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Patterns in Winter Stonefly Distribution Along a River Continuum and Land-Use Gradient in Northwest Arkansas Streams

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

by

Zachary Tipton University of Arkansas Bachelor of Arts and Science in Environmental, Soil and Water Science, 2018

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Freshwater ecosystems are facing a crisis with extinction rates of aquatic species exceeding those of their terrestrial counterparts by up to fivefold. This decline is predominantly attributed to evolving land use patterns within watersheds, leading to chemical and physical transformations in freshwater habitats. Northwest Arkansas (NWA) represents one of the fastest-growing regions in the United States, undergoing substantial shifts in land use. Consequently, the status of aquatic life in this region remains uncertain. Addressing this concern, the latest Arkansas Wildlife Action Plan emphasizes the necessity of distribution and population data to guide conservation efforts for Species of Greatest Conservation Need (SGCN). Among these species are three Allocapnia stonefly species—A. jeanae, A. ozarkana, and A. warreni—historically found in NWA during their winter emergence periods. In this study, I conducted an analysis combining fine-scale, sitespecific surveys, and species-specific assessments to provide insights into the presence and richness of *Allocapnia* along three major river systems in NWA: the White River, Clear Creek, and West Fork White River. To inform my analyses, I utilized historical stonefly surveys conducted 50 and 30 years ago. I conducted Allocapnia surveys at 22 sites during winter emergence periods in 2020-2021 and 2021-2022, with an average of 10 visits per site. Specimens were collected from emergent structures and identified in the laboratory. To gain an understanding of the relationships between independent variables (including land use, latitude, draining area, and Strahler stream order) and the presence of each Allocapnia species, I employed orthogonal partial least square regression. Additionally, univariate threshold analyses were conducted to pinpoint critical points in land use values that resulted in significant declines in Allocapnia richness. My findings reveal that Allocapnia were predominantly found in forested streams, with no occurrences recorded at highly urbanized sampling sites. Notably, A. rickeri and A. mohri displayed high prevalence and abundance in large, high-order sections within the West

Fork White River basin, suggesting a greater resilience to land use changes compared to other *Allocapnia* species. *Allocapnia jeanae* demonstrated successful dispersion within the NWA landscape over the past five decades, albeit primarily restricted to tributaries, with limited occurrences within the mainstem of sampled rivers. *Allocapnia ozarkana*, although persisting, were collected in low numbers. *Allocapnia warreni* was not detected in Clear Creek and has not been recorded in this area for over half a century, indicating a likely extirpation. Land use thresholds were identified at 64.3% (\pm 25.17) for forest, 26.19% (\pm 5.18) for agriculture, 8.56% (\pm 0.74) for urban, and 1.51% (\pm 0.16) for impervious surface cover. These thresholds highlight the critical importance of concentrating conservation efforts on the protection of forested headwaters to ensure the preservation of vital habitats for aquatic shredding macroinvertebrates, such as *Allocapnia*. Additional actions in agricultural and urban areas should include the maintenance of riparian zones and stormwater management. In the face of an evolving landscape, understanding the effects of land use on aquatic life remains imperative to safeguard our natural resources and ensure the sustainability of our ecosystems.

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Dedication

To my incredibly talented daughter, Judith Sude-Tipton. Your presence in our family has filled us with immense joy and has provided me with the strength and purpose to overcome life's challenges.

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1. Introduction

Freshwater biodiversity is declining around the world (Allan, 2004; Booth et al., 2016; Sala et al., 2000; Walsh et al., 2005), with extinction rates of aquatic species up to 5 times greater than terrestrial fauna (Ricciardi & Rasmussen, 1999). Changing land uses within the watershed resulting in chemical and physical alterations to freshwater ecosystems is a major contributing factor leading to this decline (Allan, 2004; Sala et al., 2000; Walsh et al., 2005). These disturbances often occur disproportionately near waterways (Foley et al., 2005). Even in areas of low population densities, anthropogenic effects on stream biodiversity can be severe (Sala et al., 2000). There is a continued need to understand the effects of changing land use on lotic ecosystems for the conservation of aquatic species.

Sedimentation and excessive nutrient inputs into freshwater systems resulting from land use changes have been identified as two predominant causes of lake, river, and stream impairment (EPA, 1998; EPA, 2008). Sedimentation resulting from construction activities (Wolman, 1967) and the removal of vegetation (Weigel & Robertson, 2007) can lead to the deposition of sediment on the respiratory organs of ecologically important benthic taxa such as macroinvertebrates (Lemly, 1982). Additionally, sedimentation has the adverse effect of eliminating aquatic habitat by filling in benthic pore space and reducing the availability of disturbance refugia (Collier & Quinn, 2003). This sedimentation process can elevate the drift of macroinvertebrates downstream and cause harm to organisms due to the presence of fast-moving water currents that carry significant amounts of sediment (Collier & Quinn, 2003).

Excessive nutrient inputs affect macroinvertebrate assemblages through direct and indirect chemical effects. Inorganic fertilizers and organic chemicals are often used in agricultural and urban systems, which may lead to high runoff of these chemicals into nearby

waterways (Dodds, 2007; Weigel & Robertson, 2007). These inputs may negatively impact invertebrate communities via direct effects of toxicity, for example through excessive exposure to nutrients, such as NO₃⁻ and NH₃ (Camargo & Alonso, 2006), salts (Kaushal et al., 2005), or pesticides (Schäfer et al., 2012). Nutrient enrichment may also lead to excessive microbial growth and organic matter decomposition rates (Cross et al., 2007), which may reduce dissolved O₂ levels in streams, especially during summer nights or after high levels of precipitation (Hilsenhoff, 1987). Often different pollutant mechanisms act synergistically to produce more significant damage to an ecosystem than the individual pollutants would alone (Lemly, 1982). Combined, these effects may contribute to a decrease in the diversity of macroinvertebrate assemblages by altering dissolved elemental concentrations and the quality and quantity of particulate basal food resources, ultimately resulting in communities dominated by a few taxa exhibiting greater tolerance to nutrient pollution (Gafner & Robinson, 2007; Singer & Battin, 2007).

While the mechanistic effects of land use change on stream biota may be identified for an individual stream, regional management efforts must often rely on more rapid and cost-effective monitoring approaches (Czajkowski & Lawrence, 2013). These efforts are enabled by GIS products able to remotely delineate stream watersheds and land use categories and the development of rapid bioassessments protocols for stream macroinvertebrates (Barbour et al., 1999). Researchers have consistently revealed robust correlations between agricultural and urban land use to decreasing biotic indices, highlighting the value of combining land use and biological indicators for assessing the effects of land use changes on aquatic ecosystems. (Gergel et al., 2002). When analyzing these relationships, macroinvertebrate responses may exhibit either linear or non-linear patterns across land use gradients (Allan, 2004). Non-linear responses are of

particular interest as they may signify the presence of a threshold, or a sudden and catastrophic loss of biodiversity along gradients of land use and water quality (Allan, 2004; Baker & King, 2010; Evans-White et al., 2009; Groffman et al., 2006). Discovery of ecological thresholds before they are crossed may allow for more rapid conservation efforts and the development of effective best management practices (Groffman et al., 2006; Valle Junior et al., 2015).

For this project, I conducted intensive spatio-temporal distribution surveys of the detritivorus, winter-emerging stonefly Allocapnia across land use/landscape gradients in three rivers in Northwest Arkansas (NWA). Residential development in the NWA region has experienced significant growth, with an average population increase of 32% per decade since 1980 (U.S. Census Bureau, 2020), which was the last time a major stonefly survey was conducted in the area (Poulton & Stewart, 1991). The most recent Arkansas Wildlife Action Plan (AWAP, 2015) indicates a need for baseline distribution and population status information for conservation planning of listed Species of Greatest Conservation Need (SGCN) including 3 endemic species of *Allocapnia* that have been historically found in the NWA area: A. jeanae, A. ozarkana, A. warreni (AWAP, 2015). I hypothesized that (H1) Allocapnia species presence would be related to land use, specifically, that Allocapnia presence and species richness would be positively related to percent forest cover and would decrease with agricultural and urban land use. With my sample sites arranged along a south-to-north trajectory, spanning from forested areas in the south to urban land use in the north, I hypothesized that the presence and species richness of Allocapnia would be influenced by landscape characteristics. Specifically, I hypothesized a relationship with the following factors: Strahler stream order, watershed draining area, and latitude (H2). I predicted that Allocapnia presence and species richness would decrease with increasing stream order, draining area, and latitude. This study aimed to fill critical

knowledge gaps in understanding the distribution and ecological responses of *Allocapnia* to changing land use in NWA, ultimately contributing key findings for the conservation and management of these winter stoneflies in a rapidly developing region.

2. Background, Materials, and Methods

2.1 Taxonomic and Life-history Information

Allocapnia (Order: Plecoptera, Family: Capniidae) are detritivorus primary consumers and one of the most abundant winter stonefly genera in temperate North America (Ross & Ricker, 1971). *Allocapnia* and other benthic primary consumers link riparian and aquatic nutrient cycling (Cao et al., 2018). Stoneflies also provide food for predators such as fish and riparian spiders, further contributing to energy flows through food webs (Barbour et al., 1999; DeWalt et al., 2010). Plecoptera nymphs persist within a relatively limited range of environmental conditions and are commonly used as biological indicators (Borchardt & Statzner, 1990).

Allocapnia life cycles are univoltine and hemimetabolous (Stewart & Stark, 2008). Nymphs are aquatic shredder-detritivores that hatch after 3-4 weeks of embryonic development from eggs deposited into bodies of water by gravid females and require 6 months of growth to reach maturity (Stewart & Stark, 1988). After several instars depending on species (10 - 22+)(Stewart & Stark, 1988), nymphs crawl from the water onto emerging structures (i.e., sticks, rocks, bridge pilings, leaf packs) where they molt into terrestrial adults (DeWalt et al., 2010). Nymphs disperse downstream through flood events; however, the dispersal of later instar stages is more strongly related to population density (Webb, 2002).

There are 47 species of *Allocapnia* described in North America (Stark et al., 2009). Identification of *Allocapnia* species requires sampling adult specimens, as nymphs are visually indistinguishable from each other (Poulton & Stewart, 1991; Ross & Ricker, 1971). Female

adults are slightly larger than males and have wings extending beyond the abdomen, while the male wing length is reduced and varies across species (Ross & Ricker, 1971). Males are identified to species via their tergum 7 or 8 process along with apical and basal sections of the epiproct, and females are identified to species via the configuration of sternum 7 and 8 (Poulton & Stewart, 1991). Adult dispersal ability is limited by reduced wing and flight muscle development in both sexes, with males limited to crawling and females capable of crawling and gliding upstream to deposit eggs (Covich et al., 1999; Ross & Ricker, 1971).

2.2 Historical Regional Species Distribution and Conservation Status

As of 2018, 12 *Allocapnia* species have been found either currently or historically within Arkansas (Robison & McAllister, 2018). The last survey of the Boston Mountains reported that this ecoregion had the second highest number of *Allocapnia* species including *A. granulata*, *A. jeanae*, *A. mohri*, *A. oribata*, *A. ozarkana*, and *A. rickeri* (Poulton & Stewart, 1991; Ross & Ricker, 1971). The Ozark Highlands was listed as the third highest in number of Plecopteran species including *A. granulata*, *A. jeanae*, *A. mohri*, *A. mystica*, *A rickeri*, *A. sandersoni*, *A. vivipara*, and *A. warreni* (Poulton & Stewart, 1991; Ross & Ricker, 1971). The Ouachita Mountains were found to have the greatest Plecopteran diversity in Arkansas (Poulton & Stewart, 1991); however, this region was not included in my study because my major concern was the effect of land use changes in the rapidly developing NWA area.

This development has led to substantial land use changes in between sampling periods of these previous stonefly collections that continue into the present. Widespread collections of *Allocapnia* in North America began in 1956 (Ross & Ricker, 1971) and coincided with a period of second-growth timber harvesting where forested land use in Arkansas declined by one-eighth (Van Sickle, 1970). The most significant declines in forested land use in NWA, however,

occurred between 1973-1980, as timber harvesting in the Boston Mountains and the development of the poultry and cattle industries in the region led to an increase in pastoral land use, particularly in the Ozark Highlands (Karstensen, 2009, 2010). This activity slowed during the collection period of Poulton and Stewart (1983-1988), due to agricultural recession and forest replanting efforts and partial forest recovery by the early 90's (Karstensen, 2009, 2010). Economic growth in the region since this time has led to rapid population increases and urbanization (Reynolds et al., 2017), primarily through the conversion of agricultural lands to subdivisions beginning in the early 2000's (*Northwest Arkansas Open Space Plan*, 2016).

Together, these land-use changes require updated information on the distributions and habitat requirements of SGCN Allocapnia species in the region. Allocapnia jeanae has been given an Arkansas conservation priority score of 50 (out of 100, with 0 being low conservation concern and 100 being of highest conservation concern), although it is a regional-endemic species dispersed to multiple river systems in the Ozark regions of OK, MO, and AR (Ross, 1964). A. jeanae inhabits intermittent $1^{st} - 3^{rd}$ order streams within the Ozark uplift (Ross, 1964; Ross & Ricker, 1971) and are often found in cool, fast, and rocky streams (Ross & Ricker, 1971). Allocapnia ozarkana was likewise given a priority score of 50 for similar reasons (AWAP, 2015). The scarcity of historical records pertaining to A. ozarkana indicates that the species is rarer and has a more restricted distribution compared to A. jeanae, which consequently leaves its habitat requirements relatively understudied. Allocapnia warreni is an even rarer AR endemic, as only one specimen of Allocapnia warreni was collected in 1962 from Clear Creek in NWA, and it has not been collected since (Ross & Yamamoto, 1966). Allocapnia warreni has a priority score of 80 and is classified as possibly extirpated in Arkansas since 1988 after Clear Creek became an outlet for treated municipal waste from Fayetteville (AWAP, 2015).

2.3 Study design and site description

The purpose of my study was to assess the status of these three SCGN along with any other co-occurring *Allocapnia* species in NWA. I collected *Allocapnia* from 22 sites across two river systems located in the Boston Mountain ecoregion (White River, West Fork White River), and Clear Creek, which is located in the Ozark Highlands (Figure 1; Table 1). These sites aligned with other previous sampling efforts in the area (Ross and Ricker 1971; Brown et al., 2003; Poulton & Stewart, 1991) and included 1st - 4th order tributary and mainstream sites spread across gradients ranging from high levels of forest cover to agricultural – primarily pasture – land use, and ultimately to suburban land development. There is a notable gap in sampling distances at all three river systems due to an inability to access the channels without the landowner's permission. Sites were repeatedly sampled for an average of 10 visits between November 23, 2020, to March 19, 2021, and November 22, 2021, to March 3, 2022 (Table 1) during known periods of *Allocapnia* emergence (Poulton & Stewart, 1991).

2.4 Sample Collection

Adult stoneflies were hand-collected from emergent structures such as rocks, sticks, leaf packs, and bridge piles both within stream riffles and adjacent stream banks (Phillipsen et al., 2015; Poulton & Stewart, 1991). Timed searches began at the same riffle for each visit for a duration of 20 minutes. Specimens were transported in vials containing 95% ethanol to the laboratory for identification. Species were identified to species using published keys (Poulton & Stewart, 1991).

Watershed land-use and landscape variables were collected using shapefiles constructed using the website modelmywatershed.org (Stroud Water Research Center, 2023) and then moved into QGIS 3.4.4. Landscape variables (% forest, % agriculture, % urban, area) were obtained

from these shapefiles. Percent imperviousness and watershed drainage area were calculated using ArcGIS 2.9.3 and the Multi-Resolution Land Characteristics Consortium's National Land Cover Database (NLCD) 2019 (Dewitz, 2021). Strahler stream order information was taken from the Arkansas Department of Environmental Quality's Stream Order Classification (line) shapefile. *2.5 Statistical Analyses*

To explore relationships between land use and landscape variables and stonefly distributions, I first used orthogonal partial least squares regressions (OPLS). I chose this method as it is a robust inferential framework for highly colinear datasets and allows for simultaneous prediction of both x-predictor variables (land use/landscape) and multiple y-response (species presence/absence) variables (Carrascal et al., 2009; Wold et al., 2001). The OPLS framework also provides a mechanism for gauging the importance of individual variables on x and y responses using variable importance scores. Unlike multivariate models assuming independence, OPLS models assume that both x and y variables highly dependent and influenced by a few underlying "latent" variables, which cannot be directly measured but instead inferred through modeling (Wold et al., 2001). Like most models, OPLS models also assume there are no major outlying points and that regression residuals are homogeneously distributed (Wold et al., 2001).

To test these assumptions, I first modeled the effects of land use and landscape variables on individual species using the 'ropls' package (Thévenot et al., 2015) in R (R Core Team, 2022). Permutation tests confirmed the presence of 2-3 significant latent variables in individual species datasets, for all taxa besides *A. ozarkana* (see below), confirming the dependency of xand y-variables in each OPLS model. Diagnostic plots revealed only one "orthogonal outlier" from a highly urbanized watershed in the WFWR basin, which I chose to retain, as by definition, these outliers have no effect on interpretations of other datapoints within this multivariate subspace (Hubert et al., 2005). Residuals were homoscedastic for *A. rickeri*, but less so for *A. mohri* and *A. jeanae*. Therefore, land use data were arcsine transformed to control for the compositional nature of these measurements and landscape data were ln transformed. All models were rerun and yielded improved residuals and model predictions. A viable single-species model for *A. ozarkana* could not be constructed as their presence was only recorded at two sites. Nevertheless, as a primary goal of my study was to explicitly predict the distributions of this and other rare taxa, all four species were included as y-variables in the final OPLS model, and *A. ozarkana* distributions with respect to predictor variables are interpreted with caution.

While OPLS models are useful for making inferences from multivariate data, one of their downsides is that they are not capable of testing individual hypotheses or determining the significance of individual predictors. Therefore, to confirm inferences from OPLS regressions and provide more quantitative predictions of declines in *Allocapnia* species richness across the region, I conducted univariate threshold analyses. To determine which threshold modeling to conduct, I ran Pearson's and Spearman's correlations testing relationships between *Allocapnia* species richness along land use variables (% forest, % agriculture, % urban, % impervious surfaces; Evans-White et al., 2009). The results of this analysis indicated a relatively stronger linear relationship (Table 2); thus, a parametric threshold analysis was chosen. Threshold models are designed to provide an easily interpretable figure to capture the relationship between predictors and outcomes where there may be an abrupt change in slope within the data. The parametric threshold analysis was conducted using the 'chngpt' package (Fong et al., 2017) in R (R Core Team, 2022). The "step" method was used for the threshold models along with a bootstrap value of 1000 for generating confidence intervals around threshold estimates.

3. Results

Allocapnia rickeri and *A. mohri* were the most common species in the region, representing 54% and 43% of specimens collected, respectively (Table 3). Relative abundances of these species differed temporally with *A. mohri* emerging before *A. rickeri* (Figure 2). *Allocapnia mohri* was found Nov 23, 2020 – Mar 11, 2021, and Nov 27, 2021 – Mar 3, 2022. *Allocapnia rickeri* was found Dec 26, 2020 – Mar 19, 2021, and Dec 8, 2021 – Mar 3, 2022. While the presence of *A. rickeri* was noted 2 to 4 weeks before *A. mohri*, it was 6 – 8 weeks after I began sampling before the two species were found in relatively equal abundance (Figure 2). *Allocapnia jeanae* accounted for 2.8% of specimens collected, and *Allocapnia ozarkana* was the rarest species, only found at 2 sites (Table 3). *Allocapnia jeanae* was found Nov 23, 2020 – Mar 4, 2021, and Dec 22, 2021 – Jan 11, 2022. *Allocapnia ozarkana* was found Dec 17, 2020 – Dec 26, 2020, and on Dec 10, 2021. No *Allocapnia* were found in Clear Creek, including the target species of concern, *A. warreni*.

Over half the variation in site variables and species occurrences was explained by land use across the first OPLS axis, with all species found in predominantly forested WFWR and WR streams (Figure 3A). Clear Creek is located north of these watersheds in a heavily urbanized and agriculturally developed region (Table 1; Figure 1), potentially explaining the absence of *Allocapnia* in this stream (Figure 3A). Watershed area and stream order further separated sites and species across OPLS-2. *Allocapnia rickeri* and *A. mohri* were more commonly found in relatively large, high-order watersheds in the WFWR basin compared to *A. jeanae* and *A. ozarkana*, which were more often found in headwater streams of the WF and WFWR. While all species were positively related to forested land use, moderate agricultural land use did not preclude the presence of *Allocapnia* species in these streams (Figure 3B).

Analysis of Pearson's and Spearman's correlation coefficients were used to test significance between land use/landscape variables and species richness. Both tests revealed significant relationships, statistically confirming patterns seen in the OPLS (Table 2). Pearson correlation coefficient provided a better fit with the data compared to the Spearman correlation coefficient so linear thresholds were examined for each land use/landscape category. Threshold analysis indicated a significant increase in species richness with increasing percent forest (threshold = $64.39\% \pm 25.17$; p < 0.001; Figure 4A) land use and a corresponding decrease in species richness along increasing percent agriculture (threshold = $26.19\% \pm 5.18$; p < 0.001; Figure 4B), percent urban (threshold = $8.56\% \pm 0.74$; p < 0.001; Figure 4C), and percent imperviousness (threshold = $1.51\% \pm 0.16$; p < 0.001; Figure 4D).

4. Discussion

These results demonstrate the strong influence of land use and landscape variables on the distribution and abundance of *Allocapnia* species in three major stream networks in NWA. All species were found most often in forested streams, and somewhat surprisingly, continued to persist in areas of moderate (18% - 26%) agricultural land use. No species were found in heavily urbanized (40% - 51%) watersheds. In terms of watershed characteristics, *A. rickeri* and *A. mohri* presence was strongly associated with large, high-order watersheds, while *A. jeanae* and *A. ozarkana* were more associated with headwater streams. *Allocapnia warreni*, nor any other species was collected during any visit to Clear Creek.

Allocapnia rickeri is widely distributed along the eastern region of the U.S. and was the most commonly collected *Allocapnia* species in my survey. This species has been found in high abundance in other surveys conducted across twenty-one U.S. states, Washington D.C., and Ontario, Canada (Ross & Ricker, 1971; A. L. Sheldon & Warren Jr, 2009). Due to its extensive

range, *A. rickeri* appears to have evolved life-history strategies allowing it to inhabit streams less favorable to other *Allocapnia* species (Ross & Ricker, 1971). For example, *A. rickeri* displays flexible development cycles which may make it more resilient to stream warming and hydrological variability associated with climate change (Webb, 2002). *Allocapnia rickeri*, like the other species in my study, were most strongly associated with forest land use, but it was also found in high abundances in relatively large watersheds influenced by agriculture. While it was the least sensitive species to land use differences out of all taxa in my study, a survey in Illinois suggests that *A. rickeri* may have been extirpated several watersheds due to extensive land conversion from forested to pastural areas (Webb, 2002). Additionally, I did not collect samples of *A. rickeri*, nor any other *Allocapnia* species, at any of my most agriculturally developed and urbanized sites in Clear Creek, meaning that, although it is not an SGCN taxa, *A. rickeri* may still be sensitive to the rapid land use changes occurring across the region.

Allocapnia mohri is an endemic species widely distributed in the Ozark-Ouachita region encompassing Arkansas, Missouri, and Oklahoma (Poulton & Stewart, 1991; Ross & Ricker, 1971). As opposed to *A. rickeri, A. mohri* appears to have more narrowly adapted to conditions in this region and may be unable to disperse outside this range (Ross & Ricker, 1971). *Allocapnia mohri* was the second-most collected species in my study and found in conjunction with *A. rickeri* at all but one sampling location, suggesting similar sensitivities to regional landuse development. A recent study of *A. mohri* in Arkansas (Annaratone et al., 2023) found that landscape factors, most importantly urban land use, was the strongest predictor of *A. mohri* presence across four eco-regions including the Boston Mountains and Ozark Highlands. *Allocapnia mohri* presence was also negatively related to mean winter and annual temperatures. My results indicate that these taxa may have different thermal optima, with *A. mohri* emerging in abundance up to six-eight weeks earlier than *A. rickeri* (Figure 2) and both taxa emerging before *A jeanae* and *A. ozarkana*. However, there are likely additional environmental cues besides temperature, such as stream flow and light availability, driving emergence timing. These patterns are consistent with emergence patterns found by Ross and Ricker (1971) indicating that they have remained remarkably consistent over the last 50 years. As both urban land use and temperature are projected to increase in NWA over the coming years, more research into interactions between land use and climate change are necessary for understanding coexistence and species distributions of winter stonefly taxa (Annaratone et al., 2023).

Allocapnia granulata has previously been documented in the Boston Mountains and Ozark Highlands ecoregions including the WFWR and WR stream networks sampled for my study (Poulton & Stewart, 1991; Ross & Ricker, 1971). Poulton & Stewart (1991) found that *A. granulata* was the second most common taxa within the Ozark region (AR, MO, OK), and the species has a dispersal range in North America even larger than that of *A. rickeri* (Ross & Ricker, 1971). However, despite my efforts, I did not observe or collect any specimens of this species at any of my sampled sites. The emergence period of *A. granulata* is thought to overlap with that of *A. rickeri* and *A. mohri*, making it surprising that I did not encounter it in my study. *Allocapnia granulata* has recently been recorded further to the east of my sample sites in highorder streams within the Boston Mountains and in large, permanently flowing streams at low elevations in the Arkansas River Valley and Ouachita Mountains (Evans-White; unpublished data). Thus, its absence from my collections could indicate that this species is not well adapted to conditions in low-order streams in the region (Poulton & Stewart, 1991).

The geographical distribution of *A. jeanae* was historically limited to the Ozark Highlands of NWA (Ross & Ricker, 1971). However, subsequent collections found that this

species had dispersed extensively into various suitable tributary habitats in other regions, including central Arkansas, western Oklahoma, and southern Missouri (Poulton & Stewart, 1991). This expansion is noteworthy, given that *Allocapnia* is considered to be a poor disperser, with adult male overland travel limited to crawling ~100 meters per year and females with more developed wings capable of climbing and gliding several hundred meters (Ross & Ricker, 1971). Although A. jeanae was more commonly found in low-order, small watershed streams in my study (Fig. 3), downstream drift could explain some of the dispersal ability of this species as it was found in lower abundances in 3rd and 4th order mainstream sites in the WFWR and WR drainages. Given the large distances traveled, it is also possible that regional timber harvesting and agricultural development (Karstensen, 2009) followed by overland transport of these goods also played an important role in A. jeanae dispersal in Arkansas. Because of the staggered emergence behavior of *Allocapnia* in the region, it is almost certain that some A. jeanae, along with other rare *Allocapnia* species, were overlooked in previous surveys as most sites were only sampled once (68% of sites; Poulton & Stewart, 1991). However, given the extensive land use changes in Arkansas and existence of geographically isolated "relict" populations of A. jeanae and other Allocapnia species including A. ozarkana (Poulton & Stewart, 1991) and A. oribata (P. Hogan; personal communication) throughout the state, the role of human activities on the phylogeography of winter stoneflies warrants further consideration.

Allocapnia ozarkana appears to be a rare species, with only 10 specimens found during my two years of sampling in the Boston Mountains. Historically, this species was first collected and described from WR-4/Cannon Creek and found at five other tributaries in the Boston Mountains and the WR basin (Ross & Ricker, 1971). I did not find *A. ozarkana* at this site, but it was present nearby (2-6 km) at two 1st and 2nd order streams located in small watersheds (Table

3). Colonization of these streams most likely occurred through overland crawling, as to my knowledge it has never been collected outside of small tributaries, and wing development of *A. ozarkana* can be stunted in both males and females (Ross & Ricker, 1971). It is unclear why *A. ozarkana* has been extirpated from Cannon Creek, as the other three taxa in my study were present there and coexist with *A. ozarkana* at the two nearby sites. *Allocapnia ozarkana* and *A. jeanae* hold identical priority scores in the AWAP, but based on the distributional data I have collected, populations of *A. jeanae* appear to be more pervasive. *Allocapnia ozarkana* have managed to persist despite major logging operations in the region, and conversion of forested lands to moderate amounts of pastural land use. However, their low abundances and limited dispersal abilities may leave them vulnerable to inbreeding extinction and continued land development and fragmentation (Tilman et al., 1994).

For an example of the local impacts of these factors, we can examine the unfortunate case of *A. warreni*, which I did not collect despite extensive sampling efforts in Clear Creek. Only one known specimen of this species was ever collected from here in 1962 (Ross & Yamamoto, 1966), and failed repeated sampling efforts here and in surrounding sites suggest that it was extirpated from the region by the late 1980's (Poulton & Stewart, 1991). Agricultural development could be responsible for the low initial population abundances of *A. warreni*, as agricultural land use in the Ozark Highlands exceeded my calculated thresholds for species richness declines by at least 1973 (35%; Karstensen, 2009). Land use data from 1992-1993 reported 9.7% forest, 78.2% agriculture, and 8.7% combined urban residential, commercial, & industrial near the headwaters of Clear Creek flowing from Lake Fayetteville (Burns, 2001). Despite these major land use changes, surveys found *Allocapnia* nymphs here in a 1998-1999 sampling season (Burns, 2001), but these samples were not identified to species. This study

documented a general decline in Plecopteran species in Clear Creek since 1977 with subsequent increases in more pollution-tolerant taxa such as Chironomidae. Along with *A. warreni*, it appears that all *Allocapnia* species have been eliminated from this stream, as no species were collected here during my study. This includes *A. sandersoni* which was first collected and described from Clear Creek (Ross & Ricker, 1971). Land use for this watershed by 2022 was 8.53% forest, 43.36 %agriculture, and 44.42% urban, highlighting the intensification of urban land use in NWA over the last thirty years. As NWA is a biodiversity hotspot for Plecopterans (Poulton & Stewart, 1991), continued urban expansion is likely to endanger other rare *Allocapnia* species and lead to the extirpation of winter stoneflies in general from the area unless efforts are made to conserve forested land use.

As with individual species occurrences, forested land use was positively related to species richness in my study. Forested ecosystems are a source of leaf-litter inputs which provides organic matter and nutrients to detritivorus macroinvertebrates like *Allocapnia* (Covich et al., 1999). Forests additionally contribute large woody debris to stream ecosystems, which may help retain this organic material for use by aquatic biota, increase macroinvertebrate habitat, and help reduce erosional downcutting (Hilderbrand et al., 1997). Maintaining forested streambanks reduces the amount of nutrients entering streams via uptake and removal (Lowrance et al., 1984) as well as reduces general sedimentation by slowing overland water flows (Walsh et al., 2005). Reductions in these ecosystem services could explain historical changes in species distributions and richness across the region (Karstensen, 2009, 2010), although their relative importance could differ between ecoregions where reduced food availability and sedimentation may be more critical in the heavily logged Boston Mountains compared to the development of agricultural and urban land use increasing nutrient runoff in the Ozark Highlands. Restoration

modeling has indicated a need for at least 60% mid-dense forest cover in watersheds for streams to be considered moderately healthy, with an ideal of 80% forest cover in hydrologically areas near streams for good ecosystem health (F. Sheldon et al., 2012). These values are consistent with my *Allocapnia* thresholds, which indicate the potential decline in species richness below 64% forest cover. However, the estimate's variability was notably the highest among all land use categories, approximately around 25%. As urbanization within the watershed intensifies, preserving a substantial amount of forest beyond the established threshold becomes imperative to sustain aquatic life. Conversely, in cases of watershed development for agriculture, it might suffice to maintain the percentage of forest at or slightly below the threshold. This underscores the significance of not only conserving existing forested land but also contemplating transitions from forested to other land use types in the context of stream health conservation.

The impact of agricultural development on stream ecosystems can vary significantly depending on several factors, including the primary form of agriculture (e.g., pasture or row cropping), riparian conditions, and tilling practices (Moore & Palmer, 2005). In NWA, forest clearing for agricultural use has primarily resulted from the growth of the poultry and cattle industries (Haggard et al., 2017). This activity has directly resulted in the nutrient enrichment of streams in the area, particularly in the Ozark Highlands where ongoing litigation between Arkansas and Oklahoma has revolved around stream water quality degradation (Haggard et al., 2017). In my study, I found a threshold of 26.19% agricultural land use before observing significant declines in *Allocapnia* richness. This threshold is remarkably similar to agricultural land use levels of 28% above which another winter stonefly taxa, *Strophopteryx*, dropped out of regional shredding macroinvertebrates assemblages due to nutrient enrichment of detrital food resources (Prater et al. 2015). Further, my results are consistent with this work and another study

showing general declines in shredding detritivore abundance and biomass going from primarily low-nutrient forested streams in the Boston Mountains to those located in high-nutrient pastoral watersheds in the Ozark Highlands (Haggard et al., 2017; Prater et al., 2015). Thus, while *Allocapnia* and other shredding detritivores may persist under moderate agricultural land use, they are subject to displacement by other macroinvertebrate taxa with further agricultural development in the region.

As with agricultural land use, I observed a negative association between urbanization and the presence of Allocapnia. Threshold analysis indicated an urban land use value of 8.56% before a significant decrease in species richness became apparent (see Figure 4). This threshold value is slightly lower than what has been reported in other studies investigating the impact of urbanization on macroinvertebrate communities. Although no other studies have explicitly focused on Allocapnia, Wang et al., (1997) found thresholds ranging from 10% to 20%, while Roy et al., (2003) reported thresholds between 15% and 20% when comparing percent urban land use to different indexes of biotic integrity. These studies observed substantial shifts in macroinvertebrate communities, transitioning from sensitive species to more tolerant ones at high urbanization levels. Given that Allocapnia belongs to the relatively sensitive Plecoptera order (Sánchez-Bayo & Wyckhuys, 2019), it is perhaps not surprising that it its threshold value was lower than these integrated community responses. However, it is worth noting that the utility of urban thresholds as a generic metric for assessing land use changes has been a subject of debate (Wang & Lyons, 2002). One challenge lies in categorizing the percent urban value into the four developed classes as defined by the NLCD (open space, low, medium, high). This categorization can introduce complexity when attempting to conduct an accurate threshold analysis. In recent literature, there has been a shift towards reporting urbanization as percent

impervious surface cover rather than total percent urban land use (Wang & Lyons, 2002). Consequently, I have chosen to report both values in the current study to provide a comprehensive understanding of their potential impacts on the studied stream systems.

Impervious surface cover is a widely used metric in discussions about changing land use, often regarded as a 'common currency' for effective communication among diverse stakeholders, including engineers, economists, and ecologists (Schueler et al., 2009). My results, of 1.51% total watershed imperviousness thresholds are notably lower than many previous values reported regarding thresholds. Other research concerning watershed imperviousness and aquatic biota have reported values of 4-5% (May et al., 1997), 8% (Wang & Lyons, 2002), 10% (Booth & Jackson, 1997), to as high as 12% (Klein, 1979; Lee et al., 2012) before significant declines in aquatic community metrics are observed. My results align more closely to those of King et al., (2011), which reported significant threshold declines in aquatic taxa between 0.5 and 2% watershed imperviousness, with a mean value of 1.98% threshold for *Allocapnia*, specifically. Together, these results along with those of urban land use thresholds suggests that while macroinvertebrate communities may respond negatively to urbanization, *Allocapnia* appears to be especially sensitive to small changes in impervious surfaces along with the resulting alteration of stream flow and habitat loss.

In this study, I conducted an integrated analysis that combined fine-scale, site- and species-specific surveys with regional land use and threshold analysis, drawing insights from historical data. The species-specific investigations provided valuable insights into the life history and environmental sensitivity of NWA *Allocapnia*. A key finding is that while all species responded similarly to land use, species-specific responses indicate that adequate management cannot rely on genus-level monitoring for risk of losing the rarest and most sensitive species (i.e.,

A. warreni). This information is vital for shaping effective conservation strategies and directing resources toward species requiring further data, notably A. ozarkana. Additionally, my findings revealed an expansion in the distribution of A. jeanae, despite historical forest clear-cutting and agricultural development. The outcomes of my analyses, including OPLS and threshold analysis, underscore the imperative to safeguard forested headwater streams against significant changes in land use to advance aquatic species conservation goals. Allocapnia has the ability to persist in landscapes influenced by a moderate (< 26%) agricultural development but exhibited significant decreases in richness beyond this value. Additional research has shown a general decline in shredding macroinvertebrate biomass beyond 28% agriculture (Prater et al., 2015). Agricultural best management practices are important to implement for the conservation of aquatic life. These measures should include restricting animal access to streams (Vidon et al., 2008), mitigating sediment influx (Moore & Palmer, 2005), and preserving riparian zones (Palt et al., 2022). Furthermore, my research highlighted that even minor urban and impervious surface development can lead to the extirpation of species from watersheds. Given the projected growth of the Northwest Arkansas area (Northwest Arkansas Open Space Plan, 2016), proactive measures must be adopted. These measures should encompass downstream biota sampling and managed translocation for species of concern (Olden et al., 2011), as well as the preservation of dispersal corridors (Smith et al., 2009). These actions should be integral components of a comprehensive watershed management approach (Palt et al., 2022). Acknowledging the multifaceted nature and complexity of land use and its associated effects, this research offers a pragmatic approach for managing extensive surface areas and prioritizing conservation endeavors. In the face of an evolving landscape, understanding these intricate interactions

remains paramount for safeguarding our natural resources and ensuring the long-term sustainability of our ecosystems.

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Tables and Figures

Table 1. Sample site information. Sites are named according to their river system (WFWR = West Fork White River; CC = Clear Creek; WR = White River) and order in position from upstream to downstream. Drainage area indicates the size of the HUC12 watershed up until the point of sampling. Land use values were calculated from the Multi-Resolution Land Characteristics Consortium's National Land Cover Database (NLCD) 2019 dataset.

Site ¹	Visits	Latitude	Longitude	Strahler stream Order	Drainage area (km ²)	% 0	% Agriculture ³	% Urban ⁴	% Impervious
WFWR-1	16	35.81445	-94.1298	3	17.70	72.11	16.84	8.56	1.20
WFWR-2	17	35.87671	-94.0927	2	7.42	85.41	9.14	5.04	0.57
WFWR-3	9	35.84781	-94.1464	2	3.00	81.58	8.23	7.22	0.36
WFWR-4	14	35.8666	-94.1191	4	65.13	73.92	18.29	6.06	0.82
WFWR-5	14	35.88532	-94.1688	4	125.07	76.50	15.68	5.86	1.01
WFWR-6	13	35.92497	-94.181	4	156.10	76.40	15.33	6.19	1.19
WFWR-7	11	35.94622	-94.1851	4	182.48	74.28	15.85	7.08	1.48
WFWR-8	5	35.94487	-94.1856	2	182.45	74.29	15.84	7.08	1.48
WFWR-9	15	35.9627	-94.1669	3	14.87	67.05	26.19	3.38	0.53
WFWR-10	10	35.97964	-94.1741	4	214.13	71.69	18.27	7.06	1.513
WFWR-11	5	36.04358	-94.1347	3	30.60	33.30	13.50	51.84	23.330
WFWR-12	8	36.05001	-94.1171	4	303.79	64.39	19.08	13.71	4.565
CC-1	5	36.13362	-94.1408	2	24.33	8.53	43.36	44.42	20.317
CC-2	5	36.13017	-94.1538	2	25.72	9.20	41.44	45.84	21.140
CC-3	5	36.12787	-94.1651	2	26.65	9.50	40.62	46.46	21.615
CC-4	5	36.13441	-94.203	3	92.10	14.05	26.34	57.39	24.262
CC-5	5	36.10032	-94.3151	3	190.31	24.41	33.18	40.03	15.742
WR-1	5	35.81866	-93.7803	3	104.70	85.84	8.35	3.83	0.281
WR-2	15	35.77888	-93.796	1	2.86	97.12	2.15	0.72	0.183
WR-3	15	35.85475	-93.931	1	2.02	86.58	5.33	8.08	0.533
WR-4	18	35.90448	-93.947	1	6.69	89.36	7.61	2.86	0.303
WR-5	15	35.93023	-93.9623	1	7.05	74.58	18.78	4.65	0.417

¹ Site: WFWR = West Fork White River; CC = Clear Creek; WR = White River

² NLCD Class 41 and 42 combined

³ NLCD Class 81 and 82 combined

⁴ NLCD Class 21-24 combined

Table 2. Results from Pearson's and Spearman's Correlation Coefficient tests. Bold fonts indicate statistically significant relationships. Land use data (% forest, % agriculture, % urban, % impervious) are all derived from the Multi-Resolution Land Characteristics Consortium's National Land Cover Database (NLCD) 2019 dataset with % forest combining NLCD class 41 and 42, % agriculture combining classes 81 and 82, and % urban combing classes 21-24.

Dependent variable	Independent variable	Pearson r	р	Spearman rho	р
Species Richness	% Forest	0.89	< 0.001	0.75	< 0.001
	% Agriculture	-0.71	0.003	-0.57	0.074
	% Urban	-0.89	< 0.001	-0.70	0.005
	% Impervious	-0.90	< 0.001	-0.80	< 0.001
	Latitude	-0.86	< 0.001	-0.76	< 0.001
	Area	-0.24	> 0.999	-0.52	0.145
	Strahler stream order	-0.18	> 0.999	-0.23	> 0.999

Site	Allocapnia rickeri	Allocapnia mohri	Allocapnia jeanae	Allocapnia ozarkana
WFWR-1	389	167	1	0
WFWR-2	95	239	16	0
WFWR-3	45	18	29	0
WFWR-4	253	130	1	0
WFWR-5	324	384	2	0
WFWR-6	86	71	0	0
WFWR-7	204	183	1	0
WFWR-8	6	49	0	0
WFWR-9	229	226	25	0
WFWR-10	103	66	0	0
WFWR-11	0	0	0	0
WFWR-12	3	0	0	0
CC-1	0	0	0	0
CC-2	0	0	0	0
CC-3	0	0	0	0
CC-4	0	0	0	0
CC-5	0	0	0	0
WR-1	54	16	0	0
WR-2	87	16	2	0
WR-3	236	97	36	3
WR-4	113	206	15	0
WR-5	471	278	13	7

Table 3. Sum of *Allocapnia* species collected between November 23, 2020, to March 19, 2021, and November 22, 2021, to March 3, 2022, in three stream reaches. WFWR = West Fork White River; CC = Clear Creek; WR = White River. Site numbers indicate individual locations.

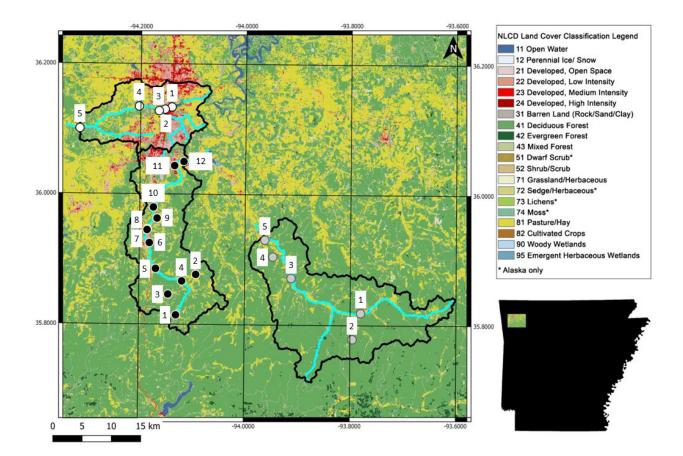


Figure 1. A site map of sample locations in Northwest Arkansas. The West Fork White River (black dots) flows from South to North, White River (grey dots) flows Southeast to Northwest. Clear Creek (white dots) flows East to West. Blue lines indicate the stream system. Black boarders were drawn around the watershed draining area to the last point of sampling. Number labels refer to site numbers in tables 1 and 3. West Fork White River 7 is a mainstem site, while West Fork White River 8 is referring to a nearby tributary.

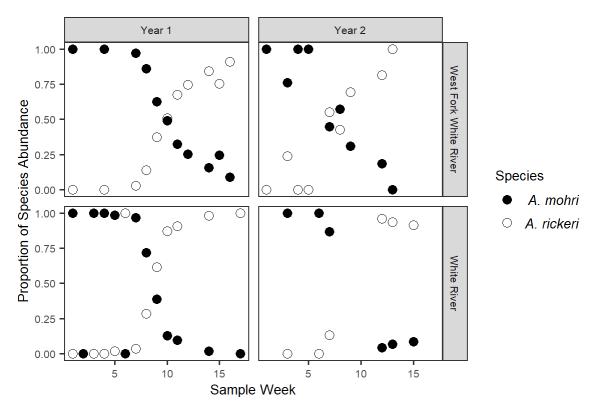


Figure 2. Temporal emergence variation between *Allocapnia mohri* (closed circles) and *Allocapnia rickeri* (open circles). The x-axis indicates sampling week. Sites were repeatedly sampled for an average of 10 visits between November 23, 2020, to March 19, 2021 (left: Year 1), and November 22, 2021, to March 3, 2022 (right: Year 2) during known periods of *Allocapnia* emergence. The y-axis shows abundance proportionally scaled between the two species separated by river system (top: West Fork White River; bottom: White River).

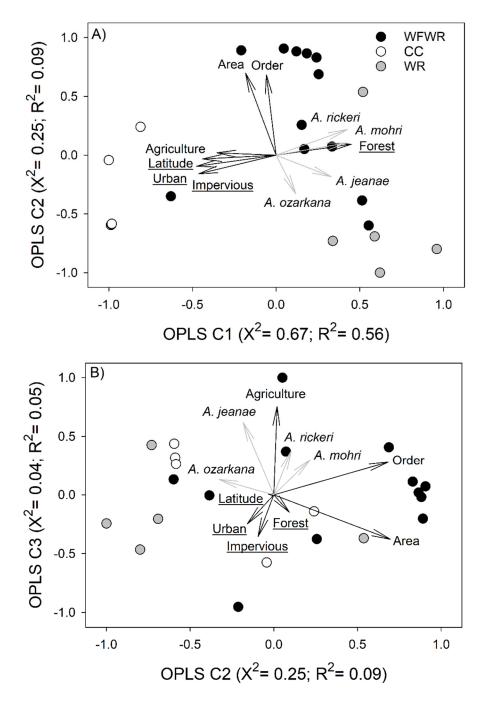


Figure 3. Land use and landscape variables relating to *Allocapnia* presence/absence. Relationships between sites (circles), predictor variables (X; black vectors), and species occurrence (Y; grey vectors) are shown from partial least squares (OPLS) regressions where correlations among variables are directly proportional to their relative positions in Euclidean space. Panel A) OPLS component (C1) vs. OPLS C2, and B) OPLS C2 vs, OPLS C3. Strong predictors of both X and Y variables are underlined and were determined by variable importance projection scores (VIP >1). Cumulative predictability of species occurrences across all PLS-axes was determined iteratively ($Q^2=0.59$; n=1000x).

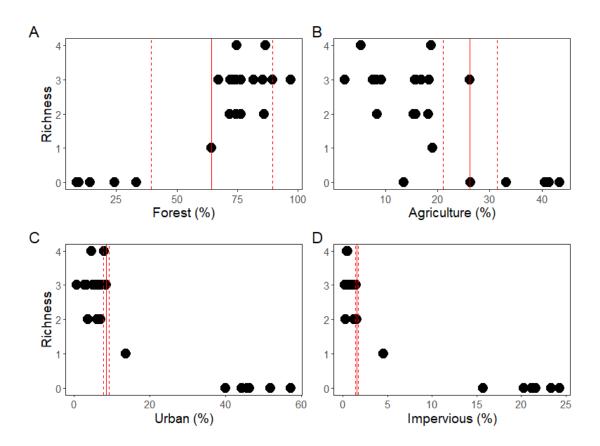


Figure 4. Threshold analyses of species richness (y-axis) across increasing gradients of land use: A) % forest, B) % agriculture, C) % urban, and D) % imperviousness. All models are statistically significant (p < 0.05). Richness thresholds are denoted by solid red lines and were found at 64.3% (±25.17) for forest, 26.19% (±5.18) for agriculture, 8.56% (±0.74) for urban, and 1.51% (±0.16) for imperviousness. Dashed red lines indicate 95% confidence intervals for thresholds.