

Changes in the Mosquito Community of Gunnison County Since 1966

Introduction

As humans further develop the lands around them, base knowledge of disease vector communities will be a vital tool to have. It is almost certain that, along with development, global warming will affect the composition of mosquito communities (Khasnis and Nettleman, 2005). As maximum temperatures in areas increase, there can be great implications for the spread of West Nile Virus, as well as other diseases that are a public health concern (Paz, 2006). Climate shifts and habitat fragmentation caused by human development can also threaten animal biodiversity, increasing the threat of endemic disease becoming epidemic due to the spread of competent vectors (Altizer et al, 2003). For these reasons, a detailed knowledge of how development and climate change affects vector spread, as well as how altitude changes affect mosquito communities is crucial to conservation biology and public health.

The presence of West Nile Virus in mosquitoes, birds and horses in counties adjacent to Gunnison (Delta, Meca and Ouray) (http://emaps.dphe.state.co.us/gis/maps/WNV_Animal_Surveillance.pdf) signifies that the monitoring of the mosquito community, especially the *Culex tarsalis* vector, is important to public health and conservation in the region. *Culex pipiens* has also been known to harbor the virus, and in both of these species, vertical transmission to offspring is a possibility (Turell et al., 2000). As global temperatures rise, it has been demonstrated that mosquito vector species are able to adapt by moving to higher altitudes, which can have detrimental effects on species previously outside of the range of the vectors (Freed et al., 2005).

Base knowledge of mosquito biology, as well as altitude, habitat and breeding preferences can assist in remote sensing mosquito communities elsewhere, assisting in the monitoring of vector populations. (Manguin and Boussinesq, 1999). Potentially,

knowledge of how climate change might affect mosquito communities at different altitudes can help predictions of when and how disease epidemics might occur in the future. In the Gunnison County area, because there have not been extensive efforts to sample the mosquito community since Marion Smith's (1966) J.R. Bowen's (1971) studies, little is known about the community's present state. The objective of the current study was to help shed light on any changes between then and now in community composition with regards to altitude.

Methodology

Carbon-dioxide baited CDC light traps were placed at four different altitudes throughout two sessions, each lasting four days. Locations for traps are: Virginia Basin (~11,200 feet); Gothic (~9,500 feet); Crested Butte (~8,800 feet) and Tomichi Creek (~7,700 feet). Two traps were placed at each site, except for Gothic, where trapping occurred on different days for another study, and where two traps were placed in each of forest, meadow and willow habitat patches. Because mosquitoes are sensitive to microclimate, mosquito species may prefer different habitat types. To ensure a representative sample of the mosquito community at each site, traps at each site were placed in a different habitat types if possible. Habitat patches in which traps were located was dependent on the available patches at each site. Site location was based on finding the most productive areas per altitude to ensure a large sample of mosquitoes from that particular elevation. Traps were placed in different habitat patches in order to ensure that species composition differences found are due to altitude and not simply factors such as tree composition in forested areas, for which some species of mosquito have been shown to have preference (Smith, 1966).

Trap Placement:

Tomichi Creek (~7,700 ft)

Both traps placed in heavily irrigated meadow

Crested Butte (~8,800 ft)

Initially both traps in placed meadow; during mid and late July trapping one trap in a lakeside meadow and one trap in a willow

Gothic (~9,500 ft)

Two traps rotated through six habitat patches of three types: mixed forest, meadow, and willow
Virginia Basin (~11,200 ft)

One trap placed in a streamside meadow, the other in an adjacent spruce forest patch.

At the Gothic site, data will be analyzed from collections trapped in forest, willow and meadow patches, and samples will be taken from the four days after the completion of trapping at Virginia Basin. This is due to the fact that there were only six CDC traps to work with, so Courtney Murdock's mosquito sampling data in Gothic from dates as close as possible to trapping sessions at other altitudes were taken into account in order to get more complete sense of altitudinal variation of species.

Half of the sampled mosquitoes (except for those from Virginia Basin, due to the unlikelihood of competent West Nile vector species at that altitude) were transferred immediately to a -80°C freezer for DNA and RNA preservation. Individuals in this half will be identified at the University of Michigan – Ann Arbor and monitored for West Nile Virus RNA. As a result, current data is still incomplete, however species presence and absence at the elevations sampled can be descriptively compared with Smith and Bowen's findings.

All trapped mosquitoes were paralyzed with peptide synthesis grade triethylamine and identified to species level under a dissection microscope. Mosquitoes not transferred to the -80°C freezer will be kept in a freezer in the RMBL Willey Laboratory where identification will take place.

Preliminary Results

Unfortunately, due to the lack of quantitative data in Marion Smith's 1966 paper on the mosquitoes around Gothic, CO, the only comparisons that can be made to her study are qualitative ones regarding the presence of species at different altitudes and the relative abundances of species (e.g. the commonness of species at different altitudes). The altitudinal ranges of mosquito species which are trapped have been compared with findings in Smith (1966) and Bowen (1971) in order to see how species ranges might have shifted in the years since then.

Mosquito identifications are still in the preliminary stages due to the large quantity of mosquitoes caught in Tomichi Creek during the first trapping session and

because the second trapping session ended on August 5th. All traps from other sites have been sampled from, with the exception of traps samples sent to Western State College to be preserved for later West Nile Virus analysis. Currently we do see evidence of possible altitudinal changes in species composition at the various altitudes since Smith's and Bowen's studies. Species lists found so far are as follows:
 (note: '*' denotes species not previously listed in the area, bold denotes important vector species of encephalitis and West Nile Virus)

Tomichi Creek

Ochlerotatus *dorsalis*
 melanimon
 spenceri idahoensis
 implicatus

Culex *tarsalis*

Crested Butte

Aedes *cinereus* Culex *tarsalis*
 *vexans**

Ochlerotatus *cataphylla* Culiseta *alaskaensis*
 excrucians *inornata*
 fitchii *impatiens*
 hexodontus
 *melanimon**
 punctor
 schizopinax

Gothic

Ochlerotatus *cataphylla* Culex *tarsalis**
 communis
 fitchii
 hexodontus
 implicatus
 intrudens
 pullatus
 (punctor)

Culiseta
 impatiens
 alaskaensis

Virginia Basin

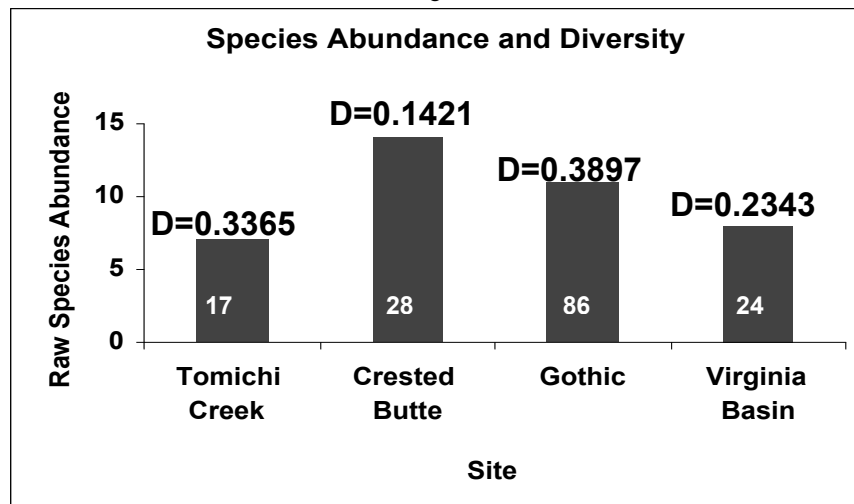
Ochlerotatus *cataphylla*
communis
hexodontus
implicatus
pullatus
punctor
schizopinax

Culiseta *impatiens*
alaskaensis

A Simpson's diversity index was undertaken to see which locations, at this stage of identification had the greatest species diversity. Simpson's diversity index describes the likelihood of any two individuals sampled from a pool (in this case, anything trapped at a given elevation) belonging to the same species.

$$D = \sum_{i=1}^s \frac{n_i(n_i - 1)}{N(N - 1)}$$

Where n_i = the number of individuals of a given species caught and N = the number of total individuals of all species caught



D represents the value of the Simpson's index for each site

The numbers in the columns represent the numbers identified at this point in time (N)

It can be seen that at this point in the identification of individuals that Crested Butte has the highest species richness as well as diversity, a measure of richness and evenness. A higher raw species abundance is expected in Tomichi Creek due to the sheer

numbers of individuals caught (~6,000 per trap per day from June 26-30), and the relatively low number of them identified so far. The low value for Simpson's diversity index at the Gothic site might be due to little identification being done on individuals trapped after mid-June. Early sessions were dominated by the presence of *Oc. implicatus* and *Oc. cataphylla*, however judging from Smith's records of the relative amounts of various species caught throughout the season, it is likely that other species of *Ochlerotatus* will emerge and their adult females will increase in numbers caught in Gothic traps. Whether this is the case or not will be decided upon further identification of the Gothic specimens, which had to be quickly frozen in a -80° freezer to preserve blood parasite DNA for later analysis.

Discussion of Preliminary Findings

There are apparent changes in mosquito communities with respect to altitude which have occurred in the period between Smith's (1966) study and the one performed in 2007. Though a lack of replication as well as a lack of quantitative data from Smith prevents a conclusive statistical analysis of mosquito species in the region, we can compare descriptive data from Smith (1966) to the current findings. There are three apparent changes in the mosquito communities of Gunnison County seen preliminarily:

- 1) Absence of *Oc. impiger* from Virginia Basin traps.
- 2) Presence of adult *Cx. tarsalis* females in Gothic.
- 3) Presence of *Ae. vexans* and *Oc. melanimon* in Crested Butte Trap sites.

1) During both Virginia trapping sessions, there was a conspicuous absence of *Ochlerotatus impiger*. This species is listed in Smith as "rarely... taken below 10,000 ft... and occurring usually with *A. communis*, *hexodontus* and *pullatus*, often as the predominant species" (1966). What was found in the current study at Virginia Basin (~11,200) feet were *Oc. implicatus* and *Oc. cataphylla* occurring as the two predominant species. This is consistent with Gothic trapping, where *Oc. implicatus* and *Oc. cataphylla* were also predominant. Individuals of the species *Ochlerotatus pullatus* and *Oc. communis* also were taken at relatively low levels, while numbers for *Oc. hexodontus*

were much higher in Virginia Basin than in Gothic. Low levels of *Oc. communis* during the first trapping session indicate a possibly poor year for the species, due to the fact that the end of June is when Smith reported peak numbers on her timelines for all three years trapping occurred (1966). Further identification of individuals sampled in Gothic will tell us whether this year was a poor year for numbers of *Oc. communis* and *Oc. pullatus* as compared to 2005. Other possibilities include an altitudinal range expansion of *Oc. implicatus* and *Oc. cataphylla* due to climate shifts or other factors, the same factors which may have caused *Oc. impiger* to have not been found at Virginia Basin.

While the absence of *Ochlerotatus impiger* is an important discovery at Virginia Basin, the prominence of *Oc. cataphylla* and *Oc. implicatus* might be a better indicator of altitudinal range shifts of species. Range shifts due to climate are described as being best detected by shifts in the mean altitude of species presence data at varying elevations (Shoo et al., 2006). The mean altitude of species presence is essentially where the optimal altitude for the species is, determined by number of individuals. While comparisons with Smith's paper may only be undertaken descriptively, due to the qualitative nature of the paper, there is much to learn from her words. Because it is described in that particular paper that *Oc. impiger* occurs predominantly with *Oc. communis*, *pullatus* and *hexodontus*, we can see that the high presence of *Oc. implicatus* and *Oc. cataphylla* might indicate a possible shift in the mean altitude of the species. Smith reports that most *Oc. implicatus* "collections were made from Gothic and its immediate vicinity, from 9500 to 9800 ft (2895-2990m)" (1966). At Virginia Basin in 2007, average numbers of *Oc. implicatus* were 17.3 individuals found in the forest trap for the early trapping session. Due to freezing for preservation of blood parasites in Gunnison, data for trapping sessions in Gothic directly before and after trapping sessions in Virginia Basin are not yet available. However, from trapping that occurred June 12-16, the average number of *Oc. implicatus* individuals found per trap was 34. This indicates that most *implicatus* individuals are still likely biting around Gothic (~9500 ft). However, we can clearly see that there were still many individuals found at a higher elevation (~11,200 ft) than Smith reports (1966). Comparisons of *Oc. cataphylla* populations between 2007 and 1966 sampling are inconclusive due to minimal reporting

of where the majority of *Oc. cataphylla* individuals were taken. The paper does mention that adults were taken from 8,900 feet to as high as 12,000 feet (Smith, 1966).

The high prevalence of *Oc. cataphylla* and *Oc. implicatus* at elevations where they weren't reported as being particularly prevalent in Smith's study, indicates a possible shift in the mean altitudinal prevalence of both of these species. The absence of *Oc. impiger* is further evidence of mosquito species exhibiting range shifts since Smith's study. However, further sampling from elevations of 11,200 feet and above are needed in order to give any conclusiveness to these presumptions.

2) In Gothic during 2007 trapping, *Culex tarsalis* females were found for the first time above 8900 feet. The finding occurred on July 31, the final day of trapping in Gothic, in which two female individuals were trapped in a forest habitat patch (Rhea Forest). This finding is of grave importance due to the fact that *Cx. tarsalis* is often the primary vector of West Nile Virus in epidemics studied west of the Mississippi River (Hayes et al., 2005). Previously, Smith had only reported seeing the larvae of *Cx. tarsalis* as high as 8900 feet (1966). Smith's sampling only extended until August in 1958, trapping ended in late July in 1959 and 1962 (Smith, 1966), so it is possible, but unlikely that as the summer progresses members of the species have traditionally gotten to elevations as high as Gothic, though it is unlikely. Another possible scenario was that *Cx. tarsalis* had been in Gothic all the while for the summer and simply made a switch from feeding on tree nesting birds to feeding on mammals on the ground. *Culex tarsalis* females are known to switch host preference late in the summer in Colorado, which could explain its presence in our traps only at the end of the season (Tempelis et al., 1967). This was refuted, however, by a study done in Alamosa, Colorado where traps were placed at varying heights. Researchers found no significant difference in the numbers of *Cx. tarsalis* visiting traps high in the canopy as opposed to those visiting ground traps or traps at an intermediate height (Poole et al., 2004).

Because the species is multiple brooded throughout the season, there are various possibilities about how individuals could have reached Gothic from lower altitudes. There is a possibility that these were mated females that had traveled a great distance from the point of their emergence, however, because two were found in the same trap, it

is unlikely that they had straggled from far away. It is more likely that these were from an emergence only slightly down valley of the Gothic trapping sites. Larval sampling was attempted after the trapping of the adult females, but it proved to be too dry with too few puddles to find any larvae.

3) The presence of *Aedes vexans* and *Ochlerotatus melanimon* in Crested Butte traps indicates a possible range shift of these two important vector species. This is due to the fact that neither of these species was reported by Smith or Bowen as being at Crested Butte altitudes (Smith, 1966; Bowen, 1971). Both species were found in Crested Butte traps in low numbers, only one *Oc. melanimon* individual and two *Oc. vexans* individuals were found. This is indicative of the elevation of Crested Butte (~8,900 ft) likely being at the upper end of the altitudinal range of each species.

Bowen describes *Oc. melanimon* as thriving in artificially irrigated areas, which can be verified by the very high numbers of the species that have been identified so far from sampling efforts at Tomichi Creek (Bowen, 1971). This species has, along with *Ae. vexans*, also been verified as a competent vector of West Nile Virus (Goddard et al., 2002). Because of the tendency for *Ochlerotatus* species to feed on mammals more than species in genera such as *Culex* or *Culiseta*, both of these species might be important in acting as bridge vectors of WNV, which primarily affects birds, to humans (Andeadis et al., 2004). Because of the potential of both of these species to be important in a cycle of transmission of encephalitis and WNV to humans, it is important that any further upward altitudinal progression of these species be carefully monitored. This is especially important when considering plans for irrigation of the Saddle Ridge between Mount Crested Butte and Crested Butte, possibly creating an ideal breeding ground for *Oc. melanimon*.

Conclusions and Further Research

This study has shown that a further monitoring of the mosquito species of Gunnison County is necessary due to the apparent upward altitudinal shifts of certain species. Regular trapping, a greater replication, and a keen observance of the mean altitude presence of species in the area may help provide more answers on whether the mean altitude of species might be increasing due to climate change.

Smaller intervals between elevations sampled might help establish a more accurate mean altitude for species presence. Though we have seen the ends of the ranges of species expanding, a consistent change of the mean altitude of any given species over multiple trapping years would give more conclusive results on whether mosquito species are moving upwards in elevation in response to climate change (Shoo et al., 2006). Because of the absence of *Oc. impiger*, further sampling at high elevations close to the peaks of mountains near Gothic is needed to see if indeed this species has been pushed up in altitude or if the species is simply absent at the Virginia Basin site but present at other areas of similar altitude.

In addition to further sampling as a way of establishing a mean altitude for species and monitoring change, polynomial regression lines may be fitted onto a graph with numbers of individuals trapped as a dependent variable and altitude as an independent variable. Over the course of multiple trapping sessions, one can monitor the way these regressions change year by year for the species with respect to snowfall and temperature data. Knowledge of how changes in yearly temperature and snowfall affect any given species at varying elevations may help in understanding how climate change might affect the altitudinal range of the species in the future.

The understanding of how mosquito species might be creeping upwards along an altitudinal gradient is critical for both understanding how disease ecology might change with a changing climate. There are also conservation implications to altitudinal shifts in vector species, especially due to the first members of *Cx. tarsalis* found in Gothic, and the profound effect the introduction of West Nile Virus has had on many American bird species (Eidson et al., 2001; Bernard et al., 2000). There are many reasons to continue study of the mosquito species in Gunnison County, as shedding light on altitudinal shifts of its mosquito species may help researchers understand how mosquito species in other areas might shift and how these shifts might affect the ecology of vectored diseases in many regions.

Works Cited

Altizer, S., Harvell, D., & Friedle, E. (2003). Rapid evolutionary dynamics and disease threats to biodiversity. *Trends in Ecology and Evolution*, 11(11), 589-594.

Bernard, K.A., Maffei, J.G., Jones, S.A., Kauffman, E.B., Ebel, G.D., Dupius II, A.P., Ngo, K.A., Nicholas, D.C., Young, D.M., Shi, P.Y., Kulasekera, V.L., Eidson, M., White, D.J., Stone, W.B., NY State West Nile Virus Surveillance Team & Kramer, L.D. (2001). West Nile Virus Infection in Birds and Mosquitoes, New York State, 2000. *Emerging Infectious Diseases*, 7(4), 679-685

Bowen, J.R. (1971). Mosquitoes of the Gunnison River Drainage, Colorado; emphasis: Ohio Creek Valley. *Master's Thesis --Western State College*.

Eidsen, M., Komar, N., Sorhage, F., Nelson, R., Talbot, T., Mostashari, F., McLean, R. & The West Nile Virus Avian Mortality Surveillance Group. (2001) Crow Deaths as a Sentinel Surveillance System for West Nile Virus in the Northeastern United States, 1999. *Emerging Infectious Diseases*, 7(4), 615-620.

Freed, L.A., Cann, R.L., Goff, M.L., Kuntz, W.A. & Bodner, G.A. Increase in Avian Malaria at Upper Elevation in Hawai'i. *The Condor*, 107(4), 753-764.

Goddard, L.B., Roth, A.E., Reisen, W.K. & Scott, T.W. (2002) Vector Competence of California Mosquitoes for West Nile Virus. (*Emerging Infectious Diseases*, 8(12), 1385-1391.

Khasnis, A. & Nettleman, M. (2005). Global Warming and Infectious Disease. *Archives of Medical Research*, 36, 689-696.

Paz, S. (2006). The west nile virus outbreak in Israel (2000) from a new perspective: The regional impact of climate change. *International Journal of Environmental Health Research*, 16(1), 1-13.

Shoo, L.P., Williams, S.E. & Hero, J.M. (2006). Detecting climate change induced range shifts: Where and how should we be looking? *Austral Ecology*, 31(1), 22-29.

Smith, M.E. (1966). Mountain Mosquitoes of the Gothic, Colorado, Area. *American Midland Naturalist*, 76(1), 125-150.

Tempelis, C.H., Washino, R.K. (1967) Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *Journal of Medical Entomology*, 4(3), 315-318.

Turell, M.J., O'Guinn, M.L., Dohm, D.J., Jones, J.W. (2000). Vector Competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus. *Journal of Medical Entomology*, 38(2), 130-134.