ABSTRACT

MCNUTT, JAMES CAMPBELL. Habitat use by *Allocapnia rickeri* and *Allocapnia wrayi* in a small North Carolina piedmont stream. (Under the direction of Samuel C. Mozley.)

The purpose of this study has been to evaluate habitat use by *Allocapnia rickeri* and *Allocapnia wrayi* in a small piedmont stream in Raleigh, North Carolina. Five different surface habitats (debris dams, riffle mineral, riffle leaf material, pool mineral and pool leaf material) were sampled to determine which were favored by actively growing *Allocapnia* larvae and to detect changes in preference of habitats during the active growth phase. Larvae preferred habitats associated with leaf material, especially debris dams and riffles prior to emergence. Smaller larvae were more commonly associated with riffle mineral habitats before shifting to leaf material habitats. The active growth phase started in late October after diapause break and ended with emergence in December and January. The hyporheic zone was sampled separately using core samples in order to evaluate length of diapause and characteristics of the hyporheic zone habitat. Larvae entered diapause in March and diapause break occurred in late October. The diapause period was longer than reported for *Allocapnia* in Canadian studies. Larvae were most dense between 10 and 20 centimeters into the hyporheic zone. Temperature did not decrease with depth during the hottest months and dissolved oxygen dropped rapidly with depth into the hyporheic zone. Larvae were collected where dissolved oxygen was near 0 % saturation. Larvae were most dense when pore space was near 15 percent of the sample layer and average particle size was between two and three millimeters. This is the first ecological study of *Allocapnia* at the southeastern edge of their distribution and in an intermittent stream.

HABITAT USE BY *ALLOCAPNIA RICKERI* AND *ALLOCAPNIA WRAYI* IN A SMALL NORTH CAROLINA PIEDMONT STREAM

By
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APPROVED BY:

Chair of Advisory Committee

Dedication

This work is dedicated to my loving wife, Stacey Hodges who supported me and made many sacrifices so this work could be completed.

Biography

I joined the Peace Corps in 1989 after deciding not to pursue a graduate degree so soon after finishing at Texas Tech. I taught biology, chemistry, physics and general sciences to middle and high school kids in Fiji. The experience was very rewarding. I met my wife there and we married. After a time traveling and visiting family we settled in North Carolina and I started work with the Durham County Health Department as an environmental specialist in on-site wastewater. We had our first child, Noa in 1995 and I then decided to pursue a graduate degree. Sam Mozely took me on as his student almost sight unseen. After completing my course work and the bulk of my data collection we had a second child, Brinn in 1999. At that time I started with the North Carolina Division of Water Quality as a basinwide planner. I estimated that I had a full two months of work left to complete this thesis. The two months were spread out over three years on weekends and days off. I do not recommend this plan of work for others, however I have no regrets. The work and study at NC State have been invaluable to me in my work protecting the state's water resources. I like to think that the investment that NC State has made in my education has also helped to protect the state's environmental resources.

Acknowledgements

I would like to acknowledge my advisory committee for sticking in there and being patient while I finished this work. Sam Mozley provided great guidance in not only helping me in selecting this line of study that is much ignored, but also in providing me with a good foundation in limnological study which I use daily. Charles Apperson provided me an opportunity to learn about mosquito movement and make a little money to support my own work. Greg Jennings helped me to see where my education could be used and was instrumental in landing me in my current position. Jeff Hinshaw provided some great advice early on about keeping the work simple no matter how complicated it may seem. I would also to thank Will Harman (then with the Water Quality Group) for the loan of some equipment. I thank Colby Meacham and Erin Struss for their assistance in the field on cold and hot days. Thanks to Boris Kondrateiff for confirming and correcting my identifications of adults. I also thank my comrades at the Division of Water Quality who constantly encouraged me to stay with it and finish. As this study was not funded I thank the Graduate School for various teaching assistantships to keep the study going. I most thank my wife for remaining gainfully employed during the early years of this work that allowed for its completion.

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Introduction

Availability of preferred surface and suitable hyporheic habitats may ultimately determine the ability of intermittent streams to support winter stonefly populations. This study evaluates the relative use of five small stream habitats by two widespread *Allocapnia* species *(A. rickeri* Frison and *A. wrayi* Ross), and explores the conditions in the hyporheic habitat during seasonal diapause. It is the first ecological study of *Allocapnia* near the southeastern edge of their distribution, and the first to look at *Allocapnia* populations in an intermittent stream. Particle size, pore space, depth, temperature and dissolved oxygen levels were selected for study as environmental factors likely to affect *Allocapnia* larval distributions in the hyporheic zone.

Review of Allocapnia

Allocapnia is an important eastern North American stonefly genus distributed from Canada to the southern piedmont of the United States (Ricker and Ross 1971). Ricker and Ross (1971) developed keys to adults and Stewart and Stark (1988) review the literature on *Allocapnia* larvae through 1987. Forty-one species of *Allocapnia* are reported from Manitoba to Louisiana in the west and from Maine to South Carolina in the east (Stewart and Stark 1988). *A. rickeri* has been collected widely from the Mississippi drainage to the east coast and as far south as Louisiana and Georgia (Stewart and Stark 1988). *A. wrayi* is common east of the Appalachian Mountains from Maryland to Georgia (Kondratieff and Kirchner 1982).

Allocapnia are univoltine with emergence patterns varying with latitude (Ernst and Stewart 1985b). *A. granulata* emerged from December to March with a peak in January and February in Indiana (Finni and Chandler 1977) and in mid March in Ontario (Harper and Hynes 1972). *A. recta* emerged in December in a non-freezing Canadian stream (Harper and Hynes 1972) and from November to mid-March in a South Carolina stream (White et al. 1979). *A. aurora* emerged from November into March in South Carolina (White et al. 1979). In a second-order Ozark stream adult *A. rickeri* started to emerge in late December (Ernst and Stewart 1985b). In a North Carolina mountain stream *Allocapnia* began to emerge in December (Huryn and Wallace 1987). Emergence lasted from March to April for *A. pygmaea* in a Canadian stream (Harper et al. 1991 and Harper and Hynes 1972). No differences in life histories were noted for *A. pygmaea* and *A. rickeri* in a study of winter stoneflies in southern Canada (Harper and Hynes 1972).

Harper and Hynes (1972) described the life history of *A. pygmaea* in a southern Ontario stream, including observations of adult movement after emergence. Adults emerged from under ice and then used visual cues to move away from the bank. Dark outlines of objects against the snow cover served as the cues. Air temperatures above freezing correlated to emergence peaks in their study. *A. pygmaea* adults fed prior to mating and this feeding was important to ovarian development (Harper and Hynes 1972). Females left the stream banks more so than males after emergence, then females flew back to the stream after eggs developed. Males remained near the stream and attempted to mate freely (Harper and Hynes 1972).

Harper and Hynes (1970) first observed diapause in winter stoneflies in Canada. Finni and Chandler (1977) collected diapausing larvae in October in an Indiana stream. In a first to second-order North Carolina mountain stream early instar *Allocapnia* larvae were collected in March, diapausing larvae in July and August and rapidly growing larvae were collected in November (Huryn and Wallace 1987). Fourteen of 91 *A. pygmaea* larvae collected in May in a small Canadian stream were in diapause and only diapausing larvae were found during the summer months (Harper et al. 1991). Diapausing larvae were shorter than the active larvae collected earlier in the year (Harper et al. 1991). Rapid growth (diapause break) started in October after an approximate four-month diapause (Harper et al. 1991). In an Ozark Mountain stream *A. rickeri* exhibited an approximatly nine-month diapause (Ernst and Stewart 1985a).

Khoo (1968) evaluated the factors causing onset and break of diapause in a European Capniid in a controlled laboratory setting. Temperature and daylight hours were noted as potential causes for onset and break of diapause (Khoo 1968). Hynes (1974) evaluated the hyporheic distribution of diapausing winter stoneflies in a Canadian stream finding most *Allocapnia* near the surface. Pugsley and Hynes (1985 and 1986) experimentally evaluated diapause habitat and depth distribution in the hyporheic zone of a Canadian stream for *A. pygmaea*. Larvae moved during diapause and at least some larvae were in diapause year around (Pugsley and Hynes 1986). Pugsley and Hynes (1986) also evaluated the body position of larvae for pre-diapause, transitional and diapause. Pre-diapause larvae were smaller and fully extended in body position, diapause larvae had the head tightly tucked and a transitional larvae were slightly bent.

Allocapnia are shredders that have strong preference for leaf pack habitats (Reice 1980 and 1981; Finni and Chandler 1977). *Allocapnia* were common colonizers of leaf packs in New Hope Creek, North Carolina (Reice 1980). *A. granulata* larvae were more common in leaf pack samplers placed over riffle areas than leaf pack samplers placed over bedrock or pools (Finni and Chandler 1977). After leaf fall organic material became entrained over riffle substrates (Finni and Chandler 1979). Riffle areas provided interstitial spaces for diapausing larvae and detritus lodged in the interstitial spaces provided an abundant food source for larvae through the fall months (Finni and Chandler 1977). Stonefly eggs were also retained by the interstices of riffle areas (Finni and Chandler 1979).

<u>Hyporheic Zone</u>

Puglsey and Hynes (1986) review the ecological importance of stream insects moving into and out of the hyporheic zone. This movement could explain temporary disappearance at different times in the life cycle, rapid recolonization after disturbances of the stream bed, fluctuations in drift and seasonal changes in vertical distributions. The hyporheic zone may serve as a refuge from high temperatures and predators (Pugsley and Hynes 1985). *Allocapnia* spend a large portion of the one year life cycle in diapause (Ernst and Stewart 1985a, Puglsey and Hynes 1985 and 1986, and Harper et al. 1991).

The hyporheic zone is important in stream ecosystems as a refuge from adverse events occurring on the streambed immediately adjacent to the water column (Williams 1984). Stocker and Williams (1972) found that pore space decreases with depth in the hyporheic zone. The hyporheic zone must have adequate pore space to allow larvae to burrow deep enough to avoid the extreme surface conditions. Suitability of the hyporheic habitat may be the most limiting aspect of all the stream habitats for *Allocapnia.*

Importance of Small Streams

Approximately 66 percent of upper Neuse River basin drainages are made up of small ephemeral channels and intermittent streams not represented on maps (Bruton 2003). Small streams are home to macroinvertebrate populations that can colonize downstream habitats after disturbances on the streambed (example Puglsey and Hynes 1985). Knowledge of the prevalence and conditions of small stream habitats in North Carolina is an important requirement for determining how to maintain healthy downstream communities (DENR-DWQ, 2002). Small streams are very susceptible to disturbances in areas where landscape modification is ongoing.

Many small streams in North Carolina have been silted out by previous farming activities. Once these activities cease, stream habitats can recover and subsequently be colonized by macroinvertebrates from nearby, undisturbed watersheds. Contributions from populations in small, headwater streams can help maintain diverse downstream communities. Unlike past agricultural activities in small watersheds, disturbances from development alter watersheds in a more permanent way that can prevent recovery of macroinvertebrate populations (DENR-DWQ 2002). More importantly, as these small watersheds are disturbed by development activities the number of macroinvertebrate populations that can colonize recovered stream reaches is reduced. It is important to preserve existing *Allocapnia* (and other macroinvertebrate) populations in small streams so that macroinvertebrate communities in other watersheds and downstream reaches can be reestablished after restoration or natural recovery. *Allocapnia* are also an important component of larger stream communities in North Carolina (Reice 1980).

To more fully understand the ecology of *Allocapnia* it is necessary to investigate habitat use. Because small streams are often not considered during land development activities, habitat use information will also be important to preservation of *Allocapnia* populations, establishing small stream refuges and in stream restoration activities.

The above studies have shed light on emergence patterns of *Allocapnia* across their distribution and provide some insights into habitats used by *Allocapnia* larvae. Several of the studies presented ecological information on *Allocapnia* populations in small streams (Harper et al. 1991 and Ernst and Stewart 1985a and 1985b). Knowing how and when *Allocapnia* use the hyporheic and surface habitats may help to reduce impacts to these populations during watershed altering activities. This information could also be used as indicators of aquatic habitat and water quality in small watersheds. More information is needed on *Allocapnia* habitat use in order to protect these habitats in small watersheds as well as in larger river systems. This expands on previous work exploring habitat use by

Allocapnia and also looks at the important hyporheic zone habitat and how it relates to the other surface habitats.

Description of Study Site

The study site is a first order stream on the North Carolina State University Centennial Campus in Raleigh, North Carolina (Figure 1). The stream emerges from the ground under a tree (34° 45' 41.26" N, 78° 41' 18.98" W –altitude 98.13 meters) at the end of a 100 meter-long ephemeral channel. The stream ends in a wetland area (35° 45' 49.97" N, 78° 41' 5.61" W – altitude 90.86 meters) at the confluence with Lake Raleigh (impoundment of Walnut Creek). The perennial/intermittent stream channel is approximately 500 meters long, draining a 16-hectare watershed. The watershed is mostly hardwood (e.g., tulip poplars) along the riparian corridor with loblolly pines in the upland regions. Parts of the watershed were farmed approximately 100 years ago but currently it is unaffected by development or other intensive land uses (NC EIS for Centennial Campus 1988). The only recent disturbance observed in the stream was from uprooted trees that fell during Hurricane Fran in September 1996. The Lake Raleigh Dam was destroyed by the hurricane allowing the study stream to flow into Walnut Creek for the first time since 1919. There are hiking/biking trails in the watershed that cross the stream near the mouth in a wetland area where there is no defined stream channel. Because of the abundance of winter stoneflies (especially *Allocapnia*) and the apparent good habitat for the two study species, the stream is unofficially known and will be referred to as Winter Creek.

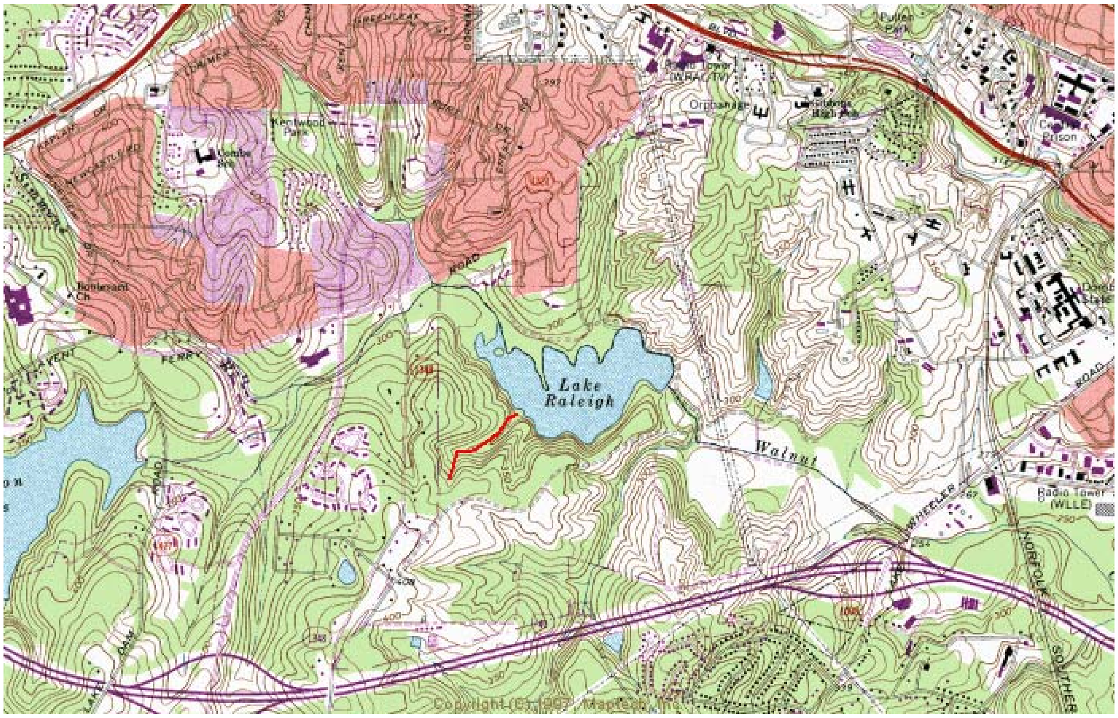

Figure 1. Winter Creek watershed. From USGS Quad Raleigh West.

An older stream bed was located below the current stream bed as evidenced by the presence of coarse material below a thick layer of silt, similar to the coarse material found in the current stream bed riffles. The extent to which the old and new channels were continuous was not known. For the purposes of this study the hyporheic zone was defined as the interstices between the free flowing surface water and the separating silt layer or bedrock (Williams 1984). In Winter Creek this zone was between 0 and 45 cm thick, and has existed since agricultural uses of the catchment ended approximately 100 years ago. Farming activities in the 19th century probably silted over the original streambed and a new channel formed on top after these activities ceased (NC EIS for Centennial Campus 1988). Winter Creek averages about 50 centimeters and expands to 2 meters wide at the largest pool. There are many pool and riffle combinations and many debris dams.

Methods and Materials

The goals of this study were to describe the seasonal abundance and habitat distributions of *Allocapnia rickeri* and *Allocapnia wrayi* in a small piedmont stream. The sampling methods used in the study were designed to evaluate habitat use by *Allocapnia* in the hyporheic zone and in five different surface habitats observed in Winter Creek. Data collection began in August 1998 and ended in July 1999. Hyporheic collections were made every four to six weeks from August 1998 to March 1999 with an extra sample collected in July 1999 when stream flow had ceased. Temperature, dissolved oxygen, pore space and particle size were evaluated in the hyporheic zone. Surface habitat collections were made approximately monthly from September 1998 to April 1999 with surveys made during the summer of 1998 and 1999 to determine if larvae were present in surface habitats. Larval body lengths were measured to determine if there were size related habitat shifts. The surveys also were used to make observations on seasonal changes in the surface habitats. Adults were collected for identification purposes and observations were made during field trips on emergence habitats and timing.

Methods for Collecting Larvae from Hyporheic Sample Layers

Beginning in August 1998, core samples were collected every four to six weeks from three different riffle areas in Winter Creek. Core sample layer collection stopped after larvae were no longer found in surface habitats in March. Another set of cores samples was collected in July 1999 to assess depth distribution at approximately mid-diapause. Substrate material was collected from at least three different depths in the core and preserved in alcohol. A total of 18 cores with 75 sample layers were collected. The depths were measured from the water/substrate interface downward. Sample layers were collected from the following depth ranges 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, and 30-35 centimeters. It was not possible to consistently sample the same depths at each location because of large rocks in the core or presence of clay/silt layers.

A 65-cm long, 10.1-cm diameter PVC pipe with a tapered and serrated front edge was used to make cores into the hyporheic zone. The corer was similar to the standpipe corer used by Pugsley and Hynes (1986) to retrieve colonization chambers buried in the hyporheic zone of the Speed River. The standpipe corer was effective for characterizing all aspects of the hyporheic community (Fraser and Williams 1997). The corer encompassed a substrate area that was approximately 30 to 50 percent of riffle widths. In the early part of the study, attempts were made to use small diameter corers (4.0 cm) in hopes of collecting smaller but more numerous samples. These smaller corers had to be removed from the stream bottom by excavating around the corer and picking up the corer by the leading edge. Due to the small size of the stream and large quantities of substrate that had to be disturbed this method was abandoned.

Because the substrate was removed from within the core, substrate disturbance was minimized in the remainder of the riffle. A small scoop was used to remove 20 to 200 cm^3 of substrate at each depth. Rocks larger than the scoops were removed by hand and the sample layer immediately below was collected. Care was taken not to contaminate deeper sample layers by removing water in the core before the next sample layer was collected. Depth of each sample layer was measured on the inside of the corer to the top of the next sample layer to be collected. All depths were measured from the point where the corer met the top of the streambed downward. Sample layers were stored in plastic containers with alcohol until further processing in the lab.

Fine particle (clay/silt) sample layers or bedrock marked the end of the hyporheic zone in Winter Creek. In many of the cores this material was found at 20-35 cm. Fine particle (clay/silt) sample layers were collected and analyzed for a few cores early in the study. No larvae were present in these sample layers in the hyporheic zone or in surface layers in pool bottoms. Since sampling was aimed at optimizing collection of diapausing larvae, sample layers were collected from riffle areas for the remainder of study.

Larvae were picked under magnification from material collected on a 100-µm sieve after elutriation of each sample layer. The organic particles and organisms were removed by pouring water and the sample layer material back and forth between large plastic containers (400 ml) then pouring the water through the 100-µm sieve. The remaining material was dried for later particle size analysis and pore space measurements. Small stoneflies were easily collected by scanning the sieved material under a dissecting microscope. The stoneflies were collected and preserved in alcohol. Abundance, total body lengths, and body positions were recorded for larvae in each sample layer. Total body length (tip of labrum to posterior end of last abdominal tergite) was measured to the nearest tenth of a millimeter using an ocular micrometer. Body position was noted as extended, half-bent or bent, corresponding to Pugsley and Hynes' (1986) descriptions of larvae in pre-diapause, transitional and diapause. Larvae were noted to be in diapause if they were bent or half-bent. Bent and half-bent larvae were straightened prior to measurement. Exuviae were also collected from sample layers but not measured.

Particle Size and Pore Space

Particle size make-up of each sample layer was also measured for the following ranges: > 2.46-mm, 1.02-2.46-mm, 0.51-1.02-mm, 0.23-0.51-mm and < 0.23-mm. A Keck Instruments SS-94 Sand Shaker was used to separate the different sizes. The dry weight of each particle size range was used to calculate the relative amount of each particle size for a sample layer. Average particle size for each sample layer was first calculated by multiplying the percent of each particle size range in each sample layer by the median particle size for that range. Size range multipliers were as follows: >2.46mm x 5mm, 1.02-2.46mm x 1.734mm, 0.51-1.02mm x 0.762mm, 0.23-0.51mm x 0.368mm and <0.23mm x 0.23mm. These products were summed and divided by 100 to obtain the average particle size for that sample layer. Regression analysis (EXCEL) was used to evaluate the relationship between particle size and depth.

Substrate (mineral material) volume for each sample layer was measured by displacement of the sample layer material in water. Pore space for each sample layer was measured by pouring water into the packed sample layer material until water reached the top of the material. The dry sample layer material was packed level into the 500-ml container to eliminate as much as possible large voids around rocks prior to adding water. A small wire was used to assure that water filled voids completely. A lid with a small hole in one corner was placed on the packed material and water. The container was inverted over a graduated cylinder and allowed to drain for three minutes. Pore space was the volume of water that drained from the container in the three-minute period. Larval densities were reported as individuals cm-3 of pore space. Regression analysis (EXCEL) was used to evaluate trends in pore space.

Temperature and Dissolved Oxygen

To evaluate physical conditions in the hyporheic zone temperature and dissolved oxygen were measured at seven sites during the most severe streambed conditions (July and August 1998). Core sample layers were collected adjacent to these sites in August 1998 to evaluate larval densities related to dissolved oxygen saturation and temperature. Measurements were made by driving a 4.0-cm diameter screened steel pipe into the substrate to 10, 20, and 30 cm. A probe (YSI 85) was lowered into the pipe and moved around in the water in the pipe. Temperature and dissolved oxygen saturation were recorded at each depth and compared to larval densities at that depth.

Methods for collecting larvae from surface habitats

To assess the surface habitats used by actively growing *Allocapnia* larvae from September 1998 to May 1999, samples from five different surface habitat types were collected. The habitats sampled were debris dams, pool leaf material, pool mineral material, riffle leaf material and riffle mineral material. Debris dams were made up of larger woody material with leaf material on the upstream side. Leaf material in pools was floating on the water near the sides or middle of the pools and on the pool bottoms. Leaf material in riffles was in smaller clumps lodged on rocks or sticks in the stream channel.

Three scoops of mineral material from a pool or riffle bottom, not deeper than 3-4cm, were placed in a bucket with water. The material was poured back and fourth between two buckets for approximately one minute to dislodge larvae from the material. Water was then poured through a 200-µm sieve to retain larvae. This process was repeated three times for each sample. Larvae were collected from debris dam and leaf material habitats by placing three handfuls of material into the buckets and repeating the procedure described above. This semi-qualitative effort was used because of difficulty in comparing available surface area of the two different substrates in a meaningful way. Also, the debris dam and leaf materials were extremely heterogeneous and no standard unit was readily defined in the natural material. The sieve residues were placed in plastic containers with 70% alcohol until further processing.

Samples were collected every four to six weeks after active larvae were detected during surveys in the near surface habitats in late September 1998. Sampling stopped after larvae were no longer detected in surface habitats in May. One set of habitat samples was collected in the upper, middle and lower reaches of Winter Creek on ten different dates from September 1998 to May 1999. In the lab, sample material was picked under magnification to collect all stonefly larvae. Each larva was measured from tip of labrum to posterior end of last abdominal tergite to evaluate larval growth and size related shifts in habitat use. Total body lengths (TBL) were compared among habitats using analysis of variance. The abundance of larvae in each habitat type was reported as a percentage of the total collected on that date for each of the surface habitats. Bent or half-bent larvae were not observed in the surface habitat samples.

Statistical Analyses

The relationship of larval density and depth was evaluated using regression analysis (EXCEL). Total body lengths (TBL) of larvae from each of the surface habitats were compared using analysis of variance (EXCEL ANOVA: single factor).

Methods for Collecting Adults

Two species of adult *Allocapnia (A. rickeri* and *A. wrayi*) were identified using keys by Ross and Ricker (1971). These identifications were confirmed by Boris Kondratieff in 2001. No attempt was made to separate the larvae by species as this can only be done with difficulty for pre-emergent larvae (Kondratieff, personal communication). Adults were collected primarily for identification purposes. General observations of emergence patterns and habitat use by adult *Allocapnia* in Winter Creek were made for comparison with studies of northern populations and to shed light distribution of larvae in stream habitats.

Results

Hyporheic Collections-Depth and Density

A total of 753 stoneflies and 179 exuviae were collected from 18 core samples (75 sample layers). All stonefly larvae collected in core sample layers were *Allocapnia* larvae in the pre-diapause, diapause, transitional or active growth phases. Densities are expressed as individuals cm^{-3} of pore space (Figure 2). The dashed outline in Figure 2 represents densities at each depth including exuviae. Analysis of body lengths does not include exuviae. Density measures reported in the remainder of the results include only larvae and not exuviae. Densities ranged from 0 to 3.66 individuals cm^{-3} of pore space. The slight increase in density of larvae with depth was not significant.

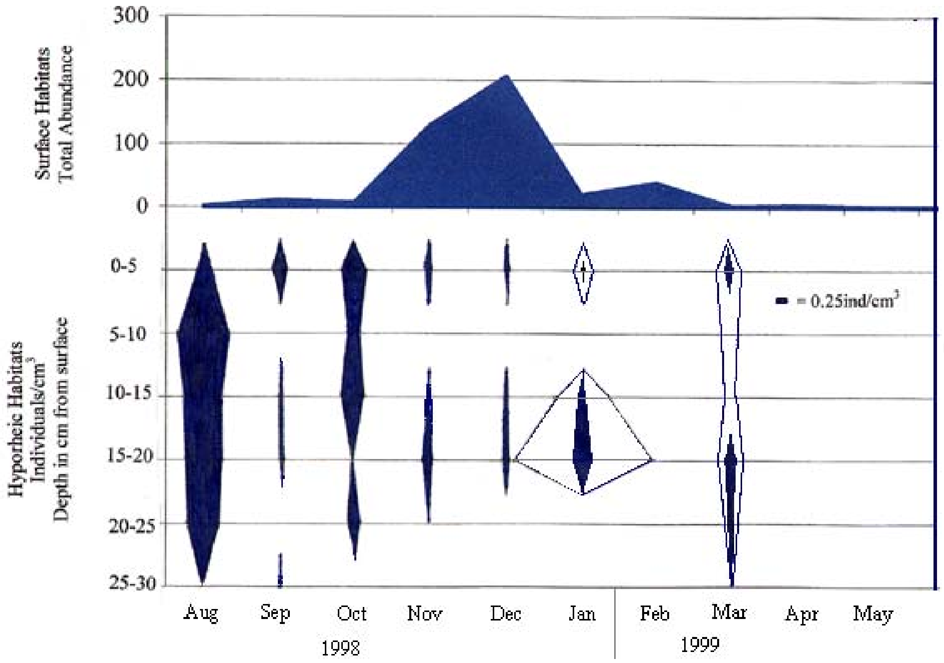

Figure 2. Summary of hyporheic densities by depth (lower portion) and total of abundance of larvae collected in surface habitats during the study. Dashed line represents densities inclusive of exuviae. Surface habitat total abundance represents all larvae collected in the five surface habitats as Individuals/ unit effort (described in methods).

All cores contained larvae except for a few in November and December, when most larvae were at the stream bed (Figure 2). Only four sample layers were collected below 25 cm due to silt layers or bedrock. No larvae were collected from those four sample layers.

High densities (greater than one individual cm^{-3} of pore space) of larvae were observed in August 1998 in five sample layers, October 1998 in one sample layer and January 1999 in one sample layer. In addition, high densities of larvae and exuviae were also observed in January 1999 in three sample layers. The lowest hyporheic larval densities were recorded in November and December when large numbers of larvae were collected in surface habitats. Only one larva was collected from core samples in July 1999, when there had been no surface flow for several weeks (not represented in Figure 2).

Hyporheic Collections- Dissolved Oxygen and Temperature

Temperature and dissolved oxygen measurements were collected in August 1998 in two riffles. Dissolved oxygen was less than 50 % saturated at 20-cm depth and was 0 % by 30-cm depth (Figure 3). Temperature did not change with depth in the two riffles where measurements were made. In three cases temperatures increased slightly with depth. At one measurement site the pipe penetrated a thick silt layer at 30 cm (end of hyporheic zone). Water temperature measured below the silt layer was 4°C cooler than the stream water above and dissolved oxygen was at 0 %.

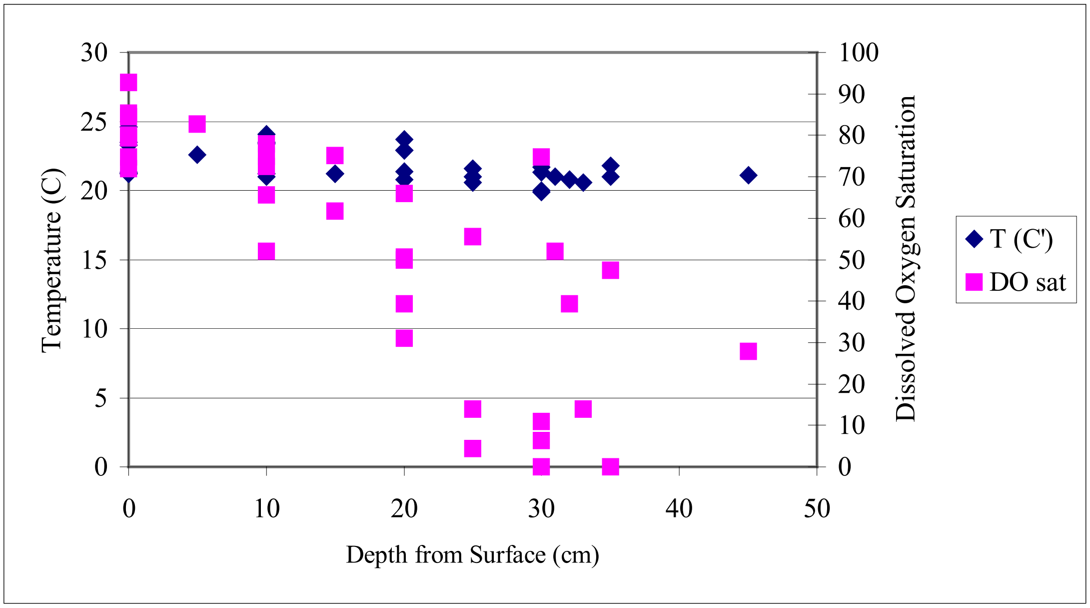

Figure 3. Temperature and dissolved oxygen saturation by depth near cores collected in August 1998.

Winter Creek was well shaded throughout the summer except for two large pools (where no samples were collected) and maximum summer temperatures were 23°C at the surface. Surface water oxygen was > 80 % saturated year round.

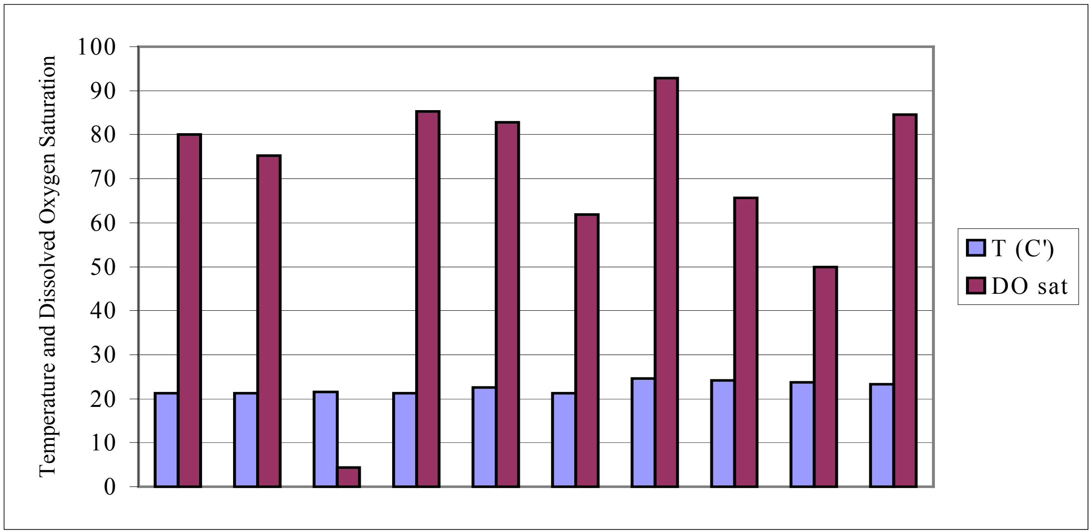

Figure 4. Temperature and dissolved oxygen saturation by depth near sample layers with larvae present in August 1998.

Three core samples were collected within one meter of the temperature and dissolved oxygen measurement sites in August 1998. Larvae were collected from these adjacent sample layers with dissolved oxygen as low 4.4 % saturation (Figure 4). Larvae found at this low dissolved oxygen level appeared to be in the same condition as larvae collected where dissolved oxygen levels were higher. Larval density at this low oxygen saturation was 1.77 individuals cm^{-3} pore space compared to a range of 0.022 to 3.58 individuals cm^{-3} at the higher dissolved oxygen levels.

Hyporheic Collections- Body Position Analysis

Three body positions extended, half-bent and bent (corresponding to Puglsey and Hynes 1986 descriptions for larvae in pre-diapause, transitional and diapause) were examined to determine the relationship between body position, season and depth. The degree to

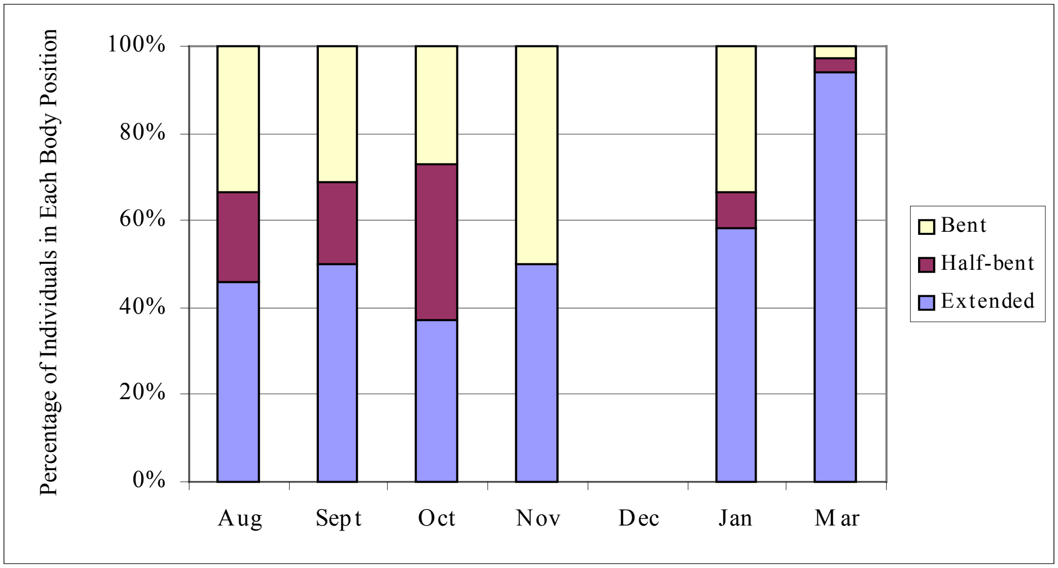

Figure 5. Percentage of larvae in each body position collected in core samples from August 1998 to March 1999. Numbers collected: Aug=243, Sept=16, Oct=81, Nov=2, Dec=0, Jan=12 and Mar=170.

which body positions were altered by processing is unknown, although few larvae appeared to be damaged beyond missing cerci and antennal segments.

Extended larvae were the highest percentage of samples in March. All larvae collected in surface habitats were extended and not characteristic of diapausing or transitional larvae (bent or half-bent). The percentage of half-bent larvae was highest in October. Percentage of bent larvae was slightly lower in October than August (Figure 5).

Percentage of extended larvae decreased with depth but was at least a third of the larvae collected at all depths. The percent of extended larvae was highest in the 0-5 cm sample layers but at least a third of larvae collected at all depths were extended (Figure 6).

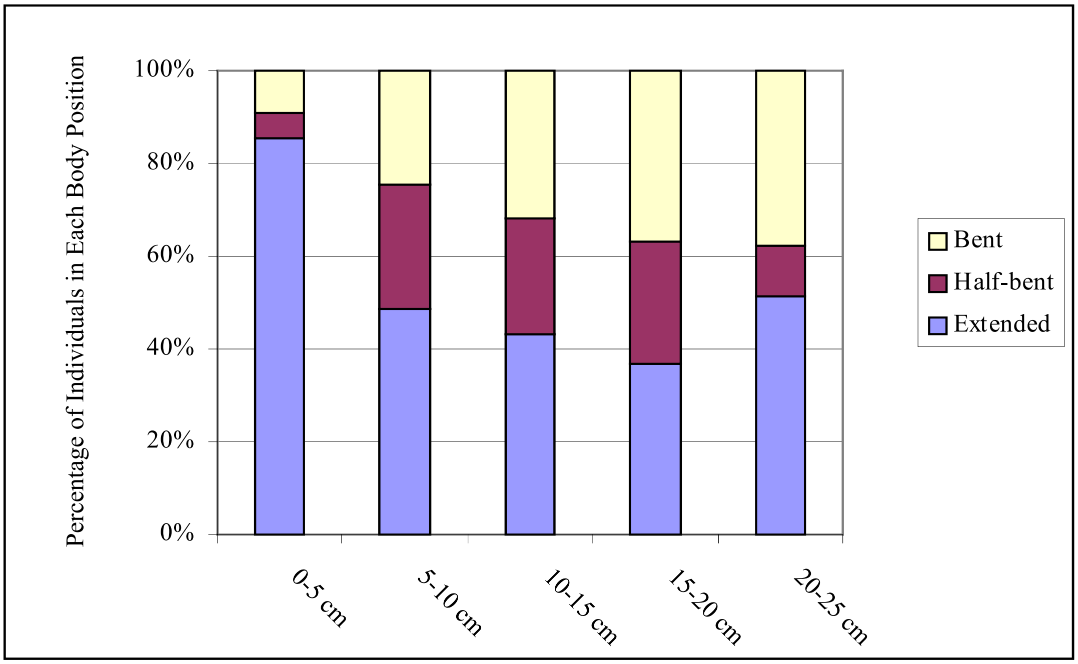

Figure 6. Percentage of larvae in each body position by depth. Numbers collected: 0-5 cm=205, 5-10 cm=101, 10-15 cm=123, 15-20 cm=54 and 20-25 cm=37.

Percentage of bent larvae increased with depth; most of the half-bent larvae were collected at the middle depths (5 –20 cm). There was no significant relationship between percent of larvae in any body position and depth. Large numbers of extended diapause (i.e., milky white) or pre-diapause larvae were collected in August 1998 and March 1999 respectively.

Hyporheic Collections- Particle Size Analysis Results

Average particle size decreased significantly with depth (*p*= 0.038) (Figure 7). Because particle size for the largest size range (>5 mm) was calculated using 5 mm (low end of range) as the multiplier, it is likely that average particle size estimates were low. Some of the silts and clays were lost during collection of sample layers, therefore the smallest particle size ranges were also underestimated.

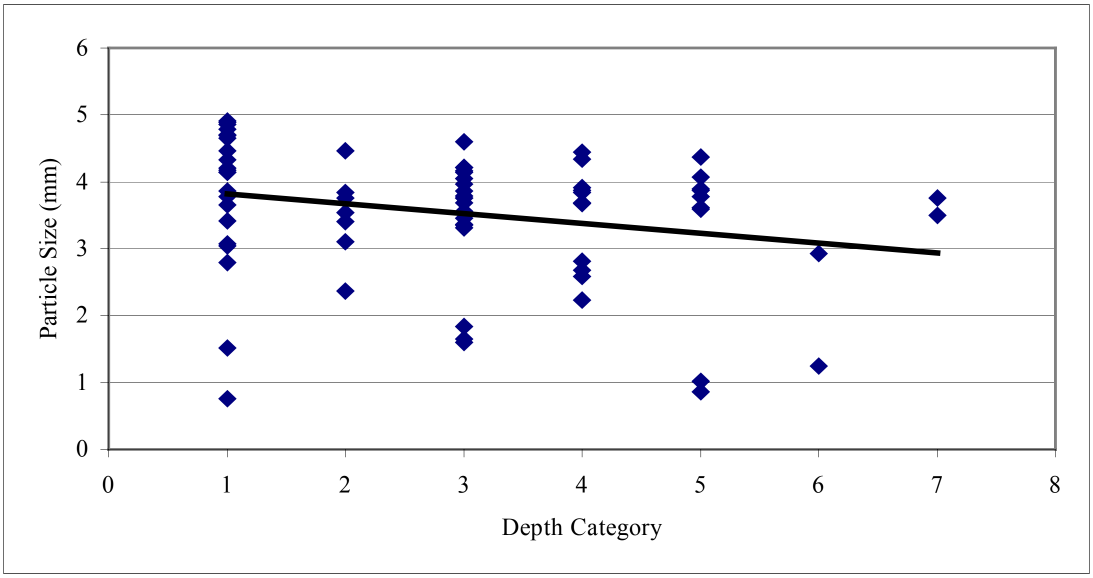

Figure 7. Particle size by depth. Particle size decreases significantly with depth (*p*= 0.038). Depth categories: 1=0-5 cm, 2= 5-10cm, 3= 10-15cm, 4= 15-20cm, 5= 20-25cm, 6=25-30cm and 7=30-35cm. R^2=0.059 df=72.

Hyporheic Collections- Pore Space Analysis Results

Percent pore space decreased significantly with depth (*p*=0.007) (Figure 8). Percent pore space decreases with depth from a mean of 21.96 % at 0-5 cm to 12.05 % at 15-20 cm. The highest measured pore space was 36 % and the lowest was 3 %.

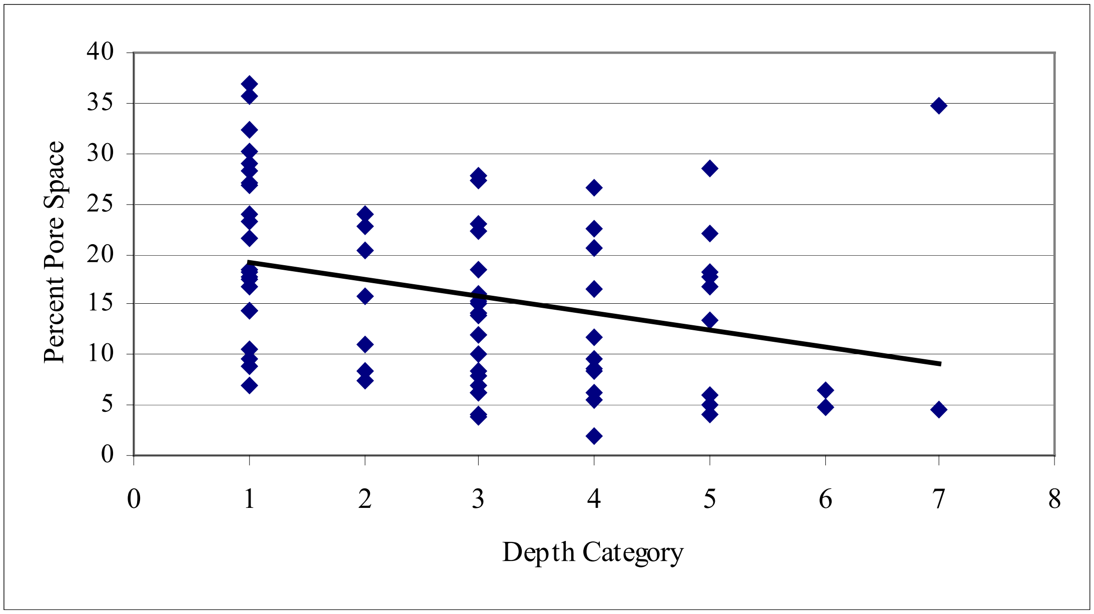

Figure 8. Pore space by depth. Pore space decreases significantly with depth (*p*= 0.007). Depth categories: 1=0-5 cm, 2= 5-10cm, 3= 10-15cm, 4= 15-20cm, 5= 20-25cm, 6=25-30cm and 7=30-35cm. R^2=0.097 df=72.

Hyporheic Collections- Particle Size, Pore Space and Larval Density

Highest densities of larvae were associated with average particle sizes between 3 and 4 mm (Figure 9). Highest densities of larvae were also associated with pore space between 20 and 25 % (Figure 10). Sample layers with no larvae had a smaller average particle size and pore space (3.07 mm and 14 % pore space) than sample layers with larvae (3.79 mm and 16.3 % pore space), though the differences were not significant.

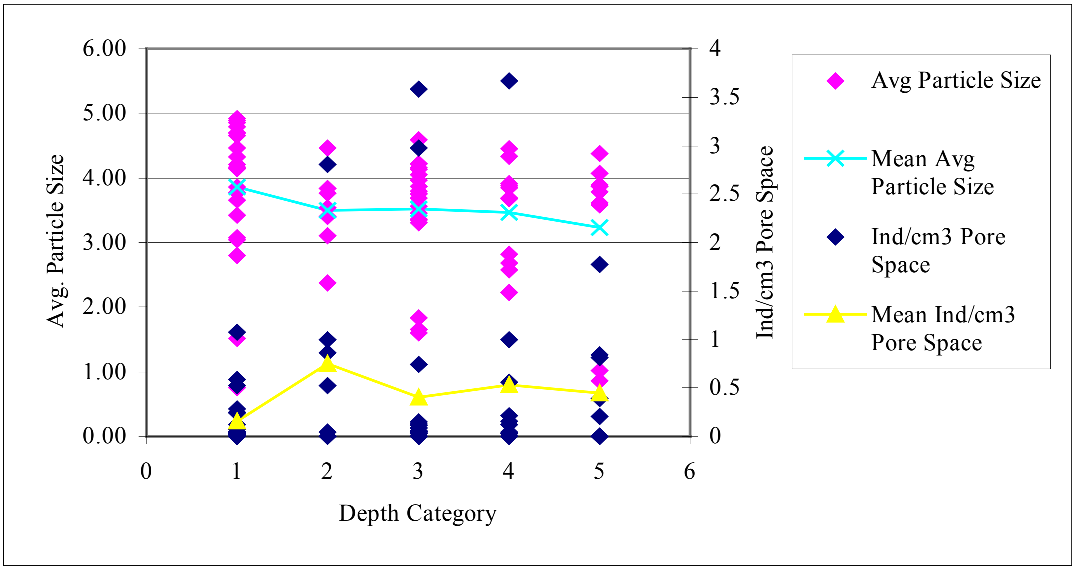

Figure 9. Particle size and larval density. Depth categories: 1=0-5 cm, 2= 5-10cm, 3= 10-15cm, 4= 15-20cm, and 5= 20-25cm,

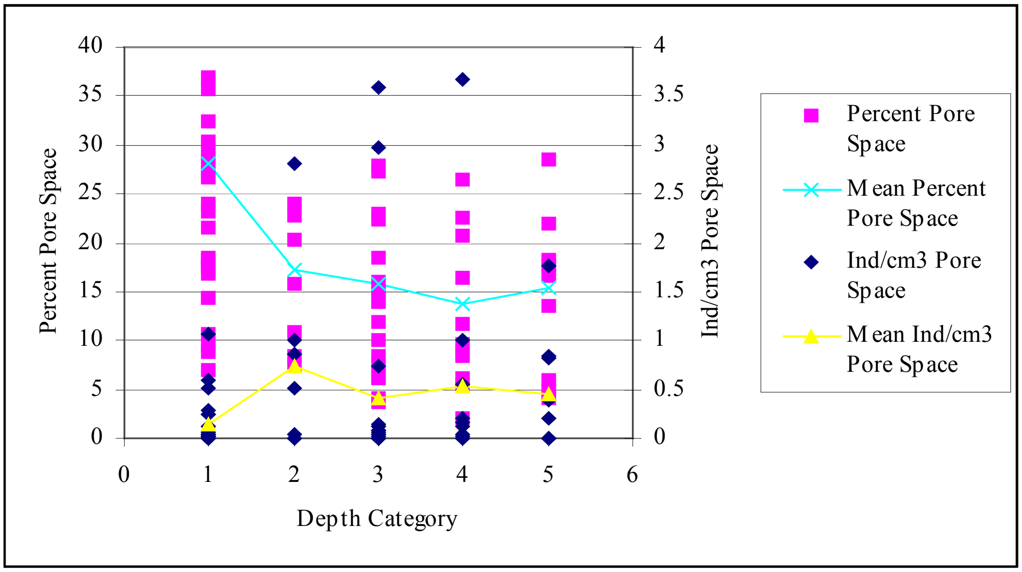

Figure 10. Mean pore space and larval density. Depth categories: 1=0-5 cm, 2= 5-10cm, 3= 10-15cm, 4= 15-20cm, and 5= 20-25cm.

Surface Habitat Collections

A total of 395 *Allocapnia* larvae were collected and measured from surface habitat samples (Table 1). Most larvae were collected in the riffle habitats in November and December, especially in November just prior to the start of emergence (Figure 2). No larvae were collected in surface habitats in September or October (compare to large numbers in hyporheic, Figure 2), and very few larvae were collected in surface habitats in April or May. No larvae were observed in surface habitats in the summer months and no collections were attempted after May, because flow ceased in June for approximately eight weeks. Very few larvae were collected from the pool habitats.

Numbers of larvae collected in surface habitats by the standardized sampling effort dropped from 129 in November and December to 76 in late December to only 19 in late

January (Table 1). This drop was coincident with large numbers of adults observed on emergent rocks and leaves and on the debris dams above the water line in January 1999.

Mean total body lengths (TBL) were largest in January (mean TBL=4.77 mm). The smallest were collected in February (mean TBL=0.95 mm) (Figure 11). Mean TBL of larvae collected in surface habitats in late November/early December was 4.18 mm, then 4.52 mm in late December and 4.77 mm by late January. The largest larvae were collected in December (TBL 6.5 mm) just prior to emergence.

Table 1. Summary of Larvae Collected in Surface Habitats from November 1998 to May 1999.

Habitat	Debris Dam			Pool Leaf Material			Riffle Leaf Material			Pool Mineral Material			Riffle Mineral Material		
Date	N	%	Mean TBL	N	%	Mean TBL	N	%	Mean TBL	N	%	Mean TBL	N	%	Mean TBL
11/5/98	3	2.3	2.47	41	31.9	2.38	62	48.1	2.13	0	0	0.0	23	17.8	1.55
11/30/98 -12/1/98	77	59.7	4.43	1	0.8	5.7	45	34.9	3.77	0	0	0.0	6	4.7	3.92
12/29/98	58	76.3	4.46	2	2.6	3.95	16	21.1	4.82	0	0	0.0	0	0.0	0.0
1/29/99	10	52.6	4.77	0	0.0	0.0	9	47.4	5.04	0	0	0.0	0	0.0	0.0
2/27/99	16	42.1	1.15	0	0.0	0.0	6	23.7	0.84	0	0	0.0	13	34.2	0.78
3/13/99	0	0.0	0.0	0	0.0	0.0	0	0	0.0	0	0	0.0	3	100.0	1.12
5/14/99	0	0.0	0.0	0	0.0	0.0	1	100.0	0.0	0	0	0.0	0	0.0	0.0
Totals	164			44			138			0			45		

There was a steady increase in mean TBL from November to January followed by a sudden decrease from January to February (Figure 10). Larvae were not found in surface habitats from April to October. During this period, the hyporheic larvae did not grow.

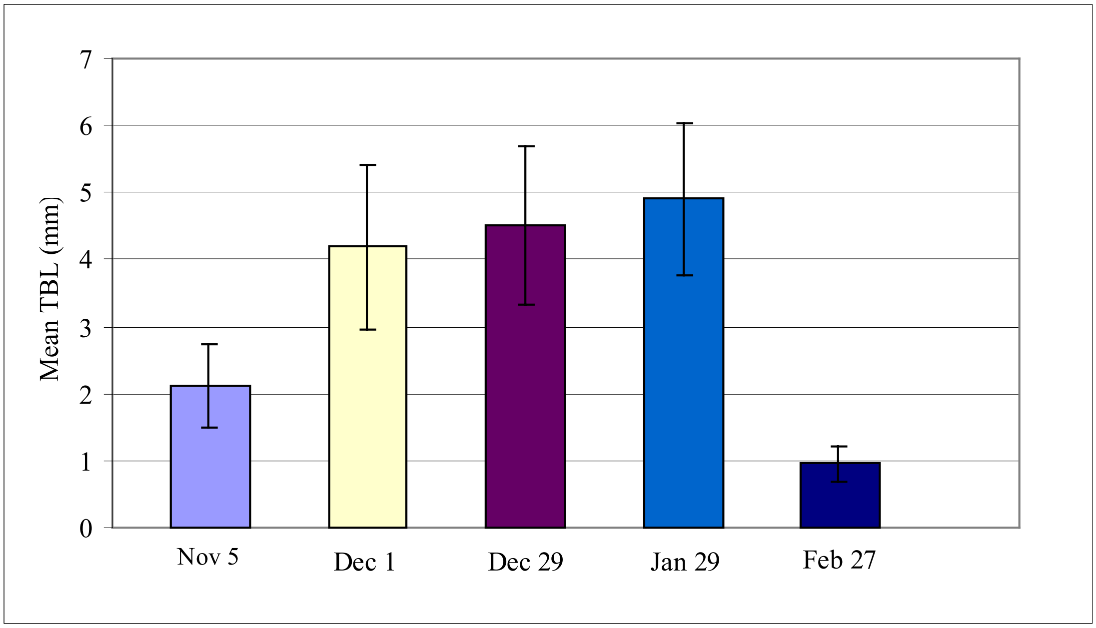

Figure 11. Mean TBL (total body length) of Larvae collected in surface habitats from November 1998 to February 1999 (+/- 1 SD).

Mean TBL for larvae collected in surface habitats in February (mean TBL=0.95 mm, last sample date with a large number larvae) and of those collected in October hyporheic sample layers (mean TBL=1.08 mm) was not significantly different.

Larval sizes progressed through the active growth period in surface habitats until most of them emerged, then disappearance of large larvae combined with appearance of tiny, new recruits caused average larval sizes to drop quickly from January to February. There were also indications that larvae moved from mineral to leaf or debris dam habitats after rising from hyporheic refuges in the riffle mineral habitat, since numbers in the habitats shifted from mineral to organic habitats from October to January, and average sizes were slightly larger, month by month, in leaf and debris dam habitats.

Figure 12 summarizes the relative use of each of the surface habitats, excluding the pool mineral habitat where no larvae were collected. Except in November, very few larvae were found in pool leaf material habitats. A high percentage of larvae were found in debris dam habitats from late November through February. Many larvae were also collected in the riffle leaf material habitats from early November into February.

In early November there was a significant difference in mean TBL ($p<0.01$) between larvae collected from riffle mineral habitats (mean TBL= 1.55 mm) and the pool leaf material (mean TBL= 2.38 mm) and riffle leaf material (mean TBL= 2.13 mm) habitats. There was also a significant difference in mean TBL between the riffle and pool leaf material habitats ($p=0.04$) in early November. Only three larvae were collected from debris dam habitats (mean TBL 2.47 mm). Refer to Table 2 for analysis of variance summary.

In late November only one larva was collected from pool leaf material habitats (mean TBL 5.7 mm), no comparisons were made between this habitat and the others. There was a significant difference ($p<0.01$) in mean TBL between the riffle leaf material (mean TBL= 3.77 mm) and the debris dam (mean TBL= 4.43 mm) habitats in late November/early December. Refer to Table 3 for analysis of variance summary.

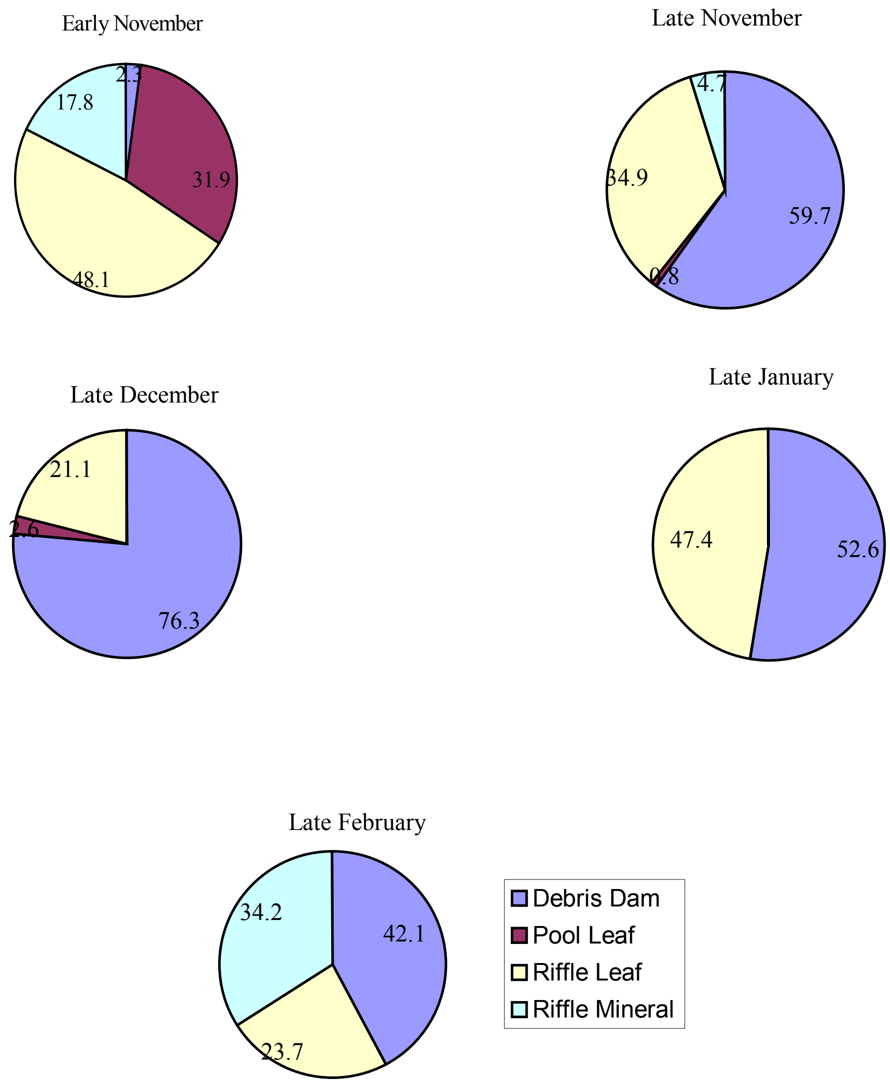

Figure 12. Relative habitat use by larvae. Numbers on charts are percent of all larvae collected in that habitat for each month. Numbers for each month are total abundance are presented in Table 1.

Too few larvae were collected from surface habitats in January (N=19) to make meaningful comparisons in habitat use and adults were numerous at that time. All but one larva were larger than 3.5 mm in January. All larvae collected in February were less than 1.6 mm with a mean TBL of 0.95 mm. All these larvae were characteristic of pre-diapause larvae (extended and clear).

Table 2. Summary of ANOVA for total body length analysis for larvae collected in surface habitats in early November.

Groups	Count	Sum	Average	Variance	total df	F	P-value
riffle min	23	35.6	1.547826	0.0642	84	23.11836	6.71E-06
riffle leaf	62	132.27	2.133387	0.315406			
riffle min	23	35.6	1.547826	0.0642	63	36.51817	9.41E-08
pool leaf	41	97.5	2.378049	0.395756			
riffle min	23	35.6	1.547826	0.0642	25	16.94152	0.000393
debris dam	3	7.4	2.466667	0.880833			
riffle leaf	62	132.27	2.133387	0.315406	102	4.254568	0.041711
pool leaf	41	97.5	2.378049	0.395756			
riffle leaf	62	132.27	2.133387	0.315406	64	0.953472	0.332572
debris dam	3	7.4	2.466667	0.880833			
pool leaf	41	97.5	2.378049	0.395756	43	0.052412	0.820029
debris dam	3	7.4	2.466667	0.880833			

Table 3. Summary of ANOVA for total body length analysis for larvae collected in surface habitats in late November.

Groups	Count	Sum	Average	Variance	total df	F	P-value
riffle min	6	23.5	3.916667	2.021667	50	0.078677	0.78028
riffle leaf	45	169.6	3.768889	1.406737			
riffle min	6	23.5	3.916667	2.021667	82	0.999009	0.320527
debris dam	77	341	4.428571	1.42312			
riffle leaf	45	169.6	3.768889	1.406737	121	8.721854	0.003784
debris dam	77	341	4.428571	1.42312			

Adult Emergence Patterns

Although emergence patterns were not rigorously analyzed, *A. wrayi* adults were first collected on December 16, 1998 and *A. rickeri* on December 25, 1998. Adults were observed in large numbers in January on rocks emerging from riffle areas and in very large numbers on debris dams. The last *A. wrayi* adult was observed and collected on

January 29, 1999 and the last *A. rickeri* adult was collected on February 23, 1999. A single adult *A. rickeri* was collected on March 12, 1997 from Winter Creek on a previous trip.

Seasonal Changes in Surface Habitats

The maximum summer stream temperature observed in Winter Creek was 23° C, partly because the stream channel is densely shaded during the summer months, and the winter minimum stream temperature observed was 5° C. The stream was not observed to freeze during the winter months, even though air temperatures were well below freezing for several days in December 1998 and January 1999. Surface water flow was sustained throughout the two previous summers, but in 1999 surface flow ceased in June and did not return until late September. Larvae and adult *Allocapnia* were observed in Winter Creek in the fall and winter of 1999-2000, so the populations were not eradicated by the flow cessation.

In late October and November, the stream channel was entirely filled with leaves. After rains in December 1998 and January 1999, the leaf material was washed out of the stream, sank to pool bottoms or formed temporary accumulations on debris dams and emergent rocks. A combination of physical and biological processes depleted the leaf accumulations almost completely by April. During the winter months large amounts of small organic particles settled in pools and accumulated temporarily on emergent rocks and debris dams. The locations and extent of mineral surface habitats remained stable over the course of this study; only a few locations had shifting sands.

Debris dams were numerous in the stream channel. Debris dams were so large that they converted the channel to a series of terraced pools. Only the larger woody material from the debris dams persisted after the leaf material was gone. Several layers of silt and leaves from past years covered the pool bottoms. Few pool bottoms had exposed rock or gravel areas. Riffle areas were a mix of large rocks (greater than 10 cm) to small gravel (2 mm), intermixed with sand and other smaller particles. Particle sizes were diverse and very few riffles were embedded with sand. Most of the riffle areas had some rocks that emerged from the water in the stream channel.

Discussion

Five different phases in the one-year life cycle of *Allocapnia* in Winter Creek were recognized, between which shifts in habitat occur. The phases are pre-diapause, diapause, immediate post-diapause, active growth and adult emergence. The first three correspond to phases defined by Pugsley and Hynes (1985) and the last two phases cover the time larvae attain surface habitats until emergence. Pugsley and Hynes (1985) also identified transitional phases occurring just before and after diapause. These transitions are discussed with the diapause phase. Pre-diapause, diapause, active growth and emergence overlap from December to March and diapause and active growth overlap again in the fall. Both sampling methods utilized in this study will be combined in the following discussion. Difficulties in identifying larvae in each phase, the hyporheic zone habitat, seasonal changes in Winter Creek habitats and the importance of small stream habitats to *Allocapnia* populations will also be discussed.

Pre-diapause Phase

The first phase, pre-diapause occurred from January to March when newly hatched or pre-diapause larvae colonized the partially processed leaf material that was closely associated with riffle mineral substrates. Here the larvae grew from just under one millimeter to just over one millimeter (Figure 10). The pre-diapause phase lasted from January into March for the two populations of *Allocapnia* in Winter Creek and overlapped with the diapause, active growth and emergence phases from the previous year. Pugsley and Hynes (1985) reported pre-diapause larvae from May into June in

Ontario. The highest numbers of pre-diapause larvae in Winter Creek were collected near the surface, which agreed with Pugsley and Hynes' (1986) findings.

Two pre-diapause larvae were collected in the first five centimeters of the hyporheic zone in late January, along with numerous exuviae. All larvae collected in surface habitats in February were characteristic of pre-diapause larvae and slightly smaller than pre-diapause larvae collected in the first five centimeters of the hyporheic zone in mid-March, indicating that some growth had occurred. Deeper in the hyporheic zone in March more larvae were in the diapause or transitional positions while the upper hyporheic zone was predominately populated by extended, pre-diapause larvae. After March no larvae were observed in surface habitats.

Diapause Phase

The second phase, diapause, occurred from March into October and possibly into January, when larvae burrowed into the substrate and transformed from the translucent extended pre-diapause position to the milky white, half-bent or bent, diapause positions. Larvae did not grow during the diapause phase of the summer months. Pugsley and Hynes (1985 and 1986) also showed no growth during this phase. However, the diapause phase in their study was only three months compared to six months or more for Winter Creek *Allocapnia*. A set of core sample layers collected in July 1999 (approximately mid-diapause) yielded no larvae at any depth. Winter Creek had been dry for several weeks before this and it is possible that larvae had moved out of the sampling area or that cores were collected in riffles where no larvae had moved into the hyporheic zone. Cores with no larvae were also found in November and December, but at that time many larvae were active in surface habitats. No larvae were active in surface habitats in July, as flow had ceased. Pugsley and Hynes (1986) also noted lower numbers in August and indicated that larvae might have moved out of the sampling area in the Speed River as well, though surface flow did not stop at that study site. In August 1998 surface flow had been maintained all summer in Winter Creek and large numbers of larvae were collected in the hyporheic zone at all depths and in all body positions. These larvae were all characteristic of milky-white diapausing larvae.

Low numbers of diapausing larvae were collected from November to January. In January numerous exuviae were also collected with diapausing larvae. As noted above pre-diapause larvae also started to appear in surface habitats at this time. It is likely that the pre-diapause larvae were newly hatched from adults that had emerged in December and the few diapausing larvae deeper in the hyporheic zone had not yet broken diapause. The many exuviae collected in January hyporheic sample layers were most likely from larvae that had broken diapause and had moved into surface habitats to enter the active growth phase in November. Although the exuviae collected in January hyporheic sample layers were not measured they appeared to be similar in size to the diapausing larvae collected from the same sample layers (1 to 1.3 mm TBL). The TBL was in the range of the smallest larvae collected in the riffle mineral surface habitats in early November and in hyporheic sample layers in October. Pugsley and Hynes (1986) indicated that some larvae may have remained in diapause year round. As no adults were observed in Winter Creek before December it seems unlikely that pre-diapause larvae from these would have already gone into diapause by January. None of the actively growing larvae observed in surface habitats in February were large enough (all pre-diapause) to be larvae that had broken diapause in January and no adults were observed after late February. There was no way to determine if the diapausing larvae found in January ever broke diapause, and the larvae may have remained in diapause another season as suggested by Pugsley and Hynes (1986).

Immediate Post-Diapause Phase

The third phase, immediate post-diapause, occurred in October when larvae broke diapause in the hyporheic zone and moved toward surface habitats. Larvae appeared to start feeding on interstitial, detrital material that remained from previous years in the hyporheic zone. Amounts of organic material were not measured but organic material was observed mixed with the mineral material in hyporheic sample layers. Finni and Chandler (1977) also observed that food was plentiful in the interstices of riffle areas and that larvae could utilize this food supply. No larvae were observed in surface habitats in October but numerous larvae were observed in November surface habitat samples. Mean TBL for hyporheic larvae was 1.08 mm in October and mean TBL for surface habitat larvae was over 2 mm in early November. Immediate-post diapause larvae associated with the riffle mineral habitat had a mean TBL of 1.55 mm, much closer to the 1.08 mm observed for the October hyporheic larvae, suggesting that larvae were colonizing leaf material habitats directly from the underlying riffle mineral and hyporheic habitats. Reice (1980) and Finni and Chandler (1977) also observed *Allocapnia* more closely associated with leaf packs over riffle habitats. Hyporheic feeding may have provided enough food for larvae to grow and allow them to wait for the larger amounts of food material associated with autumn leaf fall. Also the association of smaller larvae with the riffle mineral habitat may have provided refuge from predators in the surface habitats. By late November, leaf material habitats were extensive in Winter Creek.

Active Growth Phase

The fourth phase, active growth occurred from mid-October into January when larvae moved from the hyporheic zone and the riffle mineral surface habitats to the leaf material habitats over riffles and on debris dams. By late November a lower percentage of larvae were in the riffle mineral habitats and more larvae were collected from the growing leaf accumulations over riffle areas and on debris dams.

Pugsley and Hynes (1985) indicated rapid growth of larvae after diapause break in September, then leveling off in January until emergence started in March for *A. pygmaea*. In Winter Creek, larvae started the rapid growth phase after diapause break in October, and this phase ended in January with immediate emergence. This compressed active growth phase for Winter Creek *Allocapnia* would have allowed for larvae to go into diapause earlier before the onset of severe summer conditions.

Of the five different habitat types sampled, the pool mineral was the least preferred as no larvae were ever collected in this habitat. A thick layer of leaves and other organic material from past seasons usually covered this habitat and dissolved oxygen was lower than in the other habitats. Not only was this habitat not ideal in terms of oxygen levels but also there were few emergent rocks and sticks for *Allocapnia* to use as emergence and mating habitat. Adults were not observed at the stream bank but on emergent rocks, woody material and leaves in the stream channel.

Pool leaf material was also not a preferred habitat. Few larvae were collected in this habitat, except for 41 larvae collected in early November. At this time Winter Creek was completely covered in leaf material. In many reaches, flow was not discernible due to blockage by leaves. The 41 larvae collected in one sample in early November 1998 had most likely colonized leaf material over upstream riffle mineral habitats and drifted with this material into the pool habitat. Because larvae were not found in hyporheic core samples in pool bottom substrate, it is unlikely that larvae colonized the material from the pool mineral substrate. The lack of larvae collected in pool mineral habitat samples also supported this observation.

The largest numbers and highest percentages of larvae were found on debris dams from late November to late December. As larvae grew, they appeared to become more closely associated with the leaf material habitats. In late November, leaf material had started to accumulate on emergent rocks and debris dams. While movement of leaf material may have moved some associated larvae out of the stream reach or leave them stranded in pools a large proportion of larvae became aggregated in the forming leaf packs. These leaf accumulations also made good emergence habitats and many adult stoneflies were observed on this material above water level.

Emergence Phase

The last phase, emergence, started in mid-December and lasted into late February and early March for *Allocapnia* in Winter Creek. This emergence pattern was similar to that found for *A. granulata* in an Indiana stream (Finni and Chandler 1977), *Allocapnia* in a North Carolina mountain stream (Huryn and Wallace 1987), *A. rickeri* in an Ozark mountian stream (Ernst and Stewart (1985b) and for *A. recta* in a non-freezing stream in Ontario (Harper and Hynes 1972). This timing was different from that observed for *A. granulata* (Harper and Hynes 1972), *A. pygmaea* and *A. rickeri* (Harper et al. 1991; Pugsley and Hynes 1985; Harper and Hynes 1972) in Ontario which started in mid-March and lasted into April. As observed by Ernst and Stewart (1985b), there appears to be a latitudinal difference in emergence timing. Compared to the Canadian studies, Winter Creek *Allocapnia* have a more rapid growth phase after diapause break, no delay between the rapid growth phase and emergence and a much longer diapause (six months or more versus three months). The Winter Creek *Allocapnia* life cycle was more like that reported for *A. rickeri* (Ernst and Stewart 1985a) and *A. granulata* (Finni and Chandler 1977) in more southern streams.

Based on observations made during field trips, adults emerged from December to January from leaves, bark and rocks that extended above the water line. Mating then occurred on these same substrates. Mating couples were observed on material extending above the water line. Adults did not appear to migrate from the stream channel as observed by Harper and Hynes (1972).

Harper and Hynes (1972) indicated that many early emerging (December to February) adults would freeze before mating in Canada. Winter Creek did not freeze and adults that emerged early were less likely to die of freezing before mating in North Carolina. Also adults in Winter Creek would be able to lay eggs immediately and not have to wait for the ice to break.

Harper and Hynes (1972) reported that adults would seek refuge from cold conditions in decaying wood material above the snow. On many occasions in Winter Creek when temperatures were well below freezing adults were observed on the undersides of leaves that were just above the water surface. On these same mornings leaves on the banks were covered with ice crystals. Taking refuge from the cold over the unfrozen water of Winter Creek may have served the same purpose as that observed by Harper and Hynes (1972) for the Canadian *Allocapnia*. This behavior by Winter Creek *Allocapnia* would have allowed adults to stay in the stream channel and reduce the risk of being taken by the current while attempting to move toward the shore. This may also explain why no adults were observed outside the stream channel in Winter Creek as observed by Harper and Hynes (1972). *A. granulata* that were observed to emerge in December in a non-freezing Ontario stream (Harper and Hynes 1972) may have employed a strategy similar to that of the Winter Creek *Allocapnia*.

Difficulties in Identifying Larval Phases

As discussed above there is overlap of the pre-diapause and diapause phases in the life cycle of *Allocapni*a in Winter Creek. Analyzing body positions or posture as extended, half-bent and bent (pre-diapause, transitional and diapause after Pugsley and Hynes 1985) may not provide enough information to properly identify these phases. Difficulty arose in distinguishing extended larvae in different growth phases. Pre-diapause larvae could be readily identified because they were smaller and somewhat translucent and larvae in full diapause could be identified because they were milky-white (Hynes 1974) and clearly had their heads firmly tucked under the thorax (bent). Transitional larvae (half-bent) also were milky-white in appearance. Extended larvae, however, ranged from milky-white to translucent to brown. Brown larvae were assumed to be in the active growth phase, as they were not found below the first layer in the hyporheic zone and were active in surface habitats. The translucent and milky-white extended larvae were found at all depths and all months in the hyporheic zone. A continuum between the translucent and milky-white body appearances made it difficult to assign individual larvae to the diapause, transitional or active growth phases.

Pugsley and Hynes (1986) indicated that larvae went into a transitional phase (half-bent here) when going into and coming out of diapause. Half-bent larvae were about 33 percent of larvae collected in core samples in October and only a small percentage of larvae collected in March in Winter Creek (Figure 5). March and October appeared to be the times when larvae were going into and coming out of diapause, respectively, in Winter Creek. Pugsley and Hynes (1986) did not indicate the numbers of transitional larvae collected but since their largest number of pre-diapause larvae were found in May and June it is assumed that the transitional larvae would be found just after this period. Diapause larvae (bent) were also about one-third of the larvae collected in August, September, and October in Winter Creek. Pugsley and Hynes (1986) found the highest number of diapause larvae in August with a rapid drop in numbers coincident with the appearance of large numbers of post-diapause (immediate post-diapause and active growth) larvae from September onward. Pugsley and Hynes (1986) also found large numbers of post-diapause larvae (extended) at all depths. Extended larvae were a large proportion of larvae at all depths in Winter Creek as well (Figure 6).

Hyporheic Zone Habitat

To gain more insight into the habitat used by diapausing larvae, the hyporheic zone was sampled separately by collecting larvae from sample layers in cores. Even though riffles were targeted to maximize collection of diapausing larvae, the larvae were patchily distributed within the hyporheic zone and between riffles. Twenty-seven percent of core samples contained no larvae. This patchy distribution is perhaps best explained by adult egg laying behavior and the nature of the eggs. Although eggs were not specifically studied here it is likely that eggs are laid in sticky clumps as observed for *A. pygmaea* (Harper 1973a). Eggs are deposited in midstream especially in riffle areas (Harper and Hynes 1972). Very high densities of pre-diapause larvae collected in March in one sample layer could be explained if Winter Creek *Allocapnia* also deposit eggs in sticky clumps in riffles as observed for *A. pygmaea*.

Harper and Hynes (1972) also found very high mortality of early instar larvae in a laboratory setting, which may explain the lower densities found in the late summer sample layers. It appears that the critical factor is survival in surface habitats long enough to reach a sufficient size to burrow to diapause depth. If stream substrate characteristics were not suitable for diapause, then many *Allocapnia* larvae in Winter Creek either would be lost to desiccation when surface flow stopped or be washed down and out of the stream during spates.

Temperature did not appear to be a limiting characteristic to diapause in the hyporheic zone of Winter Creek, but increasing stream temperature in the spring may have been a cue to start diapause (Khoo 1968). Measurements made in July and August, show no decrease in temperature with depth. In Winter Creek the hyporheic zone does not appear to be a suitable refuge from high summer stream temperatures for these two *Allocapnia* populations. Burrowing deeper into the substrate did not provide a lower temperature exposure than staying near the surface. Burrowing into the hyporheic zone to diapause did, however, protect larvae from desiccation in Winter Creek. As noted earlier, Winter Creek is intermittent and surface flow ceased in July 1999 for approximately eight weeks. In many of the riffle areas the substrate was completely dry at the surface. Larvae were observed after surface flow was restored in fall 1999, indicating survival of at least some larvae over the dry period. Adult *Allocapnia* were also observed later in the winter. Pugsley and Hynes (1985) observed that diapause may have evolved as a mechanism to avoid high summer stream temperatures, reduce predation risk, or avoid desiccation. Of these, avoidance of desiccation appears to be the most important in allowing *Allocapnia* to maintain populations in Winter Creek.

Dissolved oxygen falls off rapidly with depth. Williams and Hynes (1974) found that dissolved oxygen saturation dropped 20 to 60 % in the first 10 cm of the Speed River and was only 5 % saturated by 30 cm. In July and August, dissolved oxygen was 0 % at the surface in some locations, and dropped rapidly to 0 % with depth at other locations in Winter Creek. During winter months, dissolved oxygen dropped to 0 % within the first 20 cm. Diapausing larvae were found in the hyporheic zones where dissolved oxygen was near zero (Figure 2). Pugsley and Hynes (1985) indicated that diapausing larvae may be resistant to severe conditions. During diapause, larvae may have been able to withstand low dissolved oxygen conditions in the hyporheic zone of Winter Creek for an extended period, presumably in order to avoid the more severe surface conditions (no water).

Another characteristic of the hyporheic zone is the amount of available pore space and pore size. Hyporheic substrate consisting of only small particles would have a small amount of pore volume available and the average pore size would be too small to allow larvae to burrow. Pugsley and Hynes (1985) would not have been able to evaluate the affect of particle size and pore space on diapause habitat because the particle sizes in their experimental chambers were uniform. This uniform size may have increased the densities of larvae they detected in the hyporheic zone because the larvae could move easily downward through the disturbed material to the lowest chambers. However, the Speed River bottom substrate is probably quite different in nature from that of Winter Creek. The collecting method used in this study made it difficult to rigorously analyze the particular relationship between depth and density because average particle size, dissolved oxygen saturation and pore space were not related in any consistent way to depth. However, the data collected here are likely more representative of the actual distribution of diapausing larvae in the hyporheic zone of Winter Creek than would have been found using colonization chambers. Fraser and Williams (1997) also found that standpipe samplers (model for Winter Creek corer) represented hyporheic densities better than other samplers including colonization chambers.

Measurements made in this study represent the total amount of pore space available but do not measure the average pore size in any sample layer. In order to make this measurement more sophisticated collection equipment would be needed. However, by looking at the pore space and the distribution of particle sizes in each sample layer, some insights can be gained into the nature of the hyporheic diapause habitat.

Percent pore space decreased with depth in Winter Creek. This was in agreement with Stocker and Williams (1972) findings for the Speed River. Pore space in the Speed River ranged from 21 to 35 %. Pore space in Winter Creek ranged from three to 36 %. Average particle size also decreased with depth, which caused a decrease in pore space with depth. The 0-5 cm depth range had an average pore space of nearly 22 % which dropped to 14.63 then 13.6 then 12.05 then 13.35 % by 20-25 cm. The upper five centimeters of hyporheic zone (continuous with the stream bed) was exposed to surface flow and more likely to have smaller clogging particles washed away or fall through to deeper sample layers. The upper five centimeters were also more likely to become completely dry during drought as observed in July 1999. This may also be the reason for higher densities of larvae below five centimeters. It was difficult to evaluate the effects of pore space, particle size and dissolved oxygen levels on hyporheic larval densities in Winter Creek, as these parameters are likely correlated. Whatever the relationships, it appears that the Winter Creek hyporheic zone is a suitable habitat for small *Allocapnia* larvae and that changes in one or all of these parameters may well render this stream unsuitable to these winter larvae.

Importance of Small Stream Habitats

It is apparent that Winter Creek has the necessary habitats and water quality conditions to support *Allocapnia* populations. *Allocapnia* larvae were even able to withstand severe hyporheic conditions and cessation of surface flow in Winter Creek. Increased disturbances such as increased runoff, deforestation, siltation, and oxygen depletion in the Winter Creek watershed could easily eliminate the *Allocapnia* populations thriving here.

Allocapnia require leaf material as a food source and as habitat for growth as larvae and for emergence habitats as adults. These winter stoneflies also require a suitable hyporheic habitat in order to avoid dry conditions at the streambed in summer months in Winter Creek. Degradation of the surface or hyporheic habitats may decrease or eliminate the potential for *Allocapnia* to maintain populations in Winter Creek.

Debris dam habitats would be impacted by deforestation of the watershed, removing a source of coarse woody material to maintain existing debris dams and to form new ones. Debris dams also hold organic material in Winter Creek that is important for larvae as habitat and a food source and for adults as emergence habitat.

Riffle leaf material habitats are important to Winter Creek *Allocapnia* as an initial surface habitat after diapause break that is closely associated with refuges in the underlying riffle interstices. Leaf material scattered widely over riffle habitats eventually collects on debris dams carrying associated larvae with it. Adults may also use the undersides of leaves over riffle areas as emergence habitat and as refuge from freezing.

Riffle mineral and hyporheic habitats are continuous in Winter Creek and serve as important diapause habitat. Small pre-diapause larvae and immediate post-diapause larvae also use this habitat for feeding and refuge from possible predators that are active at the same time and as a possible refuge from being displaced by the current. Interstitial spaces in this habitat also retain small organic particles that young larvae may use as a food source. These habitats must have enough pore space to allow larvae to burrow deep enough to avoid desiccation when surface flow stops in summer months. Increases in sediment, often associated with watershed disturbances, could reduce the ability of larvae to move into and out of the near surface stream bed and prevent larvae from burrowing deep into the hyporheic.

While *Allocapnia* did not favor the pool habitats in Winter Creek, these habitats are important in retaining organic material in the stream which helps maintain leafs and smaller particles in the debris dams and over riffle areas. Watershed disturbances that filled in pools would remove this important reservoir of leaf material.

Increases in impervious surfaces would make Winter Creek flashier and have longer intermittent periods. Increased storm flows would destroy all Winter Creek habitats by flushing out debris dams and other leaf material as well scouring riffle areas. Increased intermittence would increase the amount of time that larvae would need to remain in diapause, thus shortening an already compressed active growth phase.

Winter Creek habitats do not appear to be limiting to *Allocapnia,* as these two populations appear to be thriving. Because *Allocapnia* can maintain populations in this intermittent stream, Winter Creek could be used as a reference to evaluate the habitat conditions in other intermittent streams. More study is needed to determine what habitat and water quality thresholds would limit the ability of *Allocapnia* to maintain populations in intermittent streams. As many intermittent streams are being altered by development, there is also a need to locate other watersheds with *Allocapnia* populations and target these areas for preservation.

Summary of Major Findings

This was the first ecological study of *Allocapnia* at the southeastern edge of the distribution. A method was developed to collect larvae from the hyporheic zone while minimizing stream disturbance. Larvae were observed in the hyporheic zone where dissolved oxygen was near 0 % saturation. Larvae grew rapidly once leaving the hyporheic zone in late October until emergence in December and January. Larger larvae favored organic habitats and smaller larvae were more closely associated with mineral habitats.

Larvae were found in Winter Creek after a prolonged period with no surface flow indicating that the hyporheic zone serves as a suitable refuge from desiccation. This small intermittent stream is an important source pool of *Allocapnia* in the Walnut Creek watershed.

References:

Bruton, J. G. 2003. Accounting of headwater drainages in the upper Neuse River basin of North Carolina. North Carolina Department of Environment and Natural Resources, Division of Water Resouces.

Ernst, M. R. and K. W. Stewart. 1985a. Growth and dirft of nine stonelfy species (Plecoptera) in an Oklahoma Ozark foothills stream, and conformation to regression models. Ann. Entomol. Soc. Am. 78: 635-646.

Ernst, M. R. and K. W. Stewart. 1985b. Emergence patterns and assessment of collecting methods for adult stoneflies (Plecoptera) in an Ozark foothills stream. Can. J. Zool. 63: 2962-2968.

Finni, G. R. 1973. Biology of winter stoneflies in a central Indiana stream (Plecoptera). Ann. Entomol. Soc. Am. 66: 1243-1248.

Finni, G. R. and L. Chandler. 1977. Postdiapause instar discrimination and life history of the capniid stonefly, *Allocapnia granulata* (Claasen) (Insecta: Plecoptera). Am. Midl. Nat. 98: 243-250.

Finni, G. R. and L. Chandler. 1979. The microdistribution of *Allocapnia* naiads (Plecoptera; Capniidae). J. Kans. Ent. Soc. 52:93-102.

Fraser, B. G. and D. D. Williams. 1997. Accuracy and precision in sampling hyporheic fauna. Can. J. Fish. Aquat. Sci. 54: 1135-1141.

Harper, P. P. 1973a. Emergence, reproduction and growth of setipalpian Plecoptera in southern Ontario. Oikos 24: 94-107

Harper, P. P. 1979. Observations on early instars of stoneflies (Plecoptera). Gewass. Abwaess. 64: 18-28.

Harper, P. P. and H. B. N. Hynes. 1970. Diapause in the nymphs of Canadian witner stoneflies. Ecology 51: 925-927.

Harper, P. P. and H. B. N. Hynes. 1972. Life histories of Capniidae and Taeniopterygidae in southern Ontario (Plecoptera). Arch. Hydrobiol. Suppl. 40: 274-314.

Harper, P. P., M. Lauzon, and F. Harper. 1991. Life cycles of 12 species of winter stoneflies from Quebec (Plecoptera; Capniidae and Taeniopterygidae). Can. J. Zool. 69: 787-796.

Huryn, A. D. and J. B. Wallace. 1987. The exopterygote insect community of a mountain stream in North Carolina, USA: Life histories, production, and functional structure. Aquat. Insects 9:229-251.

Hynes, H. B. N. 1974. Further studies on the distribution of animals within the substratum. Limnol. Oceanogr. 19:92-99

Khoo, S. G. 1968a. Experimental studies on diapause in stoneflies I. Nymphs of *Capnia bifrons* (Newman). Proc. R. Entomol. Soc. Lond. Ser. A Gen. Entomol. 43: 40-48.

Kondratieff, B. C. and R. F. Kirchner. 1982. Notes on the winter stonefly genus *Allocapnia* (Plecoptera; Capniidae). Proc. Entomol. Soc. Wash. 84(2): 240-244.

North Carolina Department of Environment and Natural Resources, Division of Water Quality, Planning Branch. (DENR-DWQ) 2002. Assessment Report: Biological impairment in the upper Swift Creek watershed.

Pugsley, C. W. and H. B. N. Hynes 1985. Summer diapause and nymphal development In *Allocapnia pygmaea* (Burmeister) (Plecoptera; Capniidae) in the Speed River, Southern Ontario. Aquat. Insects. 7: 53-63.

Pugsley, C. W. and H. B. N. Hynes 1986. Three-dimensional distribution of winter stonefly nymphs, *Allocapnia pygmaea,* within the substrate of a southern Ontario river. Can. J. Zool. 43: 657-662.

Reice, S. R. 1980. The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. Ecology. 61(3): 580-590.

Reice, S. R. 1981. Interspecific associations in a woodland stream. Can. J. Fish. Aquat. Sci. 38: 1271-1280.

Ross, H. H. and W. E. Ricker 1971. The classification, evolution, and dispersal of the winter stonefly genus *Allocapnia*. Univ. Ill. Biol. Monogr. 45: 1-166.

Stewart, K. W. and B. P. Stark 1988. Nymphs of North American stonefly genera (Plecoptera). Thomas Say Found. Ser., Ent. Soc. Am. 12:1-460.

Stocker, Z. S. J. and D. D. Williams 1972. A freezing core method for describing the vertical distribution of sediments in a stream bed. Limnology and Oceanography 17: 136-138

Williams, D. D. 1984. The hyporheic zone as a habitat for aquatic insects and associated arthropods, pp. 430-455. In V. H. Resh and D. M. Rosenberg (eds.). The ecology of aquatic insects. Praeger, New York, NY. 625p.

Williams, D. D. and H. B. N. Hynes 1974. The occurrence of benthos deep in the substratum of a stream. Freshwater Biology 4: 233-256

White, T. R., P. H. Carlson and R. C. Fox. 1979. Emergence patterns of fall and winter stonelflies (Plecoptera: Filipalpia) in South Carolina. Proc. Entomol. Soc. Wash. 81: 379-390.