic animals as surrogates because fresh carcasses of wild animals (e.g., raptors and bats) are difficult to obtain. We present results from carcass persistence studies conducted at two sites and discuss observed differences between native and surrogate carcass types. Next, we ran simulations with the Huso Estimator using carcass persistence data from both surrogates and native animals to explore sensitivities on overall fatality estimations. Based on the results from field trials and simulations, carcass type may affect overall fatality estimates and therefore project management decisions.

# MONTANA BIOLOGICAL CHECK STATIONS: A TIME-HONORED TRADITION

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For nearly a century, Montana game check stations have been utilized to help monitor local or regional game harvest, collect biological data for management or research needs, monitor hunter activity, help disseminate information and/or answer questions and enforce hunting rules and regulations. This information is important in the ongoing design of game management programs, which provide for optimum game populations and sustainable hunting opportunity. This importance was emphasized with a state law passed in 1973 making it mandatory for hunters to stop at check stations on their routes to and from hunting. Currently, Montana Fish, Wildlife & Parks (FWP) operates at least 39 biological check stations in various ways throughout Montana. Despite its rural location in west central Montana, the Augusta check station, in operation since at least 1932, provides one unique perspective relative to the value of biological check stations. Open 7 days a week, 16 hours/day during the general fall big game rifle season, it is estimated that well over 200,000 hunters have traveled through this check station with just under 32,000 big game animals currently recognized in the database. Using data from the Augusta check station, information such as correlations to FWP hunter harvest survey estimates, deer and elk age estimates associated with game harvest weights, antler measurement data, public vs private land harvest location information and other hunter demographic information will be presented. Check stations are clearly an important tool in helping maintain Montana's game populations for the current and future hunting publics and are an excellent example of wildlife conservation and management in Montana through cooperation between hunters and FWP.

# \*\*NICHE SIMILARITIES AMONG INTRODUCED AND NATIVE MOUNTAIN

### UNGULATES

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When two ecologically similar species are sympatric, theory predicts they will occupy distinct ecological niches to reduce competition. We evaluated the niche partitioning hypothesis with sympatric mountain ungulates – native bighorn sheep (BHS; Ovis canadensis) and introduced mountain goats (MTG; Oreamnos americanus) in the northeast Greater Yellowstone Area. We characterized seasonal niches using two-stage resource selection functions with a used-available design and descriptive summaries of the niche attributes associated with used GPS locations. We evaluated seasonal similarity in niche space according to confidence interval overlap of model coefficients and similarity in geographic space by comparing model predicted values with Schoener's D metric. Our sample contained 37,962 summer locations from 53 individuals (BHS = 31, MTG = 22), and 79,984 winter locations from 57 individuals (BHS = 35, MTG = 22). Slope was the most influential niche component for both species and seasons, and showed the strongest evidence of niche partitioning. Bighorn sheep occurred on steeper slopes than mountain goats in summer and mountain goats occurred on steeper slopes in winter. The pattern of differential selection among species was less prevalent for the remaining covariates, indicating strong similarity in niche space. Model predictions in geographic space showed broad seasonal similarity (summer D = 0.88, winter D = 0.87), as did niche characterizations from used GPS locations. Our results suggest that reducing densities of mountain goats in hunted areas where they are sympatric with bighorn sheep and impeding their expansion may reduce the possibility of competition and disease transfer.

# Use of Talus and other Rock Outcrops by Bats in Western Montana

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Identifying roosts used by bats in natural features can be difficult, particularly in areas without known caves. Data on roosts is critical for monitoring populations to assess impacts of exotic emerging pathogens like *Pseudogymnoascus destructans*, which causes White-nose Syndrome, and to conserve important habitat for this taxa. Prior to our work, only 61 active season roosts were known in Montana. To determine if bats in western Montana are using talus/rock outcrops as a form of roosting sites, we completed 23 surveys of rock outcrops from mid-June to mid-July focusing on three regions of western Montana. We used three survey methods to assess bat presence: 1) acoustic detector/recorders, 2) visual encounter surveys (VES), and 3) mist net surveys in proximity to outcrops. Ten individuals were found during VES, most commonly *Myotis evotis/thysanodes*. Additionally, we used guano as an indicator of bat use and identified 35 roosts during searches. We completed 22 nights of mist netting and captured 36 individuals representing nine species associated with these outcrops. The most common capture was M. *yumanensis/lucifugus*, and all individuals were adults and

predominately male. Whether talus slopes that serve as active season roosts are also suitable for hibernation remains undetermined, but acoustic data from other locations supports this conclusion. Acoustic detectors placed in rugged landscapes record more winter activity than those in less rugged areas with presumably fewer outcrops. Future work is necessary to assess activity at rock outcrops across the year and identify the community of species that may hibernate within these features.

### \*\*Effects of Rangeland Management on Sharp-Tailed Grouse Habitat Selection in Mixed-Grass Prairies

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Temperate grasslands, including mixed-grass prairies, suffer from the greatest levels of habitat loss and degradation of any North American ecosystem. Grazing is the predominant land use on grasslands across western North America and directly affects the structure, composition, and productivity of these ecosystems. Rest-rotation grazing, which includes a year-long period of deferment, is implemented on conservation easements in Montana to improve wildlife habitat. However, this grazing system was developed in arid bunchgrass rangelands and its effectiveness has not been studied in more mesic mixed-grass prairie. Sharp-tailed grouse (Tympanuchus phasianellus) have large home ranges and require a wide range of habitat types for nesting and over-wintering, making them an ideal indicator species for grassland habitats. Rest-rotation grazing systems could influence grouse habitat selection at multiple spatial scales by providing patches of habitat that are periodically rested from disturbance. We investigated both home range selection in relation to the larger study area and selection of habitat features within the home range. We monitored 82 female sharp-tailed grouse in eastern Montana during the breeding seasons of 2016–17. Average home range size was  $503 \pm 56$  ha. Grazing system was not a good predictor of home range size or space use within the home range. Grouse selected for intact areas of grassland when choosing a home range but we did not detect third-order habitat selection within home ranges for the population. The high level of individual variation in third order habitat selection suggests an apparent plasticity in resource use at fine spatial scales.

## \*\*Evaluating Long-term Turnover of Northern Goshawks within the Minidoka Ranger District of the Sawtooth National Forest

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The Northern Goshawk is listed as a management indicator species for the Minidoka Ranger District of the Sawtooth National Forest. This distinction has enhanced research interest on goshawk population health in the region. For raptors, annual adult turnover is considered a crucial metric of population health; providing insights into mortality, fidelity, and population disturbances. Over the past 25 years of studying goshawks, the Intermountain Bird Observatory (IBO) has observed abnormally high female turnover as compared to other places the species has been studied. Their estimations are based on banding and resighting birds, and may be biased high due to undetected marked birds and unknown age of birds when banded. To increase accuracy of IBO's turnover data, we conducted parentage analyses using blood samples collected from goshawks in 2012-2016. We analyzed 32 samples from nine nest territories by examining shared alleles between adults and nestlings. With this analysis, we identified previously unknown turnover and fidelity events, increased known ages of banded birds, and quantified and removed bias from IBO's turnover estimations. Our work indicated that band-resight alone may be insufficient to produce accurate turnover estimates, and the inclusion of genetic analyses may mitigate inaccuracies. In addition, our results fundamentally altered IBO's understanding of goshawk population dynamics within the forest.

### CONVENTIONAL OIL INFRASTRUCTURE LEADS TO LOWER Abundances of Baird's Sparrow and Sprague's Pipit

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Although grassland songbird populations have steadily declined, little research has examined the effect of oil infrastructure on abundances of grassland songbirds. Even less research has identified mechanisms that explain observed effects. To evaluate this, we sampled abundance of 5 songbird species at oil well sites with different pump mechanisms, power sources, and activity levels; we also evaluated the effects of perch and road density and exotic vegetation, all of which are associated with oil development. We conducted 800m abundance transects at 42 sites twice per year in 2013 and 2014 in Alberta, Canada. Our results showed both Baird's sparrows (Ammodramus bairdii) and Sprague's pipits (Anthus spragueii) had lower abundances at all sites that contained oil infrastructure. The other 3 species, chestnut-collared longspurs (*Calcarius ornatus*), western meadowlarks (*Sturnella* neglecta), and Savannah sparrows (Passerculus sandwichensis), were relatively unaffected by oil wells, linear features, or exotic vegetation. Our research suggests that mitigation must focus on minimizing the extent of above-ground infrastructure as a reduction of human activity and noise caused little change in the effects of wells on grassland songbirds. For publication, see Nenninger and Koper (2018), "Effects of conventional oil wells on grassland songbird abundance are caused by presence of infrastructure, not noise," Biological Conservation

### ESTIMATING THE DENSITY OF LARGE CARNIVORES: EVALUATION OF SAMPLING DESIGNS AND INTEGRATED DATA SOURCES

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Understanding the variation in the abundance of animals is critical for informed management of species. Large carnivores pose a particular challenge for the estimation of population size, given their large home ranges, elusive natures, and low densities. These characteristics render traditional approaches problematic. However, methodological advances in spatial capture-recapture models that explicitly incorporate spatial information into capture probabilities have allowed for improved estimation of abundance for such animals. Yet, little information exists regarding how to best allocate resources to sampling to optimize estimation. Although recent work has suggested that additional information, e.g. hunter

harvest and telemetry data, can yield improved inference and higher precision, it is unclear how to best integrate these multiple sources of data. This is particularly true for spatially unstructured sampling designs in which captures are the result of active searching, rather than arising from fixed sampling locations such as camera traps. We conducted a simulationbased power analysis of a spatially unstructured capture-recapture study design, and identified an optimal balance of search effort and radio-collar deployments. Under the best sampling plans, abundance estimates were only slightly biased even when effort was correlated with animal densities. However, we also found that abundance estimates were strongly affected when hunter harvest information was ignored or treated as part of the sampling design, rather than modeled using a separate detection process. Finally, we demonstrated the utility of these methods by estimating the space-use patterns and density of mountain lions in western Montana.

### \*\*Can Targeted Cattle Grazing Increase Abundance of Forbs or Arthropods in Sage-Grouse Brood-Rearing Habitat?

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Suboptimal brood-rearing habitat often limits sage-grouse (*Centrocercus urophasianus*) populations in western North America. In mountain big sagebrush (Artemisia tridentata subsp. vaseyana) brood-rearing habitat, dense sagebrush canopy cover (> 25% cover) may limit the understory forbs and arthropods that sage-grouse prefer to eat during summer. We investigated whether protein supplementation could concentrate cattle browsing or trampling to reduce mountain big sagebrush canopy cover and increase the abundance of forbs or arthropods. We applied targeted cattle grazing within two large, contiguous pastures (650 ha each) in the Beaverhead Mountains of southwestern Montana. Pastures were grazed simultaneously at a light stocking rate (6.25 ha/AUM) for two weeks in mid-October 2015 and 2016. Vegetation and arthropod response was measured during the following June (2016 and 2017, respectively). Each year we evaluated vegetation and arthropod response within 16 sites where mountain big sagebrush canopy cover exceeded 30% pre-treatment. Sites were 0.008 ha to match the spatial scale at which sage-grouse broods select fine-scale habitat. In the first summer after treatment, supplemented sites averaged 78% less sagebrush canopy cover (8% vs 36%; P < 0.001) and 25% more forb canopy cover (15% vs 12%; P = 0.122). Forbs also comprised a greater proportion of the herbaceous understory in the supplemented vs. non-supplemented sites (48% vs. 36%; P = 0.002). Abundance of sage-grouse arthropods (i.e., arthropods from families documented in the literature to be eaten by sage-grouse) did not differ between supplemented and non-supplemented sites (P = 0.796). Sagebrush cover was reduced by cattle trampling, not browsing.

### **BOBCAT POPULATION RECONSTRUCTION AND HARVEST MANAGEMENT**

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We backdated ages of 31,257 bobcats (Lynx rufus) to estimate populations in each of 7 Montana trapping districts (TDs) for the years 2000-2016. To account for effects of variable effort on harvest and population estimates we established harvest scalars for each TD based on harvester effort. In all but TD 6, bobcat populations peaked in 2005, 2006 or 2007. In western Montana, TDs 1-3, adult populations were 6.8% above to 11.4% below average in 2012 and were predicted to increase to near or above average between 2012 and 2017. Adult populations in eastern Montana, TDs 4-7, were 22.3%-37.4% below average in 2012 and predicted to increase between 2012 and 2017 although remaining well below average. Lambda for adults was very low in all TDs in 2009 but improved to above or near 1.0 in most years and TDs in western Montana after 2009. In eastern Montana, lambda remained below 1.0 in most years and TDs after 2009. The number of bobcats trapped per day in year t was the best predictor of adult populations in year t+1 and number of juveniles per adult captured in year t was the best predictor of adult growth rate in t+1. Using observed relationships between total harvest and lambda of total populations we estimated a harvest level for each TD that historically was correlated with stable or increasing populations. Using predictions for population and lambda along with historic population trends managers will have information to more accurately set annual quotas.

### \*\*HABITAT CONDITIONS ASSOCIATED WITH COLONIZATION OF SUBOPTIMAL HABITATS BY BEAVERS IN SOUTHWEST MONTANA

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Awareness of beavers as integral components of healthy ecosystems has increased in recent decades, and as a result beaver-mediated habitat restoration projects are becoming more common. Beaver restoration sites are frequently located in degraded stream systems with little or no beaver activity. However, the selection of restoration sites is often based on habitat suitability research comparing well-established beaver colonies to unoccupied stream sections. This approach may over-emphasize habitat conditions that are modified by beaver activity. We conducted beaver-use surveys on streams in the upper Gallatin and Madison River drainages to investigate habitat selection by beavers starting new colonies in areas where most optimal beaver habitat is occupied. We identified new colony locations in relatively unmodified stream segments and compared them to unsettled sites to evaluate baseline conditions that promote colonization. Beavers selected areas with low gradients, high canopy cover of woody riparian vegetation, and wetland types corresponding with willow cover and low-lying areas directly adjacent to the stream channel. However, the effects of these habitat components on the probability of settlement were weak and colonization patterns were highly variable. Models developed based on well-established beaver colonies in the study area supported different interpretations of habitat selection patterns. This gives credence to the hypothesis that habitat selection studies based on active colony locations may not provide reliable information on settlement site selection by beavers starting new colonies in novel areas. Our research provides recommendations for beaver restoration practitioners to select restoration sites that will have the highest probability of successful colony establishment

## More than Fish – Incorporating Riparian and Wetland Dependent Bird and Herptile Habitat into Stream Restoration Projects in Western Montana

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Millions of dollars are spent annually on watershed restoration and habitat improvement in the Pacific Northwest to increase fish populations. In the last 20 years, Trout Unlimited (TU) alone has partnered on \$7.5 million in projects that reconstruct or improve instream, riparian, floodplain and wetland habitats. Even though more than 50% of bird species in Montana breed in riparian habitats and 16 native amphibians, 3 turtles, and 7 of Montana's snakes find essential habitat in riparian areas, TU's stream restoration work has largely been done without considering the impacts or - more importantly - the potential benefits to riparian and wetland dependent birds and herptiles. Stream restoration often involves the physical manipulation of large landscapes, with precisely engineered design plans that define stream widths and depths, earthwork grading, soil/sediment size distribution and quality, and many other factors. Working with state and federal non-game biologists, conservation groups and other resource managers, TU has begun to focus on the structural considerations of stream restoration for birds, amphibians and reptiles in projects that have primary objectives for fisheries. Our intention is to translate published research on wildlife habitat and known habitat needs for breeding, foraging, and over-wintering for native herptiles and birds into design typicals and construction plans. TU will present lessons learned from these emerging conversations and preliminary results from stream restoration activities in Ninemile Creek and other projects in western Montana.

### \*\*Responses of Songbird Populations and Breeding to Cattle Grazing Regimes in Sagebrush-Steppe of Montana

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A large portion of sagebrush-steppe across the west is used for grazing of domestic livestock, primarily cattle. We compared songbird communities over five breeding seasons in central Montana between two grazing systems: a system using a combination of rest and deferment (hereafter rest-rotation) and traditional grazing. For the purposes of our study, we define rest-rotation grazing as changing the timing of grazing in pastures each year, with some pastures alternately rested every few years. Traditional grazing is defined as grazing a pasture at the same annual season each year or all season. Recently, rest-rotation systems have been used as a conservation management tool by the Natural Resource Conservation Service's (NRCS) Sage Grouse Initiative (SGI). Their goal is to encourage private landowners to graze their livestock more sustainably in order to maintain or improve habitat for greater sagegrouse (Centrocercus urophasianus), as well as to improve rangeland productivity. We explore the effects of rest-rotation compared to traditional grazing on songbird population breeding demographics: adult abundance, nest density, and nest success. Abundance estimates are often used to assess conservation actions given the relative ease in collecting data to inform these estimates. However, information on how conservation actions influence life histories such as nest density and nest success are lacking, despite the fact that life histories inform abundance. Our goal is to understand the relationship between adult abundance, nest density, and nest success, and how land management practices, such as grazing, influence those patterns.

# TO SNIP OR NOT TO SNIP: CAN WE IMPROVE OUR UNDERSTANDING OF GARTER SNAKE GENETICS?

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In the past we utilized microsatellite analysis to understand the genetic diversity of both Wandering Garters and Common Garters across Western Montana. The results showed no isolation by distance for both species. Perhaps after a period of rapid colonization, garter snakes experienced extensive inbreeding and/or assortative mating at localized sites. Alternatively, the initial colonists may have lacked genetic diversity. Making such broad claims from our findings is difficult because the number of alleles per locus for both species was low. This is a persistent problem for snake researchers throughout the research literature. We can't be sure if microsatellite analysis is itself problematic when studying these animals. SNIPs should help elucidate this problem and may help us begin to understand the phenotypic variability of garter snake populations across the region. In 2017 we expanded sampling of both species across the breadth of Western Montana. Unfortunately, the prolonged fire season lowered ground temperatures and made some populations difficult to access. I sampled 55 Common Garters and 185 Wandering Garters during the 2017 season. Because the total number of Wandering Garter samples (N=372) was much higher than Common Garters (N=160), we decided to first genotype the Wandering Garters and compare that to the results from the earlier microsatellite analysis. I will present the findings from this endeavor while also highlighting what I learned from observations of both species during the field season.

### MIGRATORY BAT ROOST USE IN THE BITTERROOT VALLEY

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The roost-site use and migratory habits of silver-haired bats (*Lasionycteris noctivagans*) and hoary bats (*Lasiurus cinereus*) are poorly understood. This study provided some of the first documented roost locations and roost descriptions for silver-haired bats in Montana, and provided one of the first known efforts to attempt to characterize inter-annual site fidelity for these species. We used radio telemetry to track 34 silver-haired bats and 4 hoary bats to their day roost locations in 2016 and 2017. These tracking efforts resulted in discovery of 29 silver-haired bat roosts, which included a maternity colony of 43 individuals. No hoary bat roosts were documented despite extensive ground and aerial searches. Most silver-haired bat roosts (97%) were located within natural cavities of black cottonwood trees (Populus trichocarpa); however, roost trees did not differ in diameter, height, or decay stage compared to available trees. Based on radio telemetry information, hoary bats migrate through the study area in July and August, and silver-haired bats appear to migrate out of the study area in late August and early September. In addition to radio telemetry, we also marked individuals with PIT tags and recaptured 2 silver-haired bats in 2017 that were marked in 2016, suggesting some degree of inter-annual fidelity to summer habitats. The results of this study may be used to guide natural resource management decisions by providing a basic understanding of roost ecology of bats during the maternity period, and the degree of inter-annual fidelity to summer habitats

### MOTHS TO THE LIGHT: NOCTURNAL INSECT DIVERSITY AT MPG RANCH AS REVEALED BY DNA BARCODING

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We collected nocturnal insects at MPG Ranch in the Bitterroot Valley from May-August, 2017, to establish a DNA barcode reference collection for a project to investigate diet and trophic relationships of nocturnal aerial insectivores. Using mercury vapor and UV blacklights, we collected 946 specimens from nine insect Orders and obtained tissue samples for DNA analysis from 760, with 86% from Lepidoptera (moths). DNA barcode analysis produced 744 sequences that confirmed 365 insect species from 62 families and 251 genera, including 311 moth species from 25 families and 196 genera. Eighty-three percent of moth species were represented in six families: Noctuidae (43.4%), Geometridae (20.5%), Crambidae (5.8%), Pyralidae (5.8%), Tortricidae (5.5%), and Erebidae (4.8%). Pending verification, 75 species are potential new records for Montana and 162 new records for Missoula County. Moths are known to serve as herbivores, pollinators, and food resources. However, only recently has the scale and importance of the roles this taxa serves in ecosystem functions been given serious consideration. This interest has been prompted by significant declines documented in Europe, preliminary evidence of declines of large moths in northeastern United States and adjacent Canada, and the general decline of flying insects in many areas. Our initial research revealed MPG Ranch harbors a rich diversity of moth species. This diversity presents excellent opportunity for long term monitoring of population trends and further research on the role moths play in maintaining botanical diversity, community level responses to land use practices and climate change, and their importance in terrestrial food chains.

### THE DIFFICULTY OF BECOMING A BREEDING OSPREY: INSIGHT FROM A TELEMETRY STUDY BASED IN WESTERN MONTANA

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In 2006, we began testing the blood of nestling Ospreys (*Pandion haliaetus*) for mercury and other heavy metals to establish contaminant status, pinpoint pollution hotspots, and assess the success of restoration efforts associated with past mining activities along the Clark Fork River in western Montana. We began using colored leg bands with unique alphanumeric combinations in 2010 to investigate where these Ospreys travel each winter, and where juveniles disperse. We bolstered these efforts in 2012, when we began instrumenting Osprey families from three nests near Florence, Montana with GPS transmitters. To date, we have tracked 10 adult breeding Ospreys and 17 of their young from these nests. As predicted from resightings of our color-banded Ospreys, GPS tracked individuals wintered in Mexico, northern Central America, and along the Gulf Coast. Though approximately half of our GPS instrumented nestlings survive their initial meandering fall migration and first winter, we have yet to document one successfully producing a clutch of their own young. Similarly, we have yet to document a successful nesting effort by any of the 203 color-banded nestlings from our larger regional study. These results may suggest the process of becoming a breeding Osprey is longer and more arduous than we expected when we began our study.

#### MOUNTAIN GOAT: MONTANA'S BIPOLAR ALPINE UNGULATE

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Among the four states and four Canadian provinces and territories inhabited by native populations of mountain goats, Montana is unique. More than 75 years ago, the state wildlife agency began transplanting mountain goats (495 animals to 27 locations) into previously unoccupied mountain ranges. We gathered and synthesized available harvest and population data for all native and introduced populations across the state. Supplementing these data with responses to a questionnaire we developed and sent to the 18 Montana Fish, Wildlife and Parks' biologists that manage goats, we assessed past trends and the current status of Montana's mountain goats. As introduced populations have generally prospered—numbering 2,525 or 69% of the statewide population-native populations (outside Glacier National Park) have declined from an estimated 4,100 during the 1940s to about 1,160 (31%) of the statewide population in 2016. Many populations are small and potentially demographically isolated. Whereas native herds sustained 80–90% of public harvest 50 years ago, introduced populations have produced about 85% of the statewide harvest in recent years. Total harvest has declined over that period from 300-500 to ~210 goats annually. Our survey of biologists identified likely causes of population changes and a wide range of management and research needs that would benefit mountain goat management. We have recommended development of a statewide plan for the species' long-term management and conservation.

#### USING DNA BARCODING TO SHED LIGHT ON THE DIET OF NOCTURNAL INSECTIVORES

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Like diurnal animals, nocturnal species partition the environment in ways that allow for multi-species overlap. However, the cover of night obscures many of these interactions. The MPG Ranch in the Bitterroot Valley of western Montana hosts a suite of overlapping nocturnal insectivores, including Common Poorwills (*Phalaenoptilus nuttallii*), Common Nighthawks (*Chordeiles minor*), Flammulated Owls (*Psiloscops flammeolus*), and 11 bat species. All rely on healthy nocturnal insect populations for successful breeding. Though we have a general understanding of the types of insects each species eats, we suspect that dietary composition varies based on foraging behavior. We used DNA barcoding of fecal samples to examine the dietary composition and overlap within this nocturnal insectivore community. We collected 65 fecal samples from Common Poorwills, 21 from Common Nighthawks, 13 from Flammulated Owls, and 92 from seven bat species. We will discuss the amount of dietary overlap between nocturnal insectivores and how any dietary partitioning relates to observed foraging behavior. We will also evaluate the overall effectiveness of DNA barcoding as a tool to determine the diet of nocturnal insectivores and other animal species.

# \*\*Spatial Patterns of Winter Roadside Gray Wolf Sightability in Yellowstone National Park

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Daniel R. Stahler, Yellowstone Wolf Project, Yellowstone Center for Resources, Yellowstone National Park, WY

Imperfect detection is ubiquitous among wildlife research and can affect research conclusions and management. Accordingly, detection probability is often included in observation-based models. Here, we leveraged long-term research of gray wolves (Canis *lupus*) in northern Yellowstone National Park to evaluate how the probability of sighting radio-collared wolf packs from ground-based locations was affected by the characteristics of each spatial location (i.e., distance from the road and viewability [a combination of landscape "openness" and whether visible from a viewshed created from the road and nearby observation points]). To do so, we used 2,681 unique, daily observations of 17 wolf packs collected during 44 unique 30-day winter monitoring periods from 1995 - 2017 and used matched-case control logistic regression with a 1:1 sampling design between observed and random locations. We found that the probability of wolf sightings declined as wolves were farther from the road and increased when wolves were in open, viewable areas. We then evaluated whether these conclusions were affected by wolf group size or whether wolves were feeding at a carcass and found that the probability of sightings only clearly decreased when smaller groups of wolves were farther from the road. Ultimately, we used our results to build spatial predictions for seeing radio-collared wolves in northern Yellowstone National Park. These predictions are useful to managers by identifying "hot-spots" of wolf observations, and can also be incorporated into research related to wolf ecology and predator-prey dynamics that relies on ground-based observations of wolves.

#### \*\*Hen, Nest, and Poult Survival of Merriam's Wild Turkeys in the Northern Black Hills

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In South Dakota, wild turkeys (*Meleagris gallopavo*) are a high-interest species for consumptive and non-consumptive uses. Harvest records indicate that the population in the northern Black Hills may be declining. Although data on hen survival, nest survival, and early poult survival were collected for the southern Black Hills in the early 2000s, there is currently a paucity of demographic data for the northern Black Hills. We seek to inform management of wild turkeys by characterizing demography specifically for the northern Black Hills. We radio-tracked 149 turkey hens from January 2016 through January 2018 to estimate rates of hen survival, nesting, nest survival, and early poult survival. Based on our initial results, hen survival, nesting, and nest survival may be lower in the northern Black Hills than in the southern Black Hills. Annual hen survival is approximately 45.2%. In 2016 and 2017, 77.5% and 87.5% of adult hens attempted nesting, respectively. We monitored 104 nests (45 in 2016, 59 in 2017) and initial estimates of nest survival were approximately 63.5% in 2016 and 35.3% in 2017. Poult survival to 4 weeks is comparable in the northern and southern Black Hills, but rates in both areas are lower than other portions of the species' range.

Although the northern and southern Black Hills are in close proximity, climatic differences may explain reduced productivity in the northern Black Hills. Examining the role of weather (e.g., spring and summer rain, snow depth, and temperature) on vital rates likely will provide important insights.

# **POSTER ABSTRACTS**

#### Alphabetical By Presenter's Name

In Order of Presenting Author \* Denotes Presenter \*\*indicates student presentation

## \*\*Analysis of Spiny Softshell Turtle Population Structures in Five River Systems in Eastern Montana

Gabriel H. Aponte\*, Rocky Mountain College, Billings, MT Kayhan Ostovar, Rocky Mountain College, Billings, MT Andrhea Massey, Rocky Mountain College, Billings, MT Ulrich Hoensch, Rocky Mountain College, Billings, MT

The spiny softshell turtle (*Apalone spinifera*) is designated as a species of concern in the state of Montana due to a lack of knowledge regarding their conservation status, loss of habitat connectivity and anthropogenic changes in hydrology. Information on population abundance and basic population structure for this species is necessary to understand how altered hydrological regimes and catastrophic events may affect this highly aquatic species. Spiny softshell turtles were studied in five river and creek systems in southeastern Montana. Over three years a total of 553 spiny softshell turtles were captured. The proportion of females to males captured across all five systems was not significantly different between creeks and rivers, with a total of 89.69% females and 10.31% males. We developed four length / age classes (juvenile, sub-adult, reproductive adult, mature adult) based on reported age cohorts from other studies. Numbers of spiny softshell turtles in each cohort were found to be significantly different between the five systems p < 0.00001. The Musselshell and Yellowstone Rivers and Pryor Creek had evidence of juvenile age classes and a more even distribution of age classes than the other systems. The Bighorn River had mostly larger adults and the Musselshell River lacked significant numbers of mature adults. Average seasonal water temperatures, timing and magnitude of spring pulse flows, ice cover and scour in winter, and abundance of open gravel bars, should be examined as possible factors which may explain the observed differences in spiny softshell turtle demographic structures on these five systems.

## WOLVERINE MONITORING USING INTEGRATED CAMERA TRAP Station in the Helena National Forest of Montana

Kalon C. Baughan\*, Northern Rockies Conservation Cooperative, Helmville, MT

This study tested non-invasive wolverine monitoring methods designed to achieve cost effective collection of robust genetic and natural history data useful for the development of management practices. Historically, wolverine monitoring has used bait stations to collect hair samples, confirm presence, and estimate population sizes. Individual wolverines, their sex and reproductive status can, however, be consistently identified in the absence of DNA samples through photo analysis. Here, Integrated Camera Trap (ICT) stations combined strategically

placed bait and motion-activated trail cameras with construction of wooden frameworks designed to encourage wolverines into upright, bipedal positions. Photographs of the unique ventral pelage markings that distinguish individual wolverines, as well as of sex organs and signs of reproduction were consistently captured throughout the term of the study. Hair samples for later DNA analysis were also collected from gun brushes located on the wooden frameworks. Between January and May 2016, eight ICT stations were deployed evenly across six contiguous cells in a 5 x 5 square mile grid system within the Helena National Forest near Lincoln, Montana. Six of the eight stations successfully attracted two wolverines who collectively made a total of 93 visits over the course of the study. The individuals were successfully identified as male wolverine M6 and female wolverine F7 from a library of photographs. The identity of these individuals was further confirmed through DNA analysis of hair samples collected during the study. In sum, this study supported the ability of ICT stations to collect reliable information on individual wolverine presence, sex, and reproductive status. They are cost-effective and can provide wildlife managers with valuable data.

#### Estimating Natal Origins of Migratory Juvenile Northern Goshawks Using Stable Hydrogen Isotopes

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From 2004 to 2007, we collected hatch-year feathers from 44 juvenile Northern Goshawks (*Accipiter gentilis*) captured at our Rocky Mountain Front banding station near Lincoln, Montana. Due to the relative scarcity and secretive nature of goshawks, little is understood about their migration patterns or the geographic origins of birds encountered at research sites. Most studies suggest goshawks are partial migrants, often moving <100km, but select band returns and radio and satellite telemetry have shown some individuals occasionally travel thousands of kilometers. We performed a stable hydrogen isotope analysis on the feathers we collected from young goshawks to determine their predicted natal origin. We found that 68% of goshawks had predicted natal origins relatively close to our capture site, 25% from areas in northwestern Canada and eastern Alaska, and 7% somewhere significantly south or east of our capture site. We did not find any significant patterns with sex and passage date or latitudinal origin, nor did we find a meaningful relationship between latitudinal origin and passage date. Our findings support the current understandings of goshawk migration, with a majority of individuals traveling short distances from their natal grounds and a few outliers traveling great distances, not always in a southerly direction.

## \*\*The Effect of Wildfire Smoke on Migratory Bird Species Using Nocturnal Flight Call Monitoring

Natalie W. Dulac\*, Florence-Carlton High School, Florence, MT Vanessa Haflich, Florence-Carlton High School, Florence, MT Craig Kuchel, MPG Ranch, Florence, MT

Many migratory songbirds travel at night and produce unique calls while in flight. These species-specific calls are referred to as nocturnal flight calls (NFCs) and function as a way for the birds to communicate with other individuals, echolocate, and maintain flock formation in the darker night hours. This study focused on the impact wildfire smoke density had on the frequency of NFCs recorded. The NFCs were recorded using a 21C bucket microphone from Oldbird.org, and placed at Florence-Carlton High School (FCHS), the floodplain of the MPG Ranch, and Seeley-Swan High School (SSHS). The smoke data, provided by the Montana

Department of Environmental Quality (MT DEQ), were collected by monitors located in Florence and Seeley Lake. These monitors tracked the concentration of pollutant particulate matter less than 2.5 microns (PM2.5) produced by the wildfires. No previous research on the correlation between smoke and NFCs has been found. By comparing the frequency of NFCs with smoke particulate density throughout the month of August, we hope to gain a better understanding of the impact wildfire smoke has on NFC production and potentially on migration timing and patterns.

#### **\*\*BAT ROOSTING AND HIBERNATION IN TALUS SLOPES**

Sarah M Gaulke\*, University of Montana Wildlife Biology, Missoula Dan A Bachen, Montana Natural Heritage Program, Helena Erick P Greene, University of Montana Wildlife Biology, Missoula Alexis L McEwan, Montana Natural Heritage Program, Helena

Twelve species of bats are thought to overwinter in Montana, but surveys have found few individuals hibernating within caves and mines. However, bats are consistently recorded on acoustic detectors year round across the state indicating that they are hibernating in other places. There is anecdotal evidence of bats using talus slopes for roosting and foraging, and these features may serve as important hibernacula. Pilot work using mist nets, roost searches, and acoustic detectors, was conducted at 10 sites during summer 2017 to identify active summer roosts and document species within talus slopes. To continue this work and assess what species are hibernating within these features, we placed ten acoustic recorders on talus slopes to record ultrasonic calls during winter in Northwestern Montana. In warm weather during winter, bats emerge from hibernation to drink, so winter activity may indicate hibernacula in proximity to the detectors. These acoustic data will help us estimate species diversity and relative use at each site. Additionally, we assessed attributes that may influence hibernacula suitability including: aspect, talus depth, talus size, air flow from underneath the slope, and daily temperature and humidity. In February, we will retrieve the data and analyze the number and type of calls. We will determine if the average daily number of call sequences correlates with any of the talus attributes or microclimate variables. Our goal for this project is to provide baseline data to assess the impacts of White Nose Syndrome when it reaches the state, and provide previously undocumented hibernation information.

# \*\*The Effect of Varying Moon Phase on Nocturnal Flight Calls

Destini J Greer\*, Florence Carlton High School, Florence, MT Vanessa Haflich, Florence Carlton High School, Florence, MT Craig Kuchel, MPG Ranch, Florence, MT

Many avian migrants travel at night to avoid predators and reduce the risk of increased exhaustion during the heat of the day. It is well known that nocturnal flight calls (NFCs) allow songbirds to migrate in the dark while still effectively communicating among their species. What is not understood is what factors influence the production of calls over the course of the migratory season. In 2012, the MPG Ranch in Florence, Montana joined with software developer Harold Mills to design his recording program, Vesper, around their workflow. Vesper has enabled researchers to study NFCs using remote recording devices and online software that allows species to be classified both digitally and manually based on spectrograms of individual calls. By collaborating with MPG Ranch, we hoped to better comprehend the effect of luminosity, determined by varying moon phases, on the frequency of NFCs. With the autonomous recording units set up at Florence-Carlton High School and

the MPG Ranch floodplain monitor, we collected NFC data from Savannah Sparrows, Vesper Sparrows, and Wilson Warblers, and are in the process of analyzing the relationship between their call activity and luminosity.

#### **REDUCED REPRODUCTIVE SUCCESS OF GRAY CATBIRDS IN WESTERN WOODLAND HABITATS DOMINATED BY EDGE**

Sharon M. Fuller\*, University of Montana Bird Ecology Lab, Missoula Yvette K. Ortega, U.S. Forest Service, Missoula, MT Kate M. Stone, MPG Ranch, Florence, MT

In the western United States, relatively few studies have comprehensively examined songbird performance in fragmented habitat, particularly within naturally fragmented systems. For this study, we compared territory density and breeding success of Gray Catbirds (Dumetella carolinensis) from 2014-2016 in the Bitterroot Valley of Montana, between two woodland habitat types: floodplains and naturally fragmented draws. These two habitats fell within the same human-modified landscape, abutting mixed-use grasslands. Yet, they differed in configuration or their spatial distribution. When compared to floodplain birds, results showed that draw birds had larger territories, lower daily nest survival rates, delayed nest initiation patterns and reduced fledgling success. We also collected vegetation data around nests to see if this was a potential mechanism driving the differences across habitat types. We found the percentage of down woody debris and mid-shrub canopy cover were significantly higher in draws than in floodplains. However, neither vegetation variable significantly influenced catbirds' daily nest survival rates. This excluded local vegetation as the driving mechanism behind differences and provided evidence toward configuration. Draws, as thin strips of corridor habitat, contain high amounts of edge and this configuration could lead to an increase in documented "edge effects". Our results corroborate studies in the eastern U.S. which have shown negative impacts from high edge prevalence on songbird reproduction. Overall, this study's results can assist managers in understanding that increasing the amount of edge habitat in human-altered landscapes could have negative consequences on songbird reproductive success.

## Ensuring Diversified Funding into the Future by Passage of the Recovering America's Wildlife Act

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The Association of Fish and Wildlife Agencies assembled a panel of 26 national business and conservation leaders in 2015 to recommend a new mechanism to conserve all fish and wildlife. Known as the Blue Ribbon Panel on Sustaining America's Diverse Fish and Wildlife Resources, the group recommended Congress dedicate up to \$1.3 billion annually in existing revenue from the development of energy and mineral resources to the Wildlife Conservation and Restoration Program. These funds would be awarded to state wildlife management agencies for wildlife related conservation, recreation and education projects. In Montana, the funds would be used to more fully implement State Wildlife Action Plan priorities including on the ground conservation for a broad diversity of species and habitats. Federal House Bill 5650 "Recovering America's Wildlife" act was introduced during the fall of 2016 then reintroduced in December 2017. If passed and fully funded, the federal act could bring over \$29 million dollars annually to Montana but \$10 million in non-federal match would be needed each year. Proactive and partner based projects will be critical to ensure the best use of these funds but prior to that a broad based effort to pass the act is needed. Wildlife

enthusiasts, business owners, developers, researchers and others will be needed to argue it is in everyone's best interest to keep wildlife and habitat healthy, keep species from being federally listed, and maintain outdoor education and recreation opportunities.

#### \*\* CHARACTERIZING REARING HABITAT OF AMERICAN WHITE PELICANS USING PIT TAGS FROM CONSUMED FISH

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Avian predation on wild fish populations can create challenges for wildlife and fisheries managers. Frequently, managers locate and recover indigestible tags to estimate predation rates and inform decisions. We used a similar method to characterize rearing habitat for American white pelicans (Pelicanus erythrorhynchos). During summer 2017, we sought to discern the types of vegetation and substrate white pelicans use while nesting on breeding colonies in Canyon Ferry Reservoir, Montana. First, we located passive integrated transponder (PIT) tags from marked wild fish that had been deposited on the islands and recorded the vegetation or substrate type (cobble, mud, nettle, willow, water) for each tag. Most tags were found in cobble and willow (34.3% and 36.6%, respectively) and very few were in water (0.8%) and nettle (5.3%). Second, we sought to estimate detection probability of PIT tags, as some may go undetected, and determine what factors influence whether tags are found. We hid 200 PIT tags, 40 in each of the 5 vegetation/substrate types, and found 78, resulting in an overall detection probability of 0.39 (95% CI = 0.36 - 0.42). After accounting for detection probability, we estimate that pelicans deposited the most tags, and thus were most likely to use willow vegetation (59%), with all other vegetation/substrate types containing  $\leq 17\%$ of deposited tags. Increasing the proportion of PIT tags found will influence estimates of predation and could affect inferences regarding habitat use.

# \*\*Characterizing Summer Roosts of Little Brown Myotis and Evaluating the Effects of Mountain Pine Beetle Disturbance

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Mountain pine beetle (MPB, *Dendroctonus ponderosae*) outbreaks have resulted in large-scale changes in forest structure throughout the western United States. These changes can have large impacts on wildlife, but have not been studied in bats. Given that roosting sites may limit the distribution and abundance of bat populations, we aim to 1) quantify characteristics of roosts in forests during the summer and 2) evaluate how the availability of these characteristics changes with different intensities of MPB disturbance. During the summer of 2017, we mist-netted for bats in forests dominated by lodgepole pine (*Pinus contorta*) that exhibited varying degrees of tree mortality due to MPB. Three bat species comprised 76% of captures: hoary bat (*Lasiurus cinereus*, 12%), silver-haired bat (*Lasionycteris noctivagans*, 29%), and little brown myotis (*Myotis lucifugus*, 35%). Originally, we intended to tag lactating female little brown myotis to characterize maternity roosts. However, all 42 captures of little brown myotis were male. We attached radio-transmitters to 11 males and located at least 1 roost for 6 individuals (total roosts = 18). All roosts were in rock features, even though lodgepole snags were abundant and in close proximity to roosting sites. These preliminary results suggest that in lodgepole-dominated forests, male little brown myotis choose to roost in rock features over snags, regardless of the severity of MPB disturbance.

#### EXAMINING THE UTILITY OF TIME TO EVENT MODELS FOR Abundance Estimation of a Solitary Carnivore

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Abundance estimation is a common task in wildlife biology, but techniques to estimate the abundance of low density, difficult to detect species, such as cougars (*Puma concolor*), are limited, often requiring intensive field effort and incurring high costs. Remote cameras offer an effective means of detecting these species, but most abundance estimation methods using remote camera data rely on a portion of the population being marked or uniquely identifiable. Methods to estimate the abundance of populations without identifiable or marked individuals using remote cameras have assumptions that are difficult to meet in field studies. The recent application of time to event modelling to abundance estimation relaxes these assumptions, requiring only random movement with respect to the cameras, an estimate of movement rates, and a closed population. I will use simulated walk models to test the robustness of the time to event model to violations of these assumptions likely in field studies. I will apply the model to two cougar populations in central and southeastern Idaho, and compare the abundance estimates from genetic spatially explicit capture recapture.

#### \*\*Snapping Turtle Populations and Movements in South-central Montana

Andrhea Massey\*, Rocky Mountain College, Billings MT Kayhan Ostovar, Rocky Mountain College, Billings MT Gabriel Aponte, Rocky Mountain College, Billings MT Ulrich Hoensch, Rocky Mountain College, Billings MT

Snapping turtle (*Chelydra serpentina*) populations were surveyed on a number of tributaries and other water bodies in south eastern Montana along the Yellowstone River in 2016 and 2017. While records exist from incidental observations, systematic surveys for snapping turtles have not previously been conducted in Montana. Little is known about their population density and specific habitat requirements in northern parts of their range. Anthropogenic changes in land use and hydrology may affect nesting sites and hibernacula and thus snapping turtle vital rates, while also impeding metapopulation connectivity or recolonization after localized extinctions. Eighty two snapping turtles (including nine recaptures) were recorded primarily in small creeks, ponds and lakes. Only one snapping turtle was captured on the Yellowstone River though many were found in small tributaries. When analyzed separately capture success rates were significantly different amongst five creeks (p < .05) with a higher proportion of males in all populations (p < .05). After identifying several creeks with larger numbers of snapping turtles present we fitted turtles over 8 kg with radio transmitters, beginning June 2017. Movements of radio-tagged

individuals were recorded at least monthly to determine average distances moved. Maximum river miles moved per turtle were averaged (females = 0.83 miles and males = 1.21 miles). Low numbers of females could indicate higher female mortality related to risks during nesting such as crossing roads. Snapping turtle abundance was highest in creeks dominated by cattle grazing or agricultural uses. Creeks with lower abundance were dominated by urbanization and higher density of road crossings, potentially leading to increased mortality and lower hatchling success rates.

#### \*\*Understanding Differences in Nest Site Characteristics Between Juvenile and Adult Turkeys

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Wild turkeys are one of the most-actively monitored and regulated bird species in North America. Data from the northern Black Hills, South Dakota indicate that the turkey population is declining. Nest success is an important driver of turkey populations and previous nesting experience could increase the chances of selecting a nest site where at least one egg hatches. As a result, we were curious if nest site characteristics differ between adult and juvenile hens. We monitored 88 nests and recorded nest fate (success/failure) during two field seasons. We also characterized horizontal and vertical vegetation cover around each nest at the actual or projected hatch date. Of the 43 successful nests ( $\geq$ 1 egg hatched), 33 belonged to adult hens and 10 to juveniles. Of the 45 failed nests, 30 belonged to adults and 15 to juveniles. Successful nests of juvenile hens were in locations with 20.7% greater horizontal total cover (95% CI= 5.7 to 35.7) and 16.6% greater shrub cover (-3.2 to 36.4) than successful nests of adults. However, we did not detect differences in horizontal total or shrub cover for failed nests of juveniles and adults. We also did not detect a difference in vertical vegetation cover between nests of adults and juveniles, regardless of nest fate. Retaining sufficient vegetation cover might help provide habitat features ideal for nesting juvenile and adult hens.

## \*\*GRIZZLY BEAR AND HUMAN USE AT MOTH SITES IN THE GREATER YELLOWSTONE ECOSYSTEM

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In the greater Yellowstone ecosystem (GYE), alpine aggregations of army cutworm moths (*Euxoa auxiliaris*) are an important food source for grizzly bears (*Ursus arctos horribilis*). The number of grizzly bears utilizing this food source has increased since initial documentation in 1986 in the Shoshone National Forest, Wyoming. Dozens of bears congregate and feed on moths offering a unique viewing opportunity for bear-enthusiasts, professional media, and hikers. Currently, there is a limited understanding of how bears use these areas and no information on human use. The proximity of grizzly bears and humans poses a management concern for grizzly bears and human safety. Our objectives are to quantify grizzly bear and human use patterns and to identify areas of bear-human interactions. Our methods include occupancy and written surveys, GPS tracking unit deployment, and GIS analysis. Preliminary results from our first year of bear observations (n=220) showed

48% of bears foraging on moths, 20% foraging on vegetation, and 23% travelling. We recorded 5 groups and 26 groups of human use at two locations. We documented 18 bearhuman interactions, all on high-use travel routes common to bears and humans. Despite low human use all interactions between bears and humans resulted in bear avoidance of humans. At present, bear-human interactions appear to be very low but if human use increases, interactions will increase due to lack of alternate travel routes.

### CLOVER TRAPPING MULE DEER IN NORTHWEST MONTANA: LESSONS LEARNED IN A UNIQUE ENVIRONMENT

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Mule deer (*Odocoileus hemionus*) populations are in decline in many parts of western Montana, and in 2017, harvest in Montana Fish Wildlife and Parks (MFWP) management region 1 (R1) reached an all-time low. This prompts a need to better understand mule deer habitat selection behaviors in a range of ecosystems. Using resource selection functions (RSFs), we will compare how GPS-collared mule deer select nutritional resources and hiding cover at large scales (home range level) and fine scales (within-home ranges) relative to forage quality in 3 distinct ecosystems throughout western Montana. In February of 2017, we successfully captured 30 mule deer does along the Rocky Mountain Front using helicopter net-gunning, but were unsuccessful in the Whitefish Range and Fisher River drainage of R1 due to dense canopy cover. From December 2017 to present, we have relied on a crew of graduate students, MFWP game wardens and biologists, technicians, volunteers, and private citizens to scout for and clover-trap mule deer in R1. Here we present our capture success rate thus far, though trapping efforts are ongoing. We wish highlight how creative methods and recruitment of help from across a state agency can be pooled to initiate rigorous research in a thickly forested environment on a scarcely seen ungulate.

#### \*\*Assessing Habitat Quality for Four Grassland Songbird Species of Concern in Northern Mixed-Grass Prairie

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During the past 40 years grassland bird populations have declined faster than any other avian guild in North America. In northern Montana, four species are experiencing dramatic population declines, Baird's sparrow (*Ammodramus bairdii*), Sprague's pipit (*Anthus spragueii*), chestnut-collared longspur (*Calcarius ornatus*), and McCown's longspur (*Rynchophanes mccownii*). In 2017, we began a 2-year field study to evaluate abundance, nest density, and nest survival of these species in relation to local vegetative conditions with the goal of identifying important breeding season habitat conditions to inform species and land management. We conducted fixed-radius point-counts at 50 sites to estimate local abundance, rope drag surveys to estimate nest density and vegetation surveys to estimate vegetation structure and community across grassland habitats in Phillips County, MT. Discovered nests

were monitored to estimate daily nest survival. Habitat conditions including vegetation composition and structure and herbaceous biomass were assessed at both the nest-site and plot scale. We will present preliminary information from our first year of study including effects of local and plot-level vegetation conditions on nest survival for our focal species, effects of vegetation composition and structure on local bird abundance and nest density, and the functional relationships among abundance, nest density, and nest survival of sentinel grassland birds in northern mixed-grass prairie habitat. Initial models suggest that abundance is influenced by visual obstruction, grass cover, slope, and shrub cover, and nest density was influenced by visual obstruction, grass cover, and shrub cover.

#### SAMPLING THE GRAINS: POLLEN SAMPLES FROM HUMMINGBIRDS

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Hummingbirds transmit pollen both actively and passively while feeding or moving through the landscape. They play a largely undocumented role in plant pollination on their breeding grounds. Many hummingbirds also migrate thousands of miles, potentially transporting pollen greater distances than other pollinators. To investigate the role of hummingbirds in both short- and long-distance pollen transfer, we collected pollen samples from the heads and bills of hummingbirds during migration and throughout the breeding season. We identified the pollen from 44 hummingbirds captured during the 2016 and 2017 field season in western Montana. We also solicited and analyzed 9 samples from southwestern Idaho. Pollen analysis revealed 18 different genera of pollen on sampled hummingbirds, including genera (e.g., Pinus, Larix) that passively broadcast pollen. We detected some pollen from plant species not locally available, suggesting that hummingbirds do transfer pollen long distances, and may serve as vectors for plant genetic diversity. Pollen grains differed in their anatomy and potential for adhesion to hummingbird feathers and bills, suggesting that some pollen is better suited for long-distance dispersal. We plan to continue collecting pollen samples from ours and other sites in the future. We also hope to perform experiments that investigate the role pollen morphology may play in adhesion longevity.

#### **EPAULETS IN GOLDEN EAGLE PLUMAGES IN WESTERN MONTANA**

Mary Scofield\*, Raptor View Research Institute, Missoula, MT Rob Domenech, Raptor View Research Institute, Missoula, MT Adam Shreading, Raptor View Research Institute, Missoula, MT

An uncommon plumage characteristic of North American Golden Eagles (*Aquila chrysaetos*) is the epaulet, a white feather or feathers at the shoulder, often symmetrical on both sides in appearance. The size and shape of an epaulet varies by individual. This characteristic is mentioned within the literature, but is not noted how it is reflected within a population. We have observed epaulets in 17 out of 102 (16.6%) individual Golden Eagles with at least one epaulet in western Montana since 2012. Of these individuals, 13 (76.4%) were observed on the Rocky Mountain Front while on migration, and 4 (23.6%) were observed on wintering grounds in the Bitterroot Valley, Montana. Epaulets have been observed on both male and female individuals, and on both sub-adult and adult aged birds. This plumage characteristic should continue to be recorded in Golden Eagles for a better estimate of rate of occurrence within a population.

## Conservation Planning Tools; Free Resources to Facilitate Ecological Modelling and Stakeholder Engagement

Craig Thompson\*, Conservation Biology Institute, Missoula, MT Rebecca Degagne, Conservation Biology Institute, Corvallis, OR Tim Sheenan, Conservation Biology Institute, Corvallis, OR John Gallo, Conservation Biology Institute, Santa Barbara, CA

Conservation management decisions are growing progressively more challenging, requiring consideration of ecological, social, legal, and economic factors. Land managers are expected to not only consider a diverse range potential influences and impacts, they are expected to analyze increasingly complex datasets and effectively present the results to a more exacting public audience. Numerous free, online, and open-source tools are available to help with this process, however many land managers are unaware of or unfamiliar with these resources. We present examples of three such tools and demonstrate how they can be integrated to assist with conservation planning, decision-support, and data visualization. Examples include aquatic intactness modelling in the Great Basin region, alternative energy planning in the Mojave Desert region, and forest resiliency modelling in the Sierra Nevada Mountains.

#### ACOUSTIC DETECTIONS OF UNCOMMON AVIAN MIGRANTS

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During migration, many bird species emit nocturnal flight calls that can be passively recorded, allowing great insight into avian migration ecology. Since 2012, we have used acoustic recorders to look at the composition, timing, and factors influencing avian migration in the Bitterroot Valley of western Montana. Hidden amongst the thousands of expected detections, we found evidence of many unusual or unexpected species. These species include: Barn Owl, LeConte's Sparrow, Canada Warbler, Chestnut-sided Warbler, Grey-cheeked Thrush, Upland Sandpiper, Pectoral Sandpiper, and Greater Yellowlegs. We reviewed observations from eBird, the Montana Natural Heritage Program, and published range maps to provide context for the rarity of detections. Our results suggest that acoustic monitoring may broaden the known distribution of migratory species in ways more traditional methods have not.

# MONTANA ACADEMY OF SCIENCES

#### **2018** ANNUAL MEETING

#### APRIL 6-7, 2018

#### Montana Tech of the University of Montana - Butte, Montana

Chrissie Carpenter, President, Montana Academy of Sciences James G. Berardinelli, Executive Director, Montana Academy of Sciences

#### **INTRODUCTION**

The Montana Academy of Sciences (MAS) was incorporated on the 20th day of March, 1961 as a non-profit, educational organization. The objectives of the Montana Academy of Sciences are to encourage interest and participation in the sciences and to promote public understanding of science and its contribution to society. The Academy accomplishes its objectives by conducting meetings of those interested in sciences and the education of scientists, by publishing contributions to scientific knowledge, by supporting research, by making awards to recognize accomplishments in science, by administering gifts and contributions to accomplish these aims, by assigning and cooperating with affiliated and other organizations with similar objectives and by engaging in such other activities as deemed necessary to accomplish its objectives.

We held our 2018 Annual Meeting at Montana Tech in Butte, MT. on April 6 and 7. Over 80 registrants participated, viewing 21 contributed oral presentations and 22 poster presentations over the day and a half meeting. We present the abstracts from our meeting here so that the readers of the Intermountain Journal of Sciences can see the quality and types of science supported by MAS. Please mark your calendars for our next meeting, April 5 and 6, 2019 in Butte. Finally, the Board of Directors of MAS would like to thank the sponsors of our 2018 Annual Meeting:

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#### **PRESENTATION ABSTRACTS**

#### Alphabetical by First Author's Last Name \* Denotes Presenter

#### **ECOLOGICAL EVALUATION OF RECLAMATION SUCCESS OF MINE SITES OF THE SAPPHIRE MOUNTAINS**

Jeremy Aal \*, Biology, Montana Tech, Butte

Sapphires and gems have been mined from the Sapphire range of southwest Montana for more than 150-years. Across this region and elsewhere, an unknown count of pits and tunnels and tailing piles lay abandoned while the local ecology reclaims the disturbed earth. A historical practice of reclamation for decommissioned mines was to bulldoze the site flat, removing physical hazards from the landscape. This process mixes the soil strata and alters the composition of the topsoil. Soil compaction is also a consequence of this process. Altered composition and significant increases in soil compaction often cause native plant species to struggle or fail to thrive in an area, causing opportunistic weedy species to proliferate. Our research investigated the species richness and coverage of three sites: a disturbed mine without reclamation, a mine site with bulldoze reclamation, and a third site in the vicinity which was not mined. Our multivariate analyses confirmed that species composition was different among the three sites. The natural site had higher plant cover, however, it was not significantly different from the other two sites due to the higher coverage of exotic weeds in the reclaimed sites. A greenhouse-controlled species competition supported this as soil from the reclaimed site was significantly better for knapweed test plants. Soils from the natural sites proved to be significantly better for native bluebunch wheatgrass growth. These results show that reclamation success does depend on initial site preparation, on the presence of exotic plant species that can be further spread by inappropriate site management. Also reclamation sites need to be managed if exotic invasion could be a potential.

# EXPLORATION OF THE EFFECT OF PLATELET YIELD ON 5-7 DAY STORAGE

Aja Anderson \*, Biology, University of Providence, Great Falls, MT

Platelets are a blood component essential for normal blood clotting. Donated platelets are critical for the survival of many patients, but the currently accepted shelf life of platelets is only 5 days. Thus, maintaining an adequate supply of lifesaving platelets is an ongoing concern. Platelet supply could be increased if data supported an increased shelf life. Previous studies revealed that increasing numbers of platelets in the storage bag (platelet yield) is negatively correlated to shelf life. This study explores the effect of platelet yield on the longevity of the units and examines donor differences with respect to their platelet storage profiles. We studied 12 donor units, split into 36 experimental bags, loaded at four yield levels. Our goal was to ascertain the maximum platelet yield that maintained a bag pH above 6.2 at day 7. A platelet bag is considered "failed" when the pH drops below 6.2. We found that higher platelet yields correlated with decreasing bag pH, while donor differences were related to the magnitude of the bag failure. No bags loaded at nominal yields (the most commonly used yield) failed by day 7. Of note, none of the 18 bags loaded at high-nominal yields (a less common current practice) failed at day 5, but 3 failed before day 7. These results confirm

previous studies and support manufacturer determined yield limits and shelf life. Yields at and above manufacturer limits were associated with high failure rates, and are not recommended.

#### ANALYSIS OF ANTIMICROBIAL PEPTIDE EFFICACY AGAINST CHYTRIDIOMYCOSIS FROM SKIN SECRETIONS OF COLUMBIA SPOTTED FROGS (LITHOBATES LUTEIVENTRIS)

Emma Esposito \*, Biology, Carroll College, Helena

Amphibian populations have been declining in size in recent years. A major contributing factor to this decline is the fungal disease chytridiomycosis. Chytridiomycosis occurs when the zoospores of the fungus Batrachochytrium dendrobatidis (Bd) imbed into amphibian skin and disrupt the homeostatic functions the skin provides, leading to death in most amphibians. Amphibian skin can produce antimicrobial peptides (AMPs) that inhibit Bd infection. The goal of this experiment was to determine whether or not the amount and type of AMPs a frog produces affects its ability to defend against chytridiomycosis. Columbia spotted frogs were chosen as a model organism because they vary in susceptibility to the disease, yet their populations have not been drastically affected by the fungus. AMP samples were collected from ten Columbia spotted frogs at four different locations in western Montana (40 samples total). The AMP concentration for each sample was determined using a micro BCS assay. The minimal inhibitory concentrations (MICs) were determined using 96 well plate growth inhibition assays. In the assay a set of ten AMP dilutions from each frog (ranging from 100-1000 µg/mL) were used. The Bd was grown in each dilution and growth was measured after four days as change in absorbance measured at 492 nm. Infection load of frogs was determined using Quantitative PCR analysis. From the data collected, there was no statistically significant correlation between the MIC observed and infection load.

#### **METAL-MICROBE INTERACTIONS IN SILVER BOW CREEK**

Jordan Foster \*, Environmental Chemistry, Montana Tech, Butte

This project studies the relationship between microbes and both metals and metalloids in Silver Bow Creek. Biotic manganese nodule formation in Silver Bow Creek was identified through previous studies. The formation of these nodules is of interest to Silver Bow Creek restoration since heavy metals can bind to the nodules and potentially lower contaminants of concern. The goals of this study were to identify the organism(s) involved in the formation of manganese nodules and to identify how the nodules were formed using proteomic analyses. In addition, water chemistry data was used to study relationships between several elements in the creek and the potential effect these factors have on the microbial life. A combination of microbial culturing and LC-MS was used in an attempt to isolate the microbes involved with nodule formation and identify the proteins produced by the microbes. Currently, this work is attempting to isolate a microbe in the creek and analyze metagenomic data to predict the proteins predicted, proteomic analyses with LC-MS can proceed. This work, in its current phase, has implications upon the interactions of microbial communities with contaminants of concern in Silver Bow Creek.

#### DETECTION OF COLORADO TICK FEVER VIRUSIN DERMACENTOR ANDERSONI

Zach Hart \*, Cell Molecular Biology, Montana Tech, Butte

Colorado tick fever virus is a double stranded RNA (dsRNA) virus transmitted by Rocky mountain wood ticks (Dermacentor andersoni), which can be found at high elevations in a number of western states including Montana. Although Colorado tick fever often presents as flu-like symptoms that are usually not life threatening, twenty percent of those infected are hospitalized. Little is known about the evolution of CTFV. The reference strain, Florio, has been sequenced, but this particular strain was isolated in Colorado in 1943. We hypothesize that isolates collected in Montana would have novel mutations because of the geographic distance and time between them and the reference strain. Ninety-seven ticks were collected in a previous SURF project this summer, mostly from Maud S. Canyon. RNA was extracted from crushed ticks and reverse transcribed. PCR successfully amplified CTFV cDNA from a positive control, but none have been detected in the tick samples to date. In contrast, using primers for a tick actin gene, PCR resulted in an amplicon of the expected size. This would suggest that the nucleic acids were collected from the tick. While no positive results for CTFV in the ticks have been obtained, there is reason to believe that the protocols developed are working. Our results are inconsistent with other studies that have found the virus in 21% of ticks sampled in Wyoming.

# GROUNDWATER/SURFACE WATER INTERACTIONS ON THE CLARK FORK RIVER

Eva Hover \*, Geological Engineering, Montana Tech, Butte

Grant-Kohr's Ranch is a National Historic Site located near Deer Lodge, MT. The ranch stretches along a two mile reach of the Clark Fork River, which has been contaminated by mine tailings. Plans for remediation are in progress, and information regarding where groundwater enters and leaves the river is important in this remediation process. A handheld temperature (T) and specific conductivity (SC) probe was used to look for spatial anomalies of these parameters that may indicate where groundwater enters the river. To do this, fifteen cross sections consisting of several T and SC readings were collected perpendicular to flow, and several readings were collected along the river banks (no cross sections) between each cross section. Significant spikes in specific conductivity values are consistent with groundwater entering the river. Zones of low high water temperatures in the winter is consistent with areas where groundwater warmer than the surface water is infiltrating the river. This idea holds true in warmer months as well, as areas where low temperatures are logged may show where cooler groundwater is infiltrating the warmer surface water. Radon-222 (222Rn) was collected along both the right and left banks at all fifteen monitoring locations to verify groundwater inputs inferred from T and SC measurements. 222Rn de-gasses quickly when a river flows from a gaining to a losing reach, so this is an ideal validation. Local lithology suggests a sandy gravelly aquifer that would interact with surface water. Both 222Rn combined with T and SC suggest intermittent zones of groundwater inflow along the river banks, often only occurring along one side of the river at a time.

## INTEGRATION OF A FLUXGATE MAGNETOMETER AND GPS SYSTEM INTO AN UNMANNED AIRCRAFT SYSTEM

Sierra Luoma \*, Geophysical Engineering, Montana Tech, Butte

Magnetic method has been widely used for exploration of magnetic minerals or rocks and the detection of artificial objects such as unexploded ordinances (UXOs). However, a ground-based magnetic survey is time-consuming. Aeromagnetic surveys will be faster and more efficient. In this project, we integrated a fluxgate magnetometer and GPS units with an unmanned aerial vehicle (UAV), i.e., Matrice 600 Pro, to measure magnetic anomalies for aero-geophysical exploration. To achieve this objective, we used an Arduino microprocessor to control a triaxial fluxgate magnetometer (FGM-301/310) and two GPS units (ANT-GPS-SH-SMA). Arduino IDE codes were written for simultaneous data collection from the magnetometer and the two GPS antennas and for storing the data to a microSD card. These components will be attached to the DJI Matrice 600 Pro UAV. The whole system will be tested using a UXO sample. We will fly the UAS with the magnetometer over a sample UXO. With the two GPS antennas, the location and orientation of the magnetometer is obtained so that the three components of the magnetic field can be calculated. The data collected will be processed to create a georeferenced map of magnetic fields and will be interpreted. The accuracy of the fluxgate magnetometer and UAV system will be evaluated.

#### CHARACTERIZING EXCITED STATE DIFFUSION IN PCDTBT USING TRANSIENT ABSORPTION MICROSCOPY

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Organic semiconducting polymers (OSPs) are an attractive alternative to traditional inorganic semiconductors for use in photovoltaic devices and other optoelectronic applications because they are cost effective and solution processable. Here we describe our efforts towards under-standing excited state transport in micron-scale domains of the OSP, poly [N-9"-hepta-decanyl-2,7-carbazole-alt-5,5-(4',7'-di-2-thienyl-2',1',3'-benzothiadiazole)] (PCDTBT) utilizing transient absorption microscopy (TAM). Using TAM, we directly image excited state diffusion across mi-cron scale domains of PCDTBT thin films, reducing the effects of morphological heterogeneity in these complex polymeric systems. To further understand exciton diffusion and dissociation dynamics we have begun the development of two individual experiments. This presentation will discuss the theoretical evaluation of both experiments as well as preliminary experimental development. The first experiment has been investigated by Monte Carlo simulation of exciton dissociation at microfabricated donoracceptor interfaces. The second experiment relies on the fabrication of polymer devices so that carrier diffusion can be characterized under the influence of an electric field. Investigation and implementation of these experiments was made possible by support from the Montana Academy of Sciences and will provide a more thorough understanding of the excited state transport dynamics in micron scale regions of PCDTBT.

#### INVESTIGATING THE ROLE OF DNC-2 AND DLI-1 ON AMPA Receptor Mediated Behaviors In C. Elegans

Natalie Oberding \*, Biology, Carroll College, Helena Stefanie Otto-Hitt, Biology, Carroll College, Helena

 $\gamma$ -amino-3-hydroxy-5methylisoxazole-4-proprionic acid (AMPA) receptors (AMPARs) are protein complexes involved in excitatory neurotransmission. AMPARs are tetrameric structures consisting of the pairings of GluA1, GluA2, GluA3, and GluA4 subunits. Dctn2 and Dync1li1 were found to interact with GluA2 in an immunoprecipitation screen that was performed on postnatal day 14 rat brains. DNC-2 and DLI-1 were identified as the C. elegans homologs of Dctn2 and Dync1li1, respectively, and are components of motor proteins that transport cargo throughout the cell. The goal of this project was to determine whether knocking down DNC-2 and DLI-1 by RNAi affects AMPA Receptor-mediated behaviors, including mechanosensation and chemosensation, in C. elegans.

#### HYALURONIC ACID CONJUGATED WITH ANTIOXIDANTS FOR TREATMENT OF CMV-INDUCED HEARING LOSS

Zoe Peach-Riley \*, Hellgate High School, Missoula, MT

Cytomegalovirus (CMV) is the leading nongenetic cause of sensorineural hearing loss (SNHL) in children in the United States. Current antiviral treatments exist, however with only modest short-term improvements and uncertain long-term outcome as well as reports of complications. Because CMV causes SNHL through inflammation and oxidation of the inner ear, an anti-inflammatory compound such as hyaluronic acid (HA) can be modified and combined with an antioxidant compound like D-methionine as a potential treatment. Preliminary results indicate successful synthesis of the compound CMHA+Dmethionine as well as demonstrated cytocompatibility. Upcoming procedures will include testing the combined anti-inflammatory and antioxidant effects of the novel compound CMHA+D-methionine and investigating its potential to act as an effective treatment for CMV SNHL.

#### MECHANISTIC AND KINETIC INSIGHTS OF [CU(PDTC)L]

Dr. Matt Queen, Biological and Physical Science, Montana State University Billings, Billings Daniel Willems, Biological and Physical Science, Montana State University Billings, Billings Alexander Fryett \*, Biological and Physical Science, Montana State University Billings, Billings

Carbon tetrachloride (CT) is a known carcinogen. It is know that when pyridine-2,6bis(thiocarboxylic acid) (PDTC) forms square planer coordinate complexes with copper(II) the combination can dechlorinate CT stoichiometrically. In this study three different complexes, where the position four ligands are varied Cu(PDTC)L (L= Cl-, CN-, and PPh3), were used to probe the mechanism of the dichlorination reaction. GC-MS kinetic assays were performed to assess the aqueous dechlorination kinetics while organic solvents were used in UV-Vis assays to test organic based solvents. Results on the UV-Vis show that Cu(PDTC) PPh3 shows the most significant reaction with CT in organic solvent, followed by Cu(PDTC) CN with very limited reactivity, and finally Cu(PDTC)Cl with no reaction. Water-based kinetics were done in phosphate buffer with Cu(PDTC)Cl, Cu(PDTC)CN, and Cu(PDTC) PPh3. Cu(PDTC)Cl showed the most significant loss in CT in air and under inert gas. In addition variable temperature kinetics assays were performed to elucidate activation energies for the reaction.

# SINGLE PARENTS DESCRIBE BALANCING ROLES: PARENT AND STUDENT

Rayvn Scott \*, Highlands College A. S., Montana Tech, Butte

The purpose of this qualitative study was to explore lived experiences described by single-parent students while balancing their roles: parent and student. A qualitative design included a hermeneutic approach with interpretation that consisted of a transcript coding process. Approximately 30 hours were spent reading through transcripts, while highlighting words which identified themes. Seven themes evolved: structure, stress, not enough time, guilt, isolation, self-care, perfectionism. For example, structure, isolation, and not enough time were themes reflected as Meg stated, "I know what a priority is and what isn't. Friends are not a priority. It's like this constant balancing act. Everything is going a million miles a minute, so fast - the teaching, homework, learning, my kids, the days, studying." Oncampus single-parent students' academic support and family-friendly social activities were limited; inequities in social justice were evidenced. Following themes of structure, stress, and isolation, a homework-support group on campus was offered twice a week. There was scarce involvement by single-parent students. After the last six interviews, a different approach was used for support by following the themes of guilt and isolation. On campus, family-friendly social activities were offered and participation was successful. By offering family-friendly social activities on campus, single-parents received improved support while balancing their responsibilities of parent and student.

#### PROSOCIAL BEHAVIOR AND EMPATHY: ANALYSIS OF EMOTIONAL Responses During the Use of Ted Talk Videos in the Classroom

Robert Shelton \*, Highlands College A. S., Montana Tech, Butte

Empathy may increase when observing prosocial behavior of others. The purpose of the study was to evaluate students' emotional responses when observing prosocial behavior demonstrated in a Tedx Talk video through the lens of cognitive empathy and affective empathy. Tedx Talk videos are often shown in college classrooms to enhance learning outcomes. Student participants (N=157) completed the Interpersonal Reactivity Index pre/post assessment to examine if differences of empathy were measurable from before watching the video and after. Paired Samples t test Analyses were run to explore if there were differences between groups over time. Independent Samples t tests were used to understand differences between groups: age, gender and parental status. Results suggested statistically significant changes in empathy both over time and between groups.

# REQUIREMENTS FOR AGGRESSION: ALTERING EAAT1 EXPRESSION TO MANIPULATE GLUTAMATE UPTAKE IN DROSOPHILA MELANOGASTER

Lucy Sirrs \*, Hellgate High School, Missoula, MT

Aggression is a behavior required for survival and reproduction. Research has identified several neurons and neurotransmitters (signaling molecules) that are important for promoting aggression, but the neurotransmitter glutamate has only recently been identified, and its role is not fully understood. This research examines how manipulation of glutamate expression via altered expression of its transporter, EAAT1, alters aggression in the Drosophila model

organism. Glutamate function was reduced by increasing expression of the glutamate transporter, EAAT1 (Excitatory Amino Acid Transporter 1). EAAT1 recycles extracellular glutamate to regulate neuronal communication. By increasing the amount of EAAT1, more glutamate is recycled, reducing its ability to signal to the downstream neuron. EAAT1 expression was increased via controlled transgenic expression. Conversely, glutamate function was increased by reducing expression of EAAT1 via RNA interference. Using genetic tools and antibody labeling, it was verified that EAAT1 is widely expressed in glial cells throughout the brain. A reduction in glutamate produced male flies that were less aggressive compared to controls, similar to previous research that manipulated glutamate expression only in octopamine neurons. Specifically, experimental flies took longer to start fighting, lunged less, and produced fewer wing threats and wing extensions. Flies with increased glutamate levels were also less aggressive, though this may be due to limited function at the neuron or the neuromuscular junction, where a motor neuron and a muscle fiber meet. These results demonstrate glutamate function is required for sex-specific behavior and provide a foundation to determine the role of glutamate in aggression circuitry in any system.

#### CALIBRATION AND APPLICATION OF MONTANA TECH SHAKE TABLE Apparatus

Brent Sordo \*, Geological Engineering, Montana Tech, Butte

In the field of geotechnical engineering, a key task when designing earth structures is preparation for seismically induced loads. To directly study the impacts of these loads upon geologic structures, models or samples of the structures are tested with a seismic shake table, an apparatus that precisely accelerates back and forth according to a specific pattern. Montana Tech recently acquired a high quality, unique shake table, and this project was the first use of it. Initially, the table was installed and a booklet outlining its use and specifications created. With the table in use, the efficacy of its ability to model acceleration-induced loads was tested by subjecting a number of rock joint samples to seismic loads of varying intensity. The critical acceleration value, the value at which static friction is overcome, will then be identified and compared to other methods of frictional analysis such as tilt tests. The critical acceleration can be implemented into a calculation to find static coefficient of friction, as can a friction angle acquired from tilt tests of the same samples, allowing for direct comparison. Furthermore, samples subjected to sinusoidal wave patterns can also be compared to dynamically loaded discrete element models for further verification. This project itself is preliminary, buy with this relationship proven, the application of the shake table can proliferate to more complex simulations such as liquefaction, structural fundamental frequencies, and complex earthquake time histories.

## Comparison & Analysis of Local Environmental Metagenomics and Diversity Sequencing Data Sets

Lucas Stout \*, Software Engineering, Montana Tech, Butte

Metagenomics is the rapidly advancing field that studies genetic material extracted directly from environmental samples. Recent advances in computational and sequencing methodologies now give an array of options to biologists who seek to analyze their samples; however, these new technologies are often run independently with little comparison of the results obtained between methods on the same sample. Prior to this study, field work by local biology instructors in conjunction with the labs of Drs. Marisa Pedulla and Alysia Cox made use of two such techniques to analyze the DNA of a locally-obtained soil sample. One

technique, "shotgun," or metagenomic sequencing, sequenced all of the DNA molecules in the sample; the second technique, "diversity" sequencing, only sequenced the molecules amplified from a single gene, the 16S ribosomal subunit rRNA gene, of the prokaryotic DNA in the sample. Because the 16S gene is highly recognizable and species-specific, the latter method also provided a count of the occurrences of each prokaryotic species. Due to this feature, the use of the 16S diversity sequencing approach is commonly utilized for studies aiming to understand prokaryotic species representation in samples. When metagenomics DNA sequences are known, represented species and their number of occurrences in a sample may also be derived computationally by comparison to procured databases of known sequences of organisms. This exploratory study compared the commercially obtained results of bacteria proportions in our sample by the diversity method, along with two experimental computational methods using these sequences compared to the public databases. Our hypothesis was that metagenomics data would provide the most accurate portrayal of bacteria in the sample at the phylum taxon. Results found significant disparities in results between each method, with implications in microbiome studies of the environment and human gut.

# IN VITRO SYNTHESIS OF APATITE COATINGS ON TITANIUM ALLOY SP-700 to Improve Biocompatibility

Ben Suslavich \*, Metallurgical & Materials Engineering, Montana Tech, Butte

Titanium and its alloys are commonly used in the biomedical industry for their superior strength, corrosion resistance, machineability, fatigue resistance, elastic moduli, and biocompatibility; however, endosseous implants struggle with osteointegration due to poor adhesion between the implant and the receiver's bone. It is known that by synthesizing a bone-like apatite surface coating, integration of the implant with surrounding bone is not only quicker, promoting shorter recovery times, but also reduces interface stress concentrators creating a more robust implant. This study focused on synthesizing an apatite coating via the sodium titanate method on the titanium alloy SP-700 which is a contender to be used as an implant material having a similar elastic modulus to bone. The synthesized coatings, which formed after treatment in simulated body fluid solution were characterized for topography and elemental composition using SEM/EDS analyses. The results of this study show that it is possible to synthesis thin apatite layers on the surface of SP-700 with a topography conducive to improving bone growth onto the surface of the implant.

## CLONING GRNAS TO ENABLE CRISPR-MEDIATED HUMAN GENE KNOCKOUTS

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The human genome encodes over 80 members of the tripartite motif (TRIM) protein family. Many of the TRIM proteins are upregulated in cells responding to interferon-beta (IFNβ). Interestingly, screens have shown that approximately half of the TRIM proteins act on interferon signal transduction pathways in positive and negative feedback loops. Cell lines are being created that can be used as tools for identifying the step of IFN signaling acted upon by TRIM proteins. To create tools for this goal, we have cloned guide RNAs targeting IFNB, IFNAR1, IRF3, and IRF7 genes into the pSpCas9(BB)-2A-EGFP vector using recombination techniques. Success of cloning was confirmed by restriction digests and DNA sequencing. Human cells (293T) were successfully transfected and are currently being cultured to attain monoclonal EGFP+ lines. Screening for loss of gene expression in clonal lines will assessed by immunoblotting as well as tested functionally using luciferase reporter assays.

#### **ERGONOMIC EVALUATION OF A HIGH-PERFORMANCE GAMING INPUT DEVICE AND A COMPARISON WITH TRADITIONAL DEVICES**

Jacob Van Dyke \*, Occupational Safety and Health, Montana Tech, Butte

Objective: The goal of this study is to address the need for research on the ergonomic effects of computer gaming by providing an objective, ergonomic evaluation of a gaming input device using a simulated gaming task that compares ergonomic and performance measures with traditional computer input devices. The computer input devices included one traditional, a rollerball, and a gaming input device.

Methods: 20 participants wore sEMG sensors placed on the abductor pollicis brevis of the right hand. The subjects' maximum voluntary contraction (MVC) was recorded at the beginning of the study. Performance was measured by recording the number of correct sequences that subjects completed during their trail, while force was measured using sEMG. A Welch's one-way ANOVA (Analysis of Variance) was used to analyze statistical significance of the data; a Games-Howell Pairwise Comparison test was used for the post hoc analysis.

Results: The difference in mean force exerted by the abductor pollicis brevis for the input devices was significant (p<0.001). The number of correct sequences completed by each subject, was also significant (p<0.001). Two post-hoc comparisons were statistically significant for each data set. The %MVC for the gaming input device was significantly different from both the rollerball input device (p=0.021) and the traditional input device (p<0.001). The number of correct sequences for the traditional input device was significantly different from both the gaming input device (p<0.001) and the rollerball input device (p<0.001) and the rollerball input device (p<0.001).

Conclusion: The results of this study suggest that there is a possibility that gaming input devices increase force exertion while performing gaming tasks which may increase the risk of developing MSD's of the hand and thumb, without an apparent increase in performance within the constraints of this study. Further research is needed to determine if similar results exist during a gaming task performed by actual gamers.

## ELONGATOR FUNCTION IN THE ANTERIOR PITUITARY AND ITS Relevance to Familial Dysautonomia

Joseph Walters \*, Biological and Physical Sciences, Montana State University - Billings, Billings Joy Goffena, Biological and Physical Sciences, Montana State University - Billings, Billings Richard Buksch, Biological and Physical Sciences, Montana State University - Billings, Billings Lynn George, Biological and Physical Sciences, Montana State University - Billings, Billings

Familial Dysautonomia (FD) is a devastating neurodegenerative childhood disease characterized by a diminished number of autonomic neurons. FD children suffer from a multitude of autonomic symptoms including cardiovascular instability, gastrointestinal incoordination, and respiratory dysfunction. FD patients also exhibit an abnormal autonomic stress response, show poor growth velocity, and have difficulty gaining and maintaining weight. Treatment with growth hormone (GH) has been shown to increase growth velocity in FD patients. FD results from a mutation in the IKBKAP gene and diminished levels of the corresponding protein IKAP, a scaffolding protein that assembles a multi-subunit complex called Elongator. Elongator functions in the modification of tRNAs that mediate translation of AA- and AG-ending codons including lysine, glutamine, and glutamic acid. In the absence of Elongator, small AG biased genes are upregulated and large AA-biased genes are downregulated. IKAP is expressed throughout the autonomic nervous system and historically

FD has been considered a strictly neurological disease. Here we show that IKAP is robustly expressed in the pituitary gland, indicating a strong dependence on Elongator. We hypothesize that compromised growth in FD may actually result from dysfunction of somatotrophs in the anterior pituitary, a non-neuronal cell type. To test this hypothesis, we generated a conditional knockout (CKO) mouse where Ikbkap is selectively ablated in anterior pituitary somatotrophs. These CKO mice exhibit decreased growth compared to control littermates. Surprisingly, quantitative immunohistochemistry indicates that GH1 levels may actually be increased in the CKO pituitary. CaBP7, a calcium binding protein that negatively regulates vesicle trafficking, is also found at elevated levels in the CKO, likely because of its strong AG-bias. In combination, these results suggest that upregulation of CaBP7 may inhibit GH1 exocytosis from pituitary cells, decreasing the amount of circulating GH1 and compromising growth in FD patients.

#### MINERALOGY OF A URANIUM-COPPER BRECCIA PIPE

Zane White \*, Geological Engineering, Montana Tech, Butte Robert Hofer, Geological Engineering, Montana Tech, Butte

In northern Arizona, uranium-rich breccia pipes are common, however, these deposits are rarely found to contain 36% copper. Energy Fuels sent fifteen drill-core samples from their Canyon mine to Montana Tech. These samples contain varying amounts of copper and uranium from different areas of the deposit. The samples were cut, trimmed, placed into epoxy, and polished. A total of 36 polished "plugs" were made. The plugs were scanned using an X-Ray Fluorescence (XRF) device to determine rough estimates of element composition. Each plug was then carefully observed under a reflected-light microscope. The mineralogy and estimated amounts of minerals were noted. Plugs that were seen to have interesting features, high amounts of unusual elements, or unknown minerals were placed in a scanning electron microscope (SEM). The SEM uses electron dispersive spectroscopy (EDS) to obtain a chemical analysis of a specific point on the plugs which aids in mineral identification. Some specimens were also examined by Raman spectroscopy and X-ray diffraction (XRD). The most abundant mineral found in our samples was tennantite (Cu6[Cu4(Fe,Zn)2]As4S13) followed by chalcopyrite (CuFeS2). We also found the minerals uraninite (UO2), pyrite (FeS2), bornite (Cu5FeS4), galena (PbS), sphalerite (ZnS), chalcocite (Cu2S), covellite (CuS), and rammelsbergite (NiAs2). These minerals were deposited by hydrothermal fluids into a quartz grain matrix, in which they act as a cement holding the grains together. The discovery of rammelsbergite, a nickel mineral, was an unexpected find. The results of this project will be sent to Energy Fuels where they will use the data to help in milling and metallurgy. This mineralogy study may also help the mine understand potential sources of metal contamination for future mining wastes. One of our recommendations to Energy Fuels will be to assay their ore for recoverable nickel in addition to the other metals (Cu, U, Pb).

# **Poster Abstracts**

#### Alphabetical by First Author's Last Name \* Denotes Presenter

#### CREATING AN EFFECTIVE PUBLIC HEALTH CAMPAIGN REGARDING THE POTENTIAL HEALTH RISKS OF CHRONIC WASTING DISEASE

Kirina Amada \*, Microbiology & Immunology, Montana State University, Bozeman

Margaret (Mari) Eggers, Microbiology & Immunology, Montana State University, Bozeman

Creating an effective public health campaign regarding the potential health risks of Chronic Wasting Disease

Introduction: Chronic Wasting Disease (CWD) is a neurodegenerative disease in the family of Transmissible Spongiform Encephalopathies (TSE), also known as Prion diseases. CWD has recently been detected in cervids in Montana, posing a potential health risk to game meat consumers. Appropriate places to obtain information on CWD and test meat for infection are low profile. The purpose of this study is to identify and create an effective public health campaign about CWD.

Methods: Information regarding CWD is being obtained from the scientific literature and government websites. Additionally, presentations are being made to obtain ideas from the public. Both print and online educational materials will be made and disseminated to the MSU and Bozeman public.

Results: Recent experiments have shown that CWD can be transmitted to Macaques, genetically closely related to humans, via feeding infected muscle or brain tissue from elk and deer, including from asymptomatic cervids. Although there are no reported cases of animal to human transmission of CWD, people who have contact with and consume game meat are at possible risk of becoming infected. Prion diseases are 100% fatal. The State of Montana is offering the opportunity to get your game meat tested.

Conclusion: Despite all the risk factors, education regarding CWD in the community is limited to a couple State websites. More detailed information is needed, especially for hunters and butchers and must be disseminated to the local public.

#### Analysis of Modified Vaccinia Ankara Tropism and Growth Conditions

Synda Boumediene \*, Biological and Physical Sciences, MSU-Billings, Billings Paul Nash, Biological and Physical Sciences, MSU-Billings, Billings

Vaccinia is a large enveloped virus belonging to the poxvirus family. It has linear, double stranded DNA, averaging about 190 kbp in length. Although vaccinia's origin is unknown, it is closely related to cowpox, smallpox and horsepox. It is used as a vaccine for smallpox. Recombinant vaccinia is also used a vaccine for multiple other diseases. We employed a mutant form of the virus, modified vaccinia Ankara (MVA), which has a limited host range and limited virulence compared to the wild-type vaccinia, which infects almost all cell types. MVA was established to infect the hamster cell line BHK-21. This cell line was used to test culture conditions and to determine the tissue culture infectious dose 50% (TCID50), which is the measure of the results. This work was undertaken to establish how different culture and infection conditions affect virus growth. This study reports several factors that influence growth and preliminary results for infection rates in several cell lines.

# EFFECTS OF DOPAMINE BETA HYDROXYLASE LEVELS IN A MOUSE MODEL OF FD

Richard Buksch \*, Physical and Biological Sciences, MSUB, Billings Joy Goffena, Physical and Biological Sciences, MSUB, Billings Joseph Walters, Physical and Biological Sciences, MSUB, Billings Lynn George, Physical and Biological Sciences, MSUB, Billings

Familial dysautonomia is a severe, recessive disease that devastates the peripheral nervous system, culminating in death of most patients by age 40. The most debilitating feature of familial dysautonomia is the severe autonomic crises that occur. These crises, which can sometimes last for days, cause extreme vomiting and nausea, among other symptoms. The crises have been shown to coincide with an increased level of circulating dopamine following stress. The current hypothesis suggests that elevated levels of tyrosine hydroxylase cause an overproduction of dopamine. The chromaffin cells cannot convert this dopamine into norepinephrine quickly enough; therefore, this dopamine is released into the blood stream. We propose an alternate hypothesis in which the levels of dopamine beta hydroxylase are instead reduced. Reduction of dopamine beta hydroxylase, the enzyme that converts dopamine to norepinephrine, would result in a larger amount of dopamine being released from chromaffin cells during the response to stress. This reduction in enzyme levels is also seen in dopamine beta hydroxylase deficiency, a disease that shares many of the same symptoms of familial dysautonomia. In support of this hypothesis, we have shown through quantitative RT-PCR that dopamine beta hydroxylase transcript levels are decreased in Wnt1-Cre; IkbkapLoxP/ LoxP conditional knockout (CKO) embryos in which Ikbkap is ablated in the adrenal gland. Further analysis of the CKO using immunohistochemistry indicates that DBH protein levels may also be diminished as well as mis-localized within the cell.

# Investigating the Binding of BH31-1 Derivatives to the BCL-XL Protein

David Butler \*, Biological Sciences, Montana State University-Billings, Billings Kurt Toenjes, Biological Sciences, Montana State University-Billings, Billings Joy Goffena, Biological Sciences, Montana State University-Billings, Billings

In order to develop promising drugs for infectious disease, it is important to find molecules that specifically target the infectious agent without harming human cells. The Butler/Toenjes Lab has recently published an article showing that a small organic molecule called BH3I-1 and several of its derivatives have potent anti-fungal activity against Candida albicans, a common human pathogen. BH3I-1, however, is known to be toxic to human cells through binding to the Bcl-XL and Bcl-2 proteins. The goal of this project is to identify anti-fungal derivatives of BH3I-1 that do not bind the Bcl-XL and Bcl-2. Such BH3I-1 derivatives would have the potential for low human cell toxicity.

# THE EFFECTS OF PULSED ELECTRICAL STIMULATION ON THE QUADRICEPS FEMORIS MUSCLE PRIOR TO EXERCISE

Evalani Dahlin \*, Biology, University of Providence, Great Falls Dr. Kelly Quick, Exercise Science, University of Providence, Great Falls Jessica Martin, Biology, University of Providence, Great Falls Meredith Berthleson, Mathematics, University of Providence, Great Falls

Neuromuscular electrical stimulation (NMES) is a commonly employed modality for rehabilitation and is growing in utilization due to its physiological effect in muscular activation. Recent comparative studies have examined utilizing stimulation versus isometric exercise and differences between genders on the effects of administering NMES after physical activity for recuperative purposes, but very little information exists regarding the effects of pre-stimulation to specific muscle groups for strength enhancement. This study aimed to answer this question by pre-stimulating the quadriceps femoris muscle prior to resistance training to determine if there is greater improvement in overall strength performance of the lower body. Fifteen healthy, recreationally and/or competitively active college students (male and female, ages 21 + 3 years, weight 152.8 + 42.2 lbs, height 66.1 + 6 inches) with at least 2 years of resistance training experience performed a 8-week strength training program targeting the quadriceps femoris muscle and its synergists (gluteus maximus, adductor magnus, soleus, gastrocnemius) for three nonconsecutive days each week. Individuals were randomly selected for placement in either the experimental (n=7) or control group (n=8). The experimental group received pulsed NMES prior to the workout for 15 minutes. Training volume for each participant was collected weekly to measure participants' progress, and 1-repetition maximums were collected before and after the workout program to quantitatively determine any impact on strength from pulsed NMES. A Mann-Whitney U-test was used to statistically compare the improvements in strength performance between groups. The results indicated that pre-stimulation of the quadriceps muscle before exercise will result in enhanced strength performance. This study shows that administering NMES prior to resistance training is an efficient method of pre-activating muscle fibers, conditioning the muscle to tolerate longer and more forceful contractions during exercise, and allow for greater improvement in strength to be achieved more quickly.

# XFEB, A DIRECT TARGET OF ZIC1, IS INVOLVED IN NEURAL CREST DEVELOPMENT

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The peripheral nervous system, melanocytes and craniofacial cartilage and bone arise from neural crest cells that develop during early embryonic neural development. Transcription and signaling factors form a network to regulate this development. For example, it has been shown that Zic1 and Pax3 in conjunction are able to induce full neural crest cell development (Monsoro-Burq et al., 2005). Xfeb and Gbx2 also play roles during neural crest cell development as they are present in the same regions and developmental stages as the neural crest (Plouhinec et al., 2014; Li et al., 2009). A microarray identified Xfeb as a direct, downstream target of Zic1 (Li et al., 2006). An additional lab also identified Xfeb as a neural crest gene induced by Zic1 (Plouhinec et al., 2014). We hypothesize that Pax3, Xfeb, Gbx2 and Zic1 are all part of the same gene regulatory network controlling neural crest development. To investigate the relationship between the Xfeb, Pax3, Gbx2, and Zic1 genes, we first upregulated Xfeb gene expression with sense RNA and down regulated Xfeb gene expression with morpholino oligonucleotides (MO). We used in situ hybridization to visualize neural crest induction by staining for Slug RNA expression, a known neural crest marker. Our results showed that embryos injected with Xfeb sense RNA expanded Slug expression while those injected with Xfeb MO diminished Slug expression. Given other labs' results suggesting that Zic1 plus Pax3 or Zic1 plus Gbx2 induced ectopic Slug expression, we will determine whether Xfeb plus Pax3 or Xfeb plus Gbx2 genes can induce ectopic Slug expression. These experiments will allow us to determine whether Xfeb acts in neural crest induction and will allow us to place Xfeb into the gene regulatory network that drives neural crest development.

#### LONG-TERM VEGETATION RESPONSE TO GRAZING ON A SOUTHWEST MONTANA FOOTHILLS RANGE

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Long-term vegetation data collected in area under known management provides an opportunity for understanding the potential implications of vegetation response to grazing management. Short-term studies (2-3 years) are unable to capture vegetation response due to the ecological inertia of the system and temporary fluctuations due to weather patterns. When Montana State University purchased Red Bluff Ranch in 1956, it was heavily stocked but the range was thought to be in acceptable condition. Stocking rates were lowered nonetheless. In 1958, 74 vegetation monitoring transects were established. In 2017 we attempted to relocate transects in two pastures currently used for winter grazing. Of the 28 transects in both pastures, we were able to relocate and read 11. Using original methodology, we collected basal cover data along five five-foot subplots randomly located around each transect. Data were analyzed using a paired Mann-Whitney U test. Cheatgrass (Bromus tectorum) exhibited the greatest change in cover, increasing from 0.4% in 1958 to 16.1% in 2017 (P < 0.01), and was present at 9 of the 11 monitoring sites. Perennial grass cover increased from 4.3% to 13.0% (P < 0.01). Litter cover increased from 49.2% to 58.4% (P < 0.05). Bare ground decreased from 35.2% to 3.0% (P < 0.01). The increase in cheatgrass cover explains a majority of the increase in litter and the extreme reduction in bare ground. The data indicate that the condition of the range may not have been as high as first thought, and, while cover of perennial species can increase under moderate stocking, rangeland may still be susceptible to invasion by cheatgrass. The invasion of cheatgrass may be attributable to the early spring flush of organic nitrogen associated with winter grazing.

#### HOST SPECIALIZATION OF THE PROKARYOTIC PREDATOR Bdellovibrio Bacteriovorus

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Bdellovibrio bacteriovorus is a small gram-negative bacterium with the ability to parasitize other gram-negative bacteria via periplasmic invasion leading to host cell death. B. bacteriovorus is considered a generalist and as such has been touted as a potential "living antibiotic". To be useful in treating human/veterinary infections, antibiotics should have minimal impact on commensal microflora, yet the ability of B. bacteriovorus to specialize on specific hosts while ignoring others has not been documented. In this experiment, we

investigated the ability of B. bacteriovorus to specialize on one of two different hosts: Escherichia coli or Erwinia amylovora. Our results show that over approximately 875 generations of growth, some B. bacteriovorus populations grown only in the presence of E. coli demonstrate improved growth on E. coli and significantly diminished ability to infect and kill E. amylovora. B. bacteriovorus populations exposed only to E. amylovora show growth deficits on E. coli, but the magnitude of this effect is much smaller and perhaps reflects a longer association of the B. bacteriovorus strain used here with Erwinia spp. in nature. Future work examining the genetic basis of host preference in these populations will allow greater insight into mechanisms of host specialization and serve to inform the utility of B. bacteriovorus as a targeted rather than non-specific "living antibiotic".

# NATURAL AND SYNTHETIC DRUG ANALYSIS IN BLOWFLY LARVAE AND PIG TISSUE

Haley Fallang \*, Chemistry, University of Providence, Great Falls

Blowfly larvae (*Diptera: Calliphoridae*) has long been used in connection with human death. While most commonly used as a mechanism to determine time since death, blowfly larvae have come to be a part of drug analysis in human remains. This study investigates the presence of drugs, both natural and synthetic, in pig tissues which are consumed by blowfly larvae. The classes of drugs examined in this study include opiates, psychoactives, NSAIDs, and stimulants. These active ingredients will be extracted from natural and synthetic sources and injected into pig tissue. Blowfly larvae will then be exposed to the tissue, allowed to feed, and treated with chemical digestion to extract the active ingredients. The GC-MS will determine if there is any difference in compounds digested by the larvae when comparing the natural and synthetic drug sources in remains based on blowfly larvae subjected to drug analyses.

#### DETERMINING THE PLACE OF AQP-3B IN THE WNT/CA2+ Noncanonical Pathway

Jennifer Forecki \*, Cell Biology and Neuroscience, Montana State University, Bozeman Christa Merzdorf, Cell Biology and Neuroscience, Montana State University, Bozeman

During Xenopus laevis gastrulation, convergent extension is required for the mesoderm to extend into the embryo and shape the embryonic body plan. Recent results from our lab suggest that the inhibition of aquaporin3b (aqp3b) prevents convergent extension of the mesoderm and that aqp3b acts through noncanonical Wnt signaling. Wnt signaling is a key signal pathway for embryo and tissue development and is composed of the canonical and the noncanonical pathways. Our lab has shown that aqp3b acts through a specific noncanonical pathway, the Wnt/Ca2+ pathway, and acts upstream of the cytoplasmic Wnt signaling pathway member Disheveled (Dsh). Frizzled7 (Fz7) is a membrane receptor in the noncanonical Wnt/Ca2+ pathway which also acts upstream of Disheveled. Our question for this project is whether aqp3b acts upstream or downstream of Fz7? When Fz7 was present, protein kinase C fused to green fluorescent protein (PKC-GFP) attached to the cell membrane but when Fz7 was absent, PKC-GFP remained freely in the cytoplasm. This served as our control injections: PKC-GFP + fz7 or PKC-GFP alone, respectively. The experimental injections included a morpholino (MO), small oligonucleotide that inhibited aqp3b expression, or a control MO which did not inhibit aqp3b. We have shown that the MO has kept PKC-GFP localized to the cytoplasm despite the presence of Fz7, while the control MO is allowing PKC-GFP membrane attachment. These data demonstrate that aqp3b acts downstream of the Fz7 receptor in the noncanonical Wnt signal cascade.

#### INVESTIGATION OF ON-SITE WASTEWATER TREATMENT SYSTEM SUITABILITY FOR FLOODPLAINS (POSTER)

Hannah Monaghan \*, Montana State University, Bozeman Lori Christenson, Gallatin City/County Health Department, Bozeman Margaret Eggers, Center for Biofilm Engineering, Montana State University, Bozeman

Gallatin is the fastest growing county in Montana, hence land with high groundwater, including property within the floodplain, is under increasing pressure for development. Homes and businesses without access to municipal wastewater service are required to have a permitted on-site wastewater treatment system (OWTS) that can remove solids, nutrients and pathogens from waste before releasing the effluent into the environment. Failing OWTS can create public health risks. While drain fields for septic systems can be adapted to high groundwater by installing them in a "sand mound," limited literature indicates sand mounds cannot withstand flood events. The project goal is to determine whether sand mound systems or other OWTS have been proven to withstand flooding and hence would be appropriate technology for floodplain installation. Online and database literature searches were conducted regarding local health department regulations and policies regarding OWTS. Phone calls were also made to various health departments in the western part of the United States. There are articles from the EPA, the University of Wisconsin - Madison and the Nevada Division of Environmental Protection stating that sand mounds should not be placed in floodplains. There are some OWTS that have potential to safely work within the floodplain, which are being investigated further. A failing or inadequate system can cause a variety of environmental and public health risks. Gallatin County is quickly growing, and it is imperative that the septic systems in floodplains will not adversely affect public health because of flood events.

#### INFLUENZA GROWTH VARIATIONS WITHIN MDCK CELLS

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Regardless of the preventative measures taken to keep the seasonal influenza virus under control, millions of people still get sick and tens of thousands die each year in the United States alone. This virus does not only affect the health of our nation but is also an economic burden. One study found that \$16.3 billion is the average lost earnings due to flu-related illness or death each year. The total economic losses of annual influenza amounted to \$87.1 billion in the US on average. This is merely the standard virus that comes around on a yearly basis causing the average "flu season." During the 1918 Spanish influenza pandemic; this virus affected every age group in every region, it quickly spread worldwide. The purpose of this research study is to better understand influenza growth within a laboratory setting. This becomes important when growing high titer virus for use in challenge studies and other procedures, where the most effective dose of virus is needed to test the proposed vaccine. To be able to grow the virus, there need to be cells available for infection and propagation. For this purpose, the MDCK cell line was used. There are many conditions that can affect influenza growth in MDCK cells. The assay of virus concentration is also cumbersome and somewhat variable. We show here an analysis of some of the variables affecting growth and some comparison of alternate quantification methods. The preliminary data shown here include effects of cell confluence ('thickness") upon initial infection; the amount of virus inoculum added for infection; when and how much TPCK – trypsin to be added to the media; when to harvest; and the growth medium type and volume. We are reporting the effect of these parameters on resulting virus titer.

#### Synthesis and Characterization of [PD(PDCT)L] Coordination Compounds

Dr. Matt Queen \*, Science, MSU Billings, Billings Kelton Houston, Science, MSU Billings, Billings

Carbon Tertrachloride (CT), a popular industrial solvent, and potential environmental contaminant of water and soil. An approximate ten square Kilometer plume of soil at the department of energy's site in Hanford, Washington has been polluted with CCl4. Our research is based on the study of potential CT environmental remediation technologies. Furthermore, we are interested in 2,6-pyridinedithiocarboxylic acid, or PDTC, and the dechlorination properties of its copper based coordination compounds, [Cu(PDTC)L] X. We seek to understand the link between coordination compound electronic structure and reactivity. To further understand electronic structure and reactivity of these copper coordination compounds, we synthesized several Palladium-based (Pd) coordination compounds. In this study we present IR, UV-Vis, and NMR evidence for the successful synthesis of a series of [Pd(PDTC)L]X coordination compounds. Here L-Br, CN, PPh3 as well as the dimer species [Cu(PDTC)2].

## Synthesis and Characterization of [CU(PDTC)L] Coordination Compound Series

Dr. Matt Queen \*, Science, MSUB, Billings Dr. Tom Lewis, Science, MSUB, Billings Siana Wiles, Science, MSUB, Billings

Carbon Tertrachloride (CT) is a potential environmental contaminants in water, and soil. We are interested in studying potential CT environmental remediation technologies. Specially we are interested in 2,6-pyridinedithiocarboxylic (PDTC) and the dechlorination properties of its copper based coordination compounds, [Cu(PDTC)L]X. In the interest of creating better performing more soluble coordination compounds we seek to understand the link between coordination compound electronic structure and reactivity. With the ultimate goal in mind we present IR, UV-Vis, and NMR evidence for the successful synthesis of a series of [Cu(PDTC)yL]X coordination compounds. Here L-Br, CN, PPh3 as well as the dimer species [Cu(PDTC)2]. These experimental spectra are compared to computational data calculated using DFT optimized structures utilizing a wide range of exchange and correlation density functionals.

#### Examining the Correlation Between Coliform Bacteria and Human Wastewater in Home Well Water

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Margaret Eggers, Microbiology and Immunology, Montana State University, Bozeman

Nearly 15% of the U.S. population relies on home wells for drinking water, and approximately 34% of U.S. wells test positive for coliform bacteria. However, the presence of coliform bacteria alone does not confirm the presence of fecal matter, leaving the well users

uncertain of their health risk and which mitigation measures to take. Therefore, understanding the correlation between human waste and the presence of coliform bacteria is vital to public health. A significant correlation would inform well owners and public health practitioners that mitigation must include addressing home septic system(s) (the well owner's system as well as neighbors' upgradient systems). The goal of this project is to analyze rural residential well water on the Crow Reservation to determine the degree of correlation between coliform presence, E coli presence and markers of human wastewater. The three primary analytes we are looking for are caffeine, cotinine and urobilin. All three chemicals are biomarkers of human waste. The methodology we are using to identify and quantify analytes within our water samples is solid phase extraction to concentrate the unknowns for further analysis using Gas Chromatography Mass Spectrometry. Subsequent analysis with colleagues will determine whether there are any significant correlations between the biomarkers of human waste and (1) the presence of coliform bacteria, (2) the presence of E. coli bacteria and/or (3) the absence of either coliform or E. coli. I will present my results to team members at a monthly meeting of the Crow Environmental Health Steering Committee, whereupon my colleagues on the Crow Reservation will use the data collected to inform and work with home well owners to properly mitigate home well contamination. After the completion of this project my colleagues and I plan on presenting this project at an additional conference and publishing in a peer review journal.

#### DOES AQUAPORIN **3B** AFFECT THE NUMBER AND CHARACTERISTICS OF CALCIUM WAVES IN THE NEURAL PLATE OF XENOPUS LAEVIS EMBRYOS?

Dean Ricker \*, Chemical Engineering, Montana State University, Bozeman Christa Merzdorf, Cell Biology and Neuroscience, Montana State University, Bozeman

Early in the development of the nervous system, vertebrate embryos undergo neural tube closure. This is a process where the cells in the dorsal part of an embryo, the neural plate, constrict on their outward facing side (apical constriction) to form a tube. The Merzdorf lab has found that inhibiting expression of the protein Aquaporin 3b (Aqp3b) in Xenopus laevis embryos prevents neural tube closure from happening. Given that aqp3b is only expressed in a well-defined line along the outer edge of each side of the neural plate, this action at a distance suggests some form of intercellular communication. In fact, calcium waves are required for neural tube closure. My hypothesis is that Aqp3b triggers the calcium waves that cause neural plate cells to apically constrict. To address this question, the number and characteristics of calcium waves will be compared between normal embryos and embryos that have been inhibited from expressing Aqp3b. My specific hypothesis is that the neural plate in embryos with inhibited aquaporin expression will have fewer calcium waves with different characteristics. Working towards the goal of answering this question I have developed and finetuned a method for injecting the embryos with a calcium indicator and imaging them. I have begun collecting time lapse images of calcium activity during neural tube closure and am designing methods for analyzing the time lapses I capture. This research is significant because, while calcium transients are known to be necessary to neural tube closure, no triggers of these calcium waves are known.

#### How AQP3B INFLUENCES CONVERGENT EXTENSION THROUGH NONCANONICAL WNT SIGNALING

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Aquaporin-3b, Aqp3b, is an aquaglyceroporin, a membrane water channel, that is present during gastrulation and various other stages of embryonic development. Gastrulation organizes cells into germ layers, which will later form different body tissues. During gastrulation, cells fold into the embryo, then merge by convergent extension to form the long body axis. These cell movements are regulated by noncanonical Wnt signaling, an intercellular signaling pathway that controls the migration and polarity of tissues. When Aqp3b is inhibited using a morpholino oligonucleotide (MO), convergent extension does not occur properly, suggesting a link between Aqp3b and noncanonical Wnt signaling. To assay these defects, we use the Keller tissue explanting method to observe convergent extension. Our goal is to determine which Wnt signaling pathway(s) are influenced by Aqp3b. We conducted rescue experiments by inhibiting Aqp3b with morpholino oligonucleotides and coinjecting an RNA or DNA construct of several proteins involved in Wnt signaling. Successful rescue with Dvl1 Dix and Dvl2 Dix constructs indicated that Aqp3b is involved in noncanonical Wnt signaling, since DvlADix acts in all noncanonical Wnt signaling. Further, Aqp3b acts through the Wnt/Ca2+ subpathway, indicated by rescue by PKC and PMA, and through a branch of the Wnt/PCP pathway, indicated by successful rescue with RhoA but not with Rac1. Aqp3b does not directly affect the Wnt/Ror2 pathway. In conclusion, I have demonstrated that the ability of Aqp3b to influence convergent extension is dependent on noncanonical Wnt signaling, specifically the Wnt/Ca2+ pathway and the RhoA branch of Wnt/ PCP pathway.

#### SMALL MOLECULE INHIBITION OF GRAM-POSITIVE BACTERIA S. Aureus and C. Difficile

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With the advent of antimicrobial molecules in the mid nineteenth century countless people were saved from life threatening infections. With decades of use, antimicrobials put enormous selective pressure on targeted pathogens resulting in resistances forming in many common microbes. These resistant strains of pathogens result in over two million illnesses, 23,000 deaths every year, and billions of dollars in medical expenses. This represents a need to research new molecules that have antimicrobial properties to combat the rise of resistance and prevent undue suffering to those afflicted. A small molecule is under investigation now that displays promising antimicrobial properties in preliminary studies against Candida species and many pathogenic gram-positive bacteria. The gram-positive bacteria that are inhibited by this small molecule at include Staphylococcus aureus, Streptococcus pneumonia, Listeria monocytogenes, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus cereus and Clostridium difficile. Research is currently focused on S. aureus and C. difficile with disk assays performed on both and plate assays performed on S. aureus to narrow down the minimum inhibitory concentration. C. difficile has a zone of inhibition of 9mm,

while S. aureus has zone of inhibition at 10mm. The plate assay for S. aureus has shown a minimum inhibitory concentration to be between 150-100 uM of the small molecule, though further studies are needed. Plans are being made to find the mechanism of action of the small molecule by using mutant strains of S. aureus.

#### MATERNAL EFFECTS OF ASEPTIC AND SEPTIC INJURY ON EMBRYONIC LARVAL GENE EXPRESSION IN THE TOBACCO HORNWORM, MANDUCA SEXTA

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Cross-generational effects of physical and pathogenic stress have been demonstrated in several insect groups, including our model insect Manduca sexta. Prior studies in our laboratory have shown that maternal exposure to the soil-dwelling gram-negative bacteria, Serratia marcescens, just prior to adult eclosion alters egg morphology and larval immunity. Our goal is to identify mechanisms underlaying pathogen-associated parental effects on offspring. The current study advances this goal through measurement of embryonic size, embryonic histone modification, and both embryonic and larval gene expression. Two days prior to eclosion, parents were injected with saline, heat killed S. marcescens, or live S. marcescens. Embryos were collected at 24 (+/- 2) h or permitted to hatch for clearance assays (first instar) or measurement of fat body gene expression (fourth instar). We find that maternal, but not paternal, pathogen exposure significantly increases egg volume variability, and that maternal pathogen exposure may delay hatching. Furthermore, maternal injection with bacteria conferred on their offspring an enhanced ability to clear infection when compared to their saline injected peers. Histone analysis revealed that maternal treatment does not globally alter embryonic histones, however, several immune-related genes demonstrated altered expression in both embryos and fourth instar larvae.

# COXIELLA BURNETII'S INFECTION-SPECIFIC SMALL RNA 12 (CBSR12) TARGETS CARA AND METK TRANSCRIPTS

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Coxiella burnetii is an obligate intracellular bacterial pathogen and the etiological agent of Q fever. Previous transcriptome analysis of C. burnetii by our group revealed several novel small RNAs (sRNAs) of varying sizes and expression patterns. Sequence alignments of these sRNA's across all strains of C. burnetii show strong conservation, indicating a functional role for these RNA's in C. burnetii's intracellular lifestyle. Furthermore, during C. burnetii's biphasic life cycle of metabolically active (LCV) and inactive (SCV) states, several of the sRNA's have shown differential expression in SCV and LCV cells via RNA-Seq and Northern blot analyses. One such sRNA, termed CbsR12, showed a marked upregulation in infected Vero host cells when compared to bacteria grown in axenic media. Additionally, RNA-Seq data and qRT-PCR analyses show a marked upregulation of CbsR12 in LCV cells compared to SCV cells. Here, we show that C. burnetii RNase III cleaves CbsR12 into two fragments, an observation supported by both in silico and 5' RACE analyses. in silico sRNA target prediction programs were used to determine possible mRNA targets of Cbsr12. We subsequently determined through in vitro electrophoretic mobility shift assays (EMSAs) and in vivo luciferase reporter assays, that CbsR12 binds carA transcripts, which codes for carbamoyl-phosphate synthase subunit A, and metK transcripts, which codes for S-adenosyl methionine synthetase. These genes code for essential enzymes involved in pyrimidine biosynthesis and the methionine cycle, respectively.

#### GAP JUNCTION EXPRESSION AND FUNCTION IN XENOPUS LAEVIS Embryos

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Connexins are intermembrane proteins that form protein complexes called connexons. These complexes bind together to form intercellular pores, called gap junctions, by binding to connexons of adjacent cells. These pores allow for passive transport of small molecules and ions involved in intercellular communication. Because gap junctions are required to transport of these ions, it is hypothesized that gap junctions are involved in developmental processes such as convergent extension during the processes of gastrulation and neurulation. In order to determine the role these genes play in development we must first determine the stages where they are expressed. To do this, cDNA (DNA reverse transcribed from RNA to only contain expressed genes) was made from developmental stages of Xenopus laevis embryos. The stages used were stage 6 (prior to activation of embryonic transcription), stage 8 (blastula), stages 10 and 12 (gastrula), stages 14, 16, 18 (neurula) and stage 20 (tailbud). This cDNA was then standardized by PCR using primers specific to ef1 alpha. This series of cDNA was then used as a template for PCR reactions of Xenopus connexin genes. The PCR reactions were then analyzed via gel electrophoresis in order to determine at what stages of development each gene is expressed. Gap junction gene alpha 3 was found to be expressed throughout neurulation and Gap junction gene alpha 7 was found to be expressed at all stages that were tested. Gap junction gene beta 1 and beta 2 were found to be expressed throughout gastrulation and neurulation. Gap junction gene alpha 2 was found to be maternally expressed as well as expressed in early gastrula, while Gap junction gene alpha 5 and alpha 4 are only expressed though early gastrulation. The next step of this project will be to use In Situ Hybridization to detect localized gene expression in embryotic tissue.

## Assessment of the Occurrence and Distribution of Unpermitted Wastewater Treatment Systems Along a Section of Bozeman Creek and Identification of Recommendations to Address Fecal Contamination

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Introduction - Bozeman Creek is considered "impaired" because sediment, nutrients and E. coli are at levels that impair the use of water for beneficial purposes such as irrigation or recreation. These E. coli can come from wildlife, livestock, pets or leaking septic tanks. Previous monitoring points to wastewater from septic systems as a major source of contamination to this stream. The research goal is to assess the impact of unpermitted wastewater systems on Bozeman Creek and to identify recommendations to address aging septic systems.

Methods - Online Health Department records are being reviewed to research the following questions: 1) What percentage of septic systems along a defined area of Bozeman Creek are unpermitted and what are their locations? 2) What percentage of permitted systems along a defined area of Bozeman Creek are >25 years old, and what are their locations? Additionally, a literature search is being conducted to identify best practices for the department and partners to address the status of onsite wastewater treatment along Bozeman Creek. GIS model is being created to assess which properties are most at risk of contaminating the creek.

Results - 34 properties were identified along the portion of Bozeman creek; 47 % of these properties lack septic system permits. Of those with permits, 56% are systems 25 years or older. Development of a GIS risk assessment model is underway.

Conclusions - The results of this project will be useful to health department staff to address wastewater sources contaminating Bozeman creek and serve as a model for other waterways.

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