THE LIFE HISTORY AND CONTRIBUTIONS TO THE ECOLOGY OF *Camelobaetidius variabilis* WIERSEMA 1998 (EPHEMEROPTERA: BAETIDAE) IN HONEY CREEK, OKLAHOMA

Heather A. Perry, B.S.

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APPROVED:

James H. Kennedy, Major Professor
William T. Waller, Committee Member
Kenneth L. Dickson, Committee Member
Art Goven, Chair of the Department of Biological Sciences
Sandra L. Terrell, Dean of the Robert B. Toulouse School of Graduate Studies

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A study of the life history and ecology of *Camelobaetidius variabilis* was conducted in Honey Creek, OK from February 2003-April 2004. Nymph development was assessed using changes in external morphology. Laboratory reared nymphs were used to calculate number of degree days to complete development (772 degree days at 20.8° C ±.38° C), which was used to determine voltinism. Field collected nymph microhabitat distribution was used in assessing microhabitat distribution. Nymphal thermoregulation was assessed during the winter and spring by comparing nymphal numbers present in shaded and un-shaded habitats. *Camelobaetidius variabilis* nymphs showed preference for algal microhabitats during the spring and leaf packs in the winter. Nymphs inhabited leaf packs to increase metabolic rate during the winter. Increased temperatures aid in development of nymphs. *Camelobaetidius variabilis* exhibited a multivoltine life cycle with six overlapping generations.

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TABLE OF CONTENTS

LIST OF TABELS

LIST OF FIGURES

TRADEMARKS

Name	Reg/Non-Reg	Owner
Exo Terra Sun Glo Basking Bulb	®	Rolf C. Hagen Inc. http://www.hagen.com/
Onset Stowaway Datalogger	®	Onset Computer Corporation 470 MacArthur Blvd. Bourne, MA 02532 USA
Orion Model 250a	™	Thermo Electron Co. 500 Cummings Center Beverly, MA 01915
YSI Model 50b	™	YSI Incorporated 1700/1725 Brannum Lane Yellow Springs, Ohio 45387 USA

CHAPTER 1

INTRODUCTION

Life History

Butler (1984) states, "life-history information is of fundamental importance for virtually all ecological studies of freshwater invertebrates." Even though information on life histories is available, there is still a considerable amount of information lacking on the structure and function of aquatic communities and ecosystems. One way to elevate the level of efficiency in this area of research would be to look at common life history variables, and then find out why that particular variable exists for a particular organism (Butler 1984).

The term life history has been defined as:

- "Events that govern the reproduction (and survival) of a species or a population" including fecundity, development, longevity, and behavior (Oliver 1979).
- Life history was to include method of birth, pattern and rate of growth, feeding, locomotory and social behavior, length of life, selected habitat, response to environmental factors, mode of reproduction, and mode of death (Waters 1979).
- Life histories contain the qualitative and quantitative details of the more variable events that are associated with the life cycle (egg, larvae, nymph, and

adult). The events in a life history can vary among individuals or populations of one species (Butler 1984).

Four fundamental processes in life history information are enumeration, measurement, categorization, and observation. These four processes can be associated with biotic variables: enumeration – density, measurement – size, categorization – life cycle stages, and observation – behavior. Values for these processes collected over a period of time can provide valuable information on various parameters in a life history study such as reproductive recruitment, mortality, development, growth, reproduction, phenology, and voltinism (Butler 1984).

Changes in larval morphology were used to gauge development of *C. variabilis*. Morphological characteristics are independent of chronological age of the specimen and reflect the influence variables (i.e. temperature, food availability, flow etc.) have upon the developmental rate of aquatic insect. Studies by Bretschko (1965) and Cianciara (1979) used the method of determining developmental stages to assess the life history of ephemeropterans. Both studies used similar morphological characteristics to describe developmental stages (Hutchinson 1993). Developmental stages are an important tool in ephemeropteran life histories since it is the time of synchronized emergence that is of interest (Kosnicki and Surian 2003).

Life history information for many genera in the family Baetidae is limited or nonexistent. Members of this family are a dominant portion of the aquatic biomass in systems where they are found (Wagner 1995). *Camelobaetidius variabilis* is unique among baetids, and until Wagner (1995) there had been no attempts at describing its life history. Life histories provide baseline data that could be used in impact assessment

(Lehmkuhl 1979) to determine indicators of pollution tolerances within an ecosystem (Lenat 1993) and as an aid to resolve taxonomic questions (Oliver 1979). Life history information contributes to the understanding of ecology, conservation biology and applied sciences.

Study Organism

Ephemeroptera is one of the first winged insect orders to appear in the fossil record, appearing in the Upper Carboniferous period 280-320 million years ago (Carpenter 1992). The term Ephemeroptera can be broken down into *ephemero*, for a day; and *ptera*, wings. This classification is due to the short adult life span, generally lasting one or a few days. This order is characterized by small to medium fragile, soft bodies as both adults and nymphs. Unique to this order is the subimago stage, which is a winged, sexually immature form that lacks functional genitalia (Romoser and Stoffolano 1998). Molting of this stage to the imago produces a sexually mature adult. No other insect order molts once in a winged stage. Mayfly nymphs are aquatic, and have well-developed mouthparts that are responsible for the intake of nutrients. The adults have vestigial mouthparts. Mayflies are an important component in the transfer of energy in aquatic ecosystems (Romoser and Stoffolano 1998). Greatest diversity of mayflies is found in rocky-bottomed, low order streams (Edmunds and Waltz 1996). Ephemeropterans are considered to be potential indicators of pollution. Despite their importance in stream ecosystems, life histories are known for less than 8% of the North American fauna (Wallace and Anderson 1996). The limited life history information for

ephemeropterans hinders progress in explaining community dynamics and in monitoring and assessing impacts on aquatic ecosystems (Brittain 1982).

The order Ephemeroptera contains 21 families. The family Baetidae contains 23 genera (McCafferty *et al*. 2002). Baetids have a large geographical distribution, and are found on every continent except New Zealand (Edmunds *et al.* 1976). Baetidae is one of the most ubiquitous mayfly families. They are easily distinguished from other families by antennal length, which is two or three times longer than the width of their head. All mayflies have one tarsal claw, the exception being the Baetidae genus *Camelobaetidius* spp. This genus has evolved claws that are short and thick with "teeth" that the nymph uses to adhere to the substrate surface without being dislodged (Berner and Pescador 1988). This spatulate claw is also used to scrape algae for feeding.

There are nine species of *Camelobaetidius* in North America, which are generally western in distribution (Figure 1). *Camelobaetidius* spp. are found in streams with cobble to boulder type substrates and are frequently associated with waterfalls (Traver and Edmunds 1968). Traver and Edmunds described *Camelobaetidius* in 1968 (McCafferty *et al*. 2002). Wiersema (1998) described and separated it from other species based on tarsal claw denticulation and gills on the pro- and metathoracic segments (Wiersema 1998). *Camelobaetidius variabilis* is reported from three states: Texas, Oklahoma, and Nevada (Figure 1). Other species of *Camelobaetidius* are also reported in these states (Randolph 2002).

Effects of flow and temperature

Temperature exerts a major influence on aquatic insect life cycles. It directly affects many aspects of a life history such as metabolism, growth, emergence and reproduction (Anderson and Cummins 1979; Rossillon 1988). Temperature, together with flow (Statzner and Higler 1985; Statzner *et al.* 1988), influences distribution, diversity and abundance patterns over elevation gradients in both lotic and lentic waters (Ward 1992). Temperature is an important factor needed to understand collected field data.

Developmental Stage Criteria

Some factors useful in determining developmental stages for ephemeropterans have been established by other life history studies. Sweeney and Vannote (1981) used the appearance of wing buds and male genital primordia as a developmental indicator for *Ephemerella* sp. McCafferty and Bae (1994) used wing pad development, fore wing pad length (mm), body length (mm), and color to distinguish developmental stages for *Anthopotamus verticis* (Say 1839) (Ephemeroptera: Potomanthaidae). Another factor to take into consideration is the absence (during the first instar) or presence of abdominal gills. Fink *et al*. (1991) used these characteristics in their study of *Dolania americana* Edmunds and Traver 1959 (Ephemeroptera: Behningiidae).

In this study development stages will be based on several characteristics that are either absent or present such as caudal filaments, gills and wing pads. When these features are present further assessments such as length, color, and markings will be used to further distinguish between developmental stages.

Life history information for *Camelobatidius* is virtually unknown, and the only known attempt at a life history found was by Wagner (1995). *Camelobatidius* is a unique baetid mayfly due to the morphology of its tarsal claws, which enables it to inhabit a very specific habitat of thin sheet flow, and bedrock. This sheet flow, bedrock habitat limits the geographic distribution of *Camelobatidius* and poses a difficult task in collecting aquatic nymphs. The specific goals of this research were to describe the life history, determine voltinism, thermoregulation, and microhabitat distribution. Hypothesis for the study are as follows:

H_o: There is no difference in thermoregulation behavior of *Camelobaetidius variabilis*.

H_o: There is no preference for microhabitat distribution.

Study Area

Honey Creek (Murray Co.) (Figure 2) is located in the Arbuckle Mountain range in the southern Oklahoma Aulacogen formation (Reisen 1975). It is a limestone stream (25 km) that flows parallel to the fault trace separating the Cool Creek and McKenzie Hill formations of the Arbuckle Mountains of southern Oklahoma (Reisen 1976; Ham 1969). Streams flowing across limestone beds in the Arbuckle Mountains typically precipitate calcium carbonate (Ham 1969). The calcium carbonate precipitates in Honey Creek give it a spongy-textured travertine bed. Photosynthesizing blue-green algae assist in the precipitation of the calcium carbonate by elevating the pH. Over time, the travertine bed created Turner Falls, which at one time stood at 150 feet, but large rainfalls

during the Pleistocene era caused the stream to cut into the bed and reduced the waterfall to its current height of 75 feet (Ham 1969).

Honey Creek flows 25 km northeast into the Washita River (Reisen 1976). The upper 12 km are intermittent, while the lower 13 km, fed by two springs that drain the Arbuckle limestone aquifer, are permanent (Reisen 1975). Historically, the land upstream of Turner Falls was pastureland (Reisen 1976). Today the land use within the Honey Creek watershed is 55% herbaceous rangeland and 40% deciduous forest (Taylor and Kennedy *in press*).

CHAPTER 2

METHODS AND MATERIALS

Collection methods

Collections of *Camelobaetidius variabilis* were obtained from five stream reaches in Turner Falls Park by stratified random sampling. For a reach to be considered for sampling it was required to have 100+ m of suitable habitat. Generally, each reach contained suitable riffle with pools in between areas of prime habitat. Pools were omitted from sampling because preliminary sampling showed that *C. variabilis* did not inhabit them. Riffles were sampled to obtain 100-200 organisms per trip. Samples were preserved in 80% ethanol in the field. Sampling began February 2003 and ended April 2004. Collecting occurred every four weeks during November – February, and every two weeks during March – October.

Samples were collected in a plankton net (77μm) attached with a 30.5 cm bungee cord to a three sided plexi-glass square (14cm X 14cm) (Figure 3). The plexi-glass was 5mm thick. Foam attached to the bottom of the sampler created a seal on the substrate. The opening of the sampler contained a lip that not only aided in the moving of organisms into the net, but provided the fourth side to the bottom of the sampler so the area sampled was a complete square. A five-centimeter wide natural bristle paintbrush was used to dislodge the substrate and any organisms on the substrate into the net. The net was removed from the sampler and washed over a 180μm mesh sieve (U.S. Standard #80). Nymphal distributions were determined by the use of this substrate sampler, which

sampled 196 cm^2. Microhabitat for each discrete sample was recorded in the field and analyzed seasonally as well as for the entire study to determine if there was a microhabitat preference.

Water temperature was recorded at half-hour intervals during the study using an Onset Stowaway Datalogger® (Bourne, MA). The datalogger was located approximately 200 meters below the travertine fall. General statistics were conducted with SAS 9.1 at $\alpha = 0.1$ (SAS Institute 2003). Chi-square critical values were used to define the statistical significance of calculated chi-square values. Cluster analysis was used in determining similarity of seasonal microhabitat nymphal distributions. PRIMER 6 (PRIMER-E 2005) software was used in cluster analysis.

Thermoregulation Sampling

Thermoregulation of *Camelobaetidius variablis* nymphs was evaluated during the winter and summer. Six samples were collected employing identical sampling techniques to the rest of the study. Physical parameters of micro water temperature (^oC), micro air temperature (^oC), micro velocity (cm/s), and depth (cm) were recorded. Three replicates were collected in areas exposed to the sun and in areas shaded from the sun.

Further investigation into internal leaf pack temperature was performed to determine if the internal temperature changed when exposed to direct sunlight and shade. Fallen leaves were collected from trees located at the water's edge and placed into mesh bags. Leaf packs were collected and placed in mesh bags. Three replicates were placed in the stream; one on both edge and in the middle of the stream. Leaf packs located at the edge of the stream were exposed to direct sun continuously or shade continuously, while

the leaf pack in the middle of the stream was exposed to both shade and direct sun. Dataloggers were inserted into the center of the leaf packs to avoid contact with water. Leaf packs were placed in the stream for a total of 8.5 hours.

Developmental Analysis

The criteria for the developmental life cycle stages of *Camelobaetidius variabilis* were based upon fore and hind wing development, caudal filament development, gill development, male turbinate eyes, and coloration and markings. Seven developmental stages were delineated, and will be referred to as DSI-DSVII (Table 1). These criteria were modified from Cianciara (1980), who used a similar method to determine developmental stages to interpret the life history of ephemeropterans.

Laboratory Rearing

DSI and DSII nymphs were collected in the field and transported back to the lab for rearing. Nymphs were placed in artificial streams constructed from 38-liter aquaria located in incubators. A platform was constructed within the artificial stream to place rocks and leaf litter. Nymphs were released in this area and then aerators were placed in the stream to create a circular water flow around the platform. Twenty degree Celsius was used to mimic summer temperature; temperature was recorded using an Onset Stowaway Datalogger®. Diurnal time was mimicked with the use of 100 watt Exo-Terra Sun Glo® basking bulb. Exo-Terra Sun Glo is a broad-spectrum light used to mimic daylight for terrariums. It creates a heat gradient for thermoregulation, increases ambient air temperature, and produces UVA light. Photoperiod was adjusted for 12 hours of light

and darkness. Streams were checked daily for emergence and adults were removed and recorded. Since nymphs collected in the field varied in development, the last date of emergence from artificial streams was used in estimating degree-days.

Growth and Development Rates

Voltinism of *C. variabilis* was determined from developmental classes of field-collected nymphs and peak emergence data. Laboratory rearing data and field temperature data were used in conjunction with these data to support field findings. Degree-days were calculated using the model $\{\Sigma(T_{max}-T_{min})/2\text{-Threshold } T_{min})\}$ established by Pedigo and Zeiss (1996). T_{max} is the maximum recorded daily temperature, T_{min} is the minimum temperature at which development does not occur, and Threshold T_{min} is the minimum temperature at which development does not occur.

Physio-chemical Parameters

Parameters collected each trip included micro flow, water depth, micro temperature and distance to nearest emergent vegetation/land. This information was used to describe the micro-distribution of the *Camelobaetidius variabilis* nymphs. Depth was measured using a metal metric ruler and recorded in centimeters. Micro flow was calculated using the formula for $U_1=\sqrt{(2g)(D_2-D_1)}$. U is velocity, g is the gravitational constant, D_2 is depth measured perpendicular to water flow, while D_1 is depth measured parallel to water flow. Water temperature was continuously monitored within the stream at half-hour intervals using an Onset Stowaway Datalogger®. Water quality parameters (pH, dissolved oxygen, conductivity, and alkalinity) were measured seasonally.

Dissolved oxygen and pH were measured using YSI model 50b^{TM} (Yellow Springs, OH) and Orion model 250a^{TM} (Beverly, MA) portable electronic meters.

CHAPTER 3

RESULTS AND DISCUSSION

Physio-Chemical parameters

Physio-chemical data for Honey Creek are listed in Table 2. Dissolved oxygen remained near saturation throughout the study and ranged from 10.26 mg/l in March to 11.61 mg/l in December, with a mean of 11.20 mg/l (n=5). pH ranged from 7.76 in April to 8.6 in December (n=4). Velocity ranged from 0.03-0.176 m/s during April - November 2003 (n=3), with the highest velocity occurring in July 2003 and the lowest occurring in August 2003. Annual water temperature ranged from 0.98 ^{o}C on 6 January 2004 to 31.54 ^{o}C on 23 March 2003 (mean 17.68$\pm$ SD 6.34) (Table 3, Figure 4).

Microhabitat Distribution

Camelobaetidius variabilis inhabited bedrock riffles in Honey Creek where shallow water flowed over the bedrock surface. Distributions of *C. variabilis* changed seasonally due to microhabitats present.

The null hypothesis that *C. variabilis* nymph numbers would not be different between microhabitats was rejected at a critical X^2 value of 9.236 (Kruskal-Wallis X^2 = 9.3428, $p = 0.0961$, $\alpha = 0.1$) (Table 4). However, statistical significance for the Kruskal-Wallis is weak, and a Tukey's Multiple Comparison Test on ranked data showed the only significant difference between microhabitat types were between algae and micro

depressions (ALGAE BFPC OPNBR TRICH LFPK DIVET, $\alpha = 0.1$). When represented graphically, there was no obvious microhabitat preference by nymphs throughout the study (Figure 5). Nymphs were collected from velocities ranging from 0-100 cm/s over the course of this study. As with microhabitat preference there does not appear to be a preference for velocity when represented graphically (Figure 6).

Correlations assess the degree of association between two or more continuous variables. The value generated is a correlation coefficient (r), and can be negative, positive or zero. In correlation analyses both variables vary together, so that as one goes up/down the other can go up, go down, or do nothing. With correlations there is no assumption of cause and effect between variables, even if one does exist, and a significant correlation does not mean causation. Weak correlation coefficients could be due to statistical randomness and potentially mean nothing (Beitinger 2001). Many of the following correlations are weak and could be of no ecological importance.

Correlation analyses were performed between environmental factors and both total nymphs and nymphs by season. The environmental parameters that had statistically significant correlations were velocity in centimeters per second (cm/s) and distance to shore in meters (m). Velocity was positively correlated with total nymphs for the entire study ($r = 0.11046$, $p = 0.0891$, $\alpha = 0.1$), and for summer ($r = 0.40179$, $p = 0.0009$, $\alpha = 0.1$) (Table 5). As velocity increased, so did the total number of nymphs present. Distance to shore was negatively correlated for total nymphs for the entire study ($r = -0.19454$, $p = 0.0026$, $\alpha = 0.1$), and for spring ($r = -0.27176$, $p = 0.0050$, $\alpha = 0.1$) (Table 5). This correlation can be directly related to the previous correlation of high velocities containing higher number of *Camelobaetidius variabilis* nymphs as near shore water

velocities are generally decreased. Therefore, samples collected closer to the shore would be expected to yield fewer nymphs than samples collected further from shore in higher velocities.

Additional correlations were performed to investigate the relationships between environmental factors and the nymphal developmental stages. Analyses were conducted for the entire study as well as seasonally (Table 6). No correlations were made for DSI due to small sample size. DSII was negatively correlated to depth ($r = -0.15302$, $p = 0.0182$, $\alpha=0.1$) and distance to emergent vegetation ($r = -0.11585$, $p=0.0744$, $\alpha=0.1$). As depth increased the number of DSII decreased. This could be due to increased velocity as depth increased, but since there was no correlation between DSII and velocity this assumption cannot be supported. DSII nymphs were found further away from nearest emergent vegetation/land, which would be expected since imminent emergence was not anticipated. DSIII was negatively correlated to depth ($r = -0.20686$, $p=0.0013$, $\alpha=0.1$) and velocity ($r = -0.19724$, $p=0.0022$, $\alpha=0.1$). DSIV was negatively correlated with depth ($r = -0.18573$, $p=0.0040$, $\alpha=0.1$) and velocity ($r = -0.20599$, $p=0.0014$, $\alpha=0.1$). As both depth and velocity increased, the number of DSIII and DSIV decreased, which could be due to the inability of these developmental stages to physically withstand the forces in deeper, faster moving water. No correlation coefficients were statistically significant for DSV and DSVI. DSVII was positively correlated to depth ($r =0.16221$, $p=0.0122$, $\alpha=0.1$) and velocity ($r =0.13166$, $p=0.0420$, $\alpha=0.1$). This developmental stage was found in higher numbers in both deeper and higher velocity waters.

Winter

Seventy-six percent of the winter (December-February) samples were dominated by leaf packs (Figure 7). Nymphs were also collected from open bedrock, micro depressions, and simulid pupal cases. Winter velocity ranged from 0-75cm/s with higher number of nymphs inhabiting velocities from 0-50 cm/s (Figure 8). Depth and velocity were negatively correlated ($\alpha = 0.1$) for DSII-DSVI during the winter. Distance to nearest emergent vegetation was negatively correlated ($\alpha = 0.1$) for DSIII-DSVI (Table 5). As depth and velocity increased, the number of DSII-DSVI decreased during the winter. Velocity was affected by the presence of leaf packs within the stream. DSIII-DSVI numbers decreased the closer samples were to emergent vegetation. These developmental stages would not be expected close to emergent vegetation, as they are not nearing the emergence stage of their lifecycle.

Winter nymphal numbers were not different between microhabitats (Kruskal-Wallis Multi-sample Test, $X^2 = 0.6913$, $p = 0.8753$, $\alpha = 0.1$, Table 4). The inability of this test to show statistical significance during the winter can be attributed to sampling methods. Sampling was tailored to locating *C. variabilis* microhabitat distributions, and therefore uneven sampling occurred during the winter because nymphs were found mainly in leaf packs and not evenly distributed throughout other habitats. However, when microhabitat data were analyzed by cluster analysis (Primer 6) (Figure 9) and compared to distributions during the other seasons, leaf packs showed a 90% relatedness to spring algae preference, which was determined by Kruskal-Wallis Multi-sample Test ($X^2 = 25.2681$, $p = <.0001$, $\alpha = 0.1$, Table 4) and Tukey's Multiple Comparison Test on ranked data ($\alpha = 0.1$) to be statistically significant.

Leaf packs in Honey Creek impede the flow of water and become calcified due to calcium carbonate precipitate. This damming process diverts the water around the leaf packs, allowing for suitable habitat. Leaves are generally dark brown to black in color, with little to no water passing through them, and could be utilized to maintain metabolic functions as well as other ecological needs. Graphs, raw numbers, and cluster analysis all support the hypothesis that *C. variabilis* nymphs have a microhabitat preference for leaf packs during the winter.

Spring

Spring distributions (March-May) were dominated by both algae and micro depressions. Spring velocities ranged between 25-100 cm/s (Figure 11). DSI-III preferred algae; DSIV was equally distributed between both habitats, while DSV-VII preferred micro depressions (Figure 10). The preference of earlier instars for algal habitat suggests that during the early spring, eggs deposited could become entangled in the filamentous algae and remains there until hatching occurred. Previous work by Wagner (1995) analyzed the gut content of *C. variablis* from Honey Creek and found that *C. variabilis* nymphs feed primarily on algae.

Spring nymphal numbers were different between microhabitats (Kruskal-Wallis Multi-sample Test, $X^2 = 25.2681$, $p = <.0001$, $\alpha = 0.1$, Table 4). Ranked spring data determined that algae was different from all other microhabitats (Tukey's Multiple Comparison Test on ranked data, $\alpha = 0.1$,

ALGAE BFPC OPNBR TRICH LFPK DIVET). Spring nymphs in algae had 90% relatedness to winter nymphs in leaf pack preference (Figure 9).

The spring correlation matrix (Table 6) revealed a negative correlation to depth for DSII-DSIV, and a positive correlation for depth for DSVII. Velocity was negatively correlated for DSIV, but positively correlated for DSVI-DSVII. Distance to shore was negatively correlated for DSVI, while distance to nearest emergent vegetation/land was negatively correlated for DSII-DSIV. Distance to shore and nearest emergent vegetation/land would be expected to have a negative correlation for DSII-DSIV and DSVI because none of these developmental stages are close to the sites for adult emergence. All spring correlation coefficients were weak and could be due to sample randomness and could be of no or little ecological importance.

Summer

Summer distributions (June-August) were dominated by micro depressions (50%), but open bedrock was the other habitat of choice (Figure 12). A Kruskal-Wallis Multi-sample Test failed to reject the null hypothesis ($X^2 = 3.6181$, $p = 0.6056$, $\alpha = 0.1$, Table 4).

DSI preferred velocities of 25-50 cm/s, while all other developmental stages preferred velocities of 25-100 cm/s during the summer (Figure 13). A correlation matrix for total *C. variablis* nymphs (Table 5, $\alpha = 0.1$) showed a moderately strong positive correlation during the summer for velocity (0.40179, $p=0.0009$, $\alpha=0.1$). Further analysis with a correlation matrix by developmental stage for summer (Table 6, $\alpha = 0.1$) determined velocity to be positively correlated with DSII, but negatively correlated to DSV-DSVI. Distance to shore was negatively correlated for DSIV. All summer correlation coefficients were weak, except for the association total nymphs with velocity. Other correlations could be due to randomness and be of little ecological importance to

nymphs. Summer *C. variabilis* nymphs generally inhabited swifter moving water while utilizing micro depressions as shelter.

Fall

Fall distributions (September-November) indicate that micro depressions, leaf packs, and open bedrock were preferred habitats for all developmental stages (Figure 14). Habitats present in the fall were equally exploited: micro depression (33%), leaf pack (31%), and open bedrock (33%). Based on a Kruskal-Wallis Multi-sample Test, the null hypothesis was accepted that there were no numerical differences in *C. variabilis* nymphs between microhabitat types ($X^2 = 4.5395$, $p = 0.21$, $\alpha = 0.1$, Table 4).

Fall velocity ranged from 0-100 cm/s (Figure 15). A correlation matrix for the fall by developmental stages (Table 6) had depth negatively correlated for DSIII-DSIV, but positively correlated for DSVII. Velocity was negatively correlated for DSII-DSIV, and positively correlated for DSVII. Distance to emergent vegetation was positively correlated for DSVII. Depth and velocity were negatively correlated for DSIII-DSIV, meaning that as depth and velocity increased, the number of nymphs decreased. DSII numbers also decreased as velocity increased. Correlation coefficients for the fall were moderate in strength and could indicate that these factors are of some ecological importance to fall nymphs.

Thermoregulation

During a preliminary observation/collecting trip in the winter of 2001, *C. variabilis* nymphs were observed moving from areas of shade to un-shaded areas. It was hypothesized that they were thermoregulating during the daytime to regulate body

temperature or maximize temperatures for metabolic functions. In the winter of 2003, the first part of a thermoregulation investigation was done. It was hypothesized that higher numbers of nymphs would be found in areas of direct sun than shaded areas. Nymphs were commonly encountered in leaf packs during the winter. Samples were taken in similar flow and depth. The ratio of nymphs in un-shaded leaf packs (529) compared to shaded leaf packs (176) was 3:1. Water moving under the leaf packs remained at a constant temperature of 9.4 ^{o}C for all but one sample, which was 9.3 ^{o}C. Air temperature over shaded and un-shaded leaf packs was noticeably different (3.5 ^{o}C) (Table 7). In January 2005, internal leaf pack temperatures were recorded using dataloggers to assess temperatures with increased exposure to the sun. Figure 16 shows the internal leaf pack temperature when exposed to shade and direct sunlight. Gray horizontal bars indicate the times when the leaf pack is shaded, while the break between the bars indicates that the leaf pack is being exposed to direct sunlight. Shaded leaf pack internal temperatures remained at or below 15 ^{o}C, but once exposed to direct sunlight the internal temperature increased to 20 ^{o}C. Leaf packs with higher internal temperatures yielded higher number of nymphs. Leaf packs located in the sun not only provide warmer temperatures for maintaining metabolic functions, but also provide other ecologically important resources.

The second part of the thermoregulation study was conducted in July 2004 to evaluate if the nymphs preferred shaded habitats to un-shaded habitats. Total nymph numbers did not change between sites, with 338 un-shaded and 337 shaded (Table 8). Summer thermoregulation was not observed in *C. variabilis* nymphs. Honey Creek is a spring fed stream, and maintains flowing water year round. Nymphs would not be

subjected to extremely high water temperatures and therefore do not thermoregulate to maintain metabolic functions during the summer.

Voltinism and Seasonal Development

Camelobaetidius variables in Honey Creek have a multivoltine life cycle. Voltinism of *C. variabilis* was derived from the developmental class frequencies of field-collected nymphs and combined with laboratory rearing and field temperature data. Early field collected developmental stages (n=11) were reared at an average temperature of 20.8 ^{o}C ±0.38 ^{o}C and emerged in 772 degree-days (approximately 35 days) in artificial streams. Nymphs were not successfully maintained or had no development in artificial streams at water temperatures 10 ^{o}C or lower. *Camelobaetidius variablilis* was determined to be multivoltine, producing at least 6 overlapping cohorts in Honey Creek.

CHAPTER 4

CONCLUSIONS

Primary objectives of this study were to describe the life history of *Camelobaetidius variabilis*, determine voltinism, thermoregulation, and microhabitat distribution. The thermoregulation study examined nymphal populations during both the winter and summer. Habitats present were compared for a preference for shade or un-shaded. Microhabitat distributions were examined for overall preference as well as for seasonal preferences.

Camelobaetidius variabilis, like many other ephemeropterans in temperate regions, has a multivoltine life history, producing as many as 6 generations in a year. This overlapping of cohorts allows for continuous emergence of adults throughout the year. Seasonal microhabitats showed preference for algae during the spring and leaf pack preference during the winter. The natural progression of stream habitat and allochthonous input allows *C. variabilis* to exploit both habitats to their benefit. The algae is used as a food source, while leaf packs are exploited by all instars that over-winter as a way to maximize metabolic function. Leaf packs may also be used to acquire other ecologically important resources (i.e. shelter, food, humidity, etc.). Thermoregulation was extremely evident during the winter season (3:1), when compared to the same experiment during the summer season (1:1). Nymphal numbers increased three fold in leaf packs exposed to direct sunlight than those located in shaded areas.

Table 1. *C.variabilis* nymphal developmental stages developed for Honey Creek, OK.

Developmental Stage	Description
DSI	2 caudal filaments, no gills, no wing pads
DSII	3 caudal filaments (medial filament may be vestigal, but does not exceed ½ the length of the lateral filaments); gills and fore wing pads present
DSIII	Fore wing pads touching metanotum
DSIV	Fore wing pads not exceeding 1st abdominal segment; hind wing pad present, but with no visible folds
DSV	Fore wing pads extending onto 2nd abdominal segment; hind wing pads extending onto 1st abdominal segment with visible folds; able to distinguish ♂ eyes
DSVI	Fore wing pads extending onto 2nd abdominal segment; hind wing pad extending ¼ -¾ length of abdominal segment 1; ♂eyes developing
DSVII	Fore and hind wing pads dark; hind wing pads ¾ length of abdominal segment 1; ♂ eyes developed and turbinate

Table 2. Seasonally measured water quality parameters for Honey Creek, OK, (-) indicates no data recorded and (*) indicates meter failure.

Parameter	21-XI-2003 Site E	16-II-2004 Site B	12-III-2004 Site A	25-III-2004 Site D	09-IV-2004 Site A
D.O. (mg/L)	11.6	12.1	10.26	10.92	11.16
Conductivity (μmhos)	380	368	428	418	470
pH (s.u.)	8.6	*	7.97	8.37	7.76
Alkalinity (mg/L)	230	-	-	-	-

Table 3. Annual and seasonal temperatures (mean ± SD) for Honey Creek, OK., minimum temperature of .98°C on 06 January 2004 and maximum temperature of 31.54°C on 23 March 2003.

Date	Min (°C)	Max (°C)	Mean ± SD	n
Annual	.98	31.54	17.76 ± 6.34	20,727
Winter	.98	26.39	9.66 ± 3.96	4,709
Spring	8.63	31.54	18.80 ± 3.74	7,227*
Summer	17.52	30.39	20.02 ± 2.71	4,419
Fall	6.16	25.86	17.06 ± 4.09	4,372

* Spring sample size is larger due to sampling in both 2003 and 2004 to obtain overlap in sampling.

Table 4. Kruskal-Wallis Test (Chi-Square) results for *C. variabilis* nymphal preference for microhabitat; null hypothesis: There will be no preference of microhabitat by *C. variabilis* nymphs ($\alpha = 0.1$).

Season	DF	Cal Chi-Square	Actual Chi-Square	p	H_o
All	5	9.3428	9.236	0.0961	Reject
Fall	3	4.5395	6.251	0.2088	Accept
Spring	4	25.2681	7.779	<.0001	Reject
Summer	5	3.6181	9.236	0.6056	Accept
Winter	3	0.6913	6.251	0.8753	Accept

Table 5. Correlation matrix for *C. variabilis* total nymphs present for entire study, as well as seasonally*. Statistically significant probabilities at α=0.1.

Season	Environmental Variable	Corr Coefficient	p
All	Velocity (cm/s)	0.11046	0.0891
	Distance to shore (m)	-0.19454	0.0026
Spring	Distance to shore (m)	-0.27176	0.0050
Summer	Velocity (cm/s)	0.40179	0.0009

* Fall and Winter seasons had no significant correlations.

Table 6. Correlation matrix of developmental stages for *C. variabilis* and environmental factors present during sampling. Correlation coefficients and corresponding probabilities that were statistically significant at $\alpha = 0.1$ are shown for entire study as well as by season.

Season	Environmental Var	DSI	DSII	DSIII	DSIV	DSV	DSVI	DSVII
All	Depth (cm)		-0.15302	-0.20686	-0.18573			0.16221
			0.0182	0.0013	0.004			0.0122
	Velocity (cm/s)			-0.19724	-0.20599			0.13166
				0.0022	0.0014			0.042
	Distance to Shore (m)							
	Dist to Emergnt Veg (m)		-0.11585					
			0.0744					
Winter	Depth (cm)		-0.39844	-0.52844	-0.52608	-0.41001	-0.46873	
			0.0323	0.0032	0.0034	0.0272	0.0103	
	Velocity (cm/s)		-0.48281	-0.62636	-0.52428	-0.49311	-0.47375	
			0.008	0.0003	0.0035	0.0066	0.0094	
	Distance to Shore (m)							
	Dist to Emergnt Veg (m)			-0.34721	-0.3914	-0.4002	-0.39995	
				0.065	0.0358	0.0315	0.0316	
Spring	Depth (cm)		-0.2534	-0.18826	-0.16946			0.20347
			0.0091	0.0544	0.084			0.0374
	Velocity (cm/s)				-0.18703		0.21986	0.24707
					0.0561		0.0242	0.0111
	Distance to Shore (m)						-0.17962	
							0.0667	
	Dist to Emergnt Veg (m)		-0.21511	-0.17368	-0.35237			
			0.0275	0.0764	0.0002			
Summer	Depth (cm)							
	Velocity (cm/s)		0.21184			-0.21447	-0.24018	
			0.0903			0.0862	0.054	
	Distance to Shore (m)				-0.24667			
					0.0476			
	Dist to Emergnt Veg (m)							
Fall	Depth (cm)			-0.36252	-0.33403			0.44801
				0.0233	0.0377			0.0042
	Velocity (cm/s)		-0.38445	-0.44299	-0.32452			0.34967
			0.0157	0.0047	0.0438			0.0291
	Distance to Shore (m)							
	Dist to Emergnt Veg (m)							0.34501
								0.0315

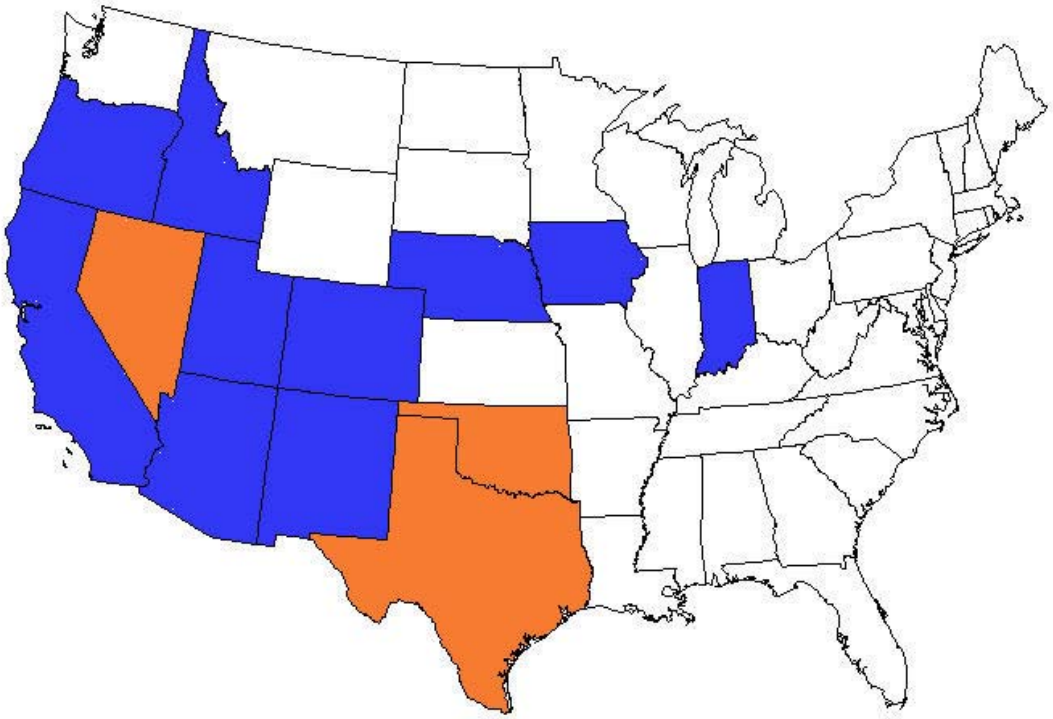
Table 7. Winter Thermoregulation data for *C. variabilis* from Honey Creek, OK.

Air Temp (^{0}C)	Depth(cm)	Flow (cm/s)	Water Temp (^{0}C)	# of nymphs	Cover
15.4	0.5	24.26	9.4	152	un-shaded
15.4	0.3	24.26	9.4	134	un-shaded
15.4	0.4	14.01	9.3	243	un-shaded
11.9	0.5	31.32	9.4	79	shaded
11.9	0.6	14.01	9.4	90	shaded
11.9	0.4	31.32	9.4	5	shaded

Table 8. Summer Thermoregulation data for *C. variabilis* from Honey Creek, OK.

Air Temp (^{0}C)	Depth(cm)	Flow (cm/s)	Water Temp (^{0}C)	# of nymphs	Cover
32.7	16	117.2	24.7	63	un-shaded
32.3	14	203	24.8	159	un-shaded
32.7	10	198.1	26.7	116	un-shaded
30.5	17	171.6	24.5	59	shaded
29.7	15	198.1	26.8	105	shaded
30.3	10	171.6	27.2	173	shaded

Figure 1. *Camelobaetidius* species distribution within the United States complied from Randolph 2002.



Camelobaetidius spp. distribution

Camelobaetidius variabilis distribution

Figure 2. Honey Creek watershed, Murray Co., OK.

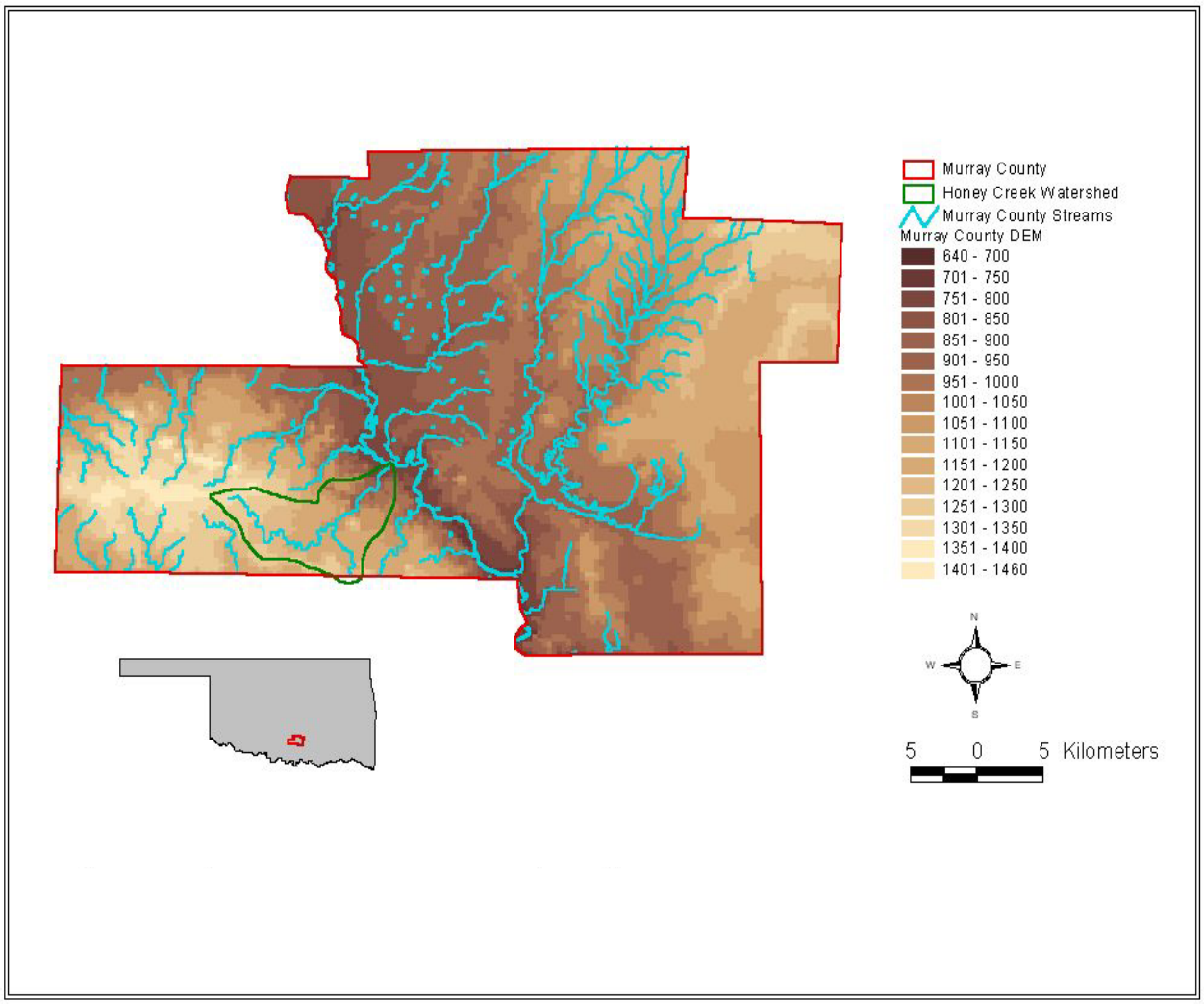
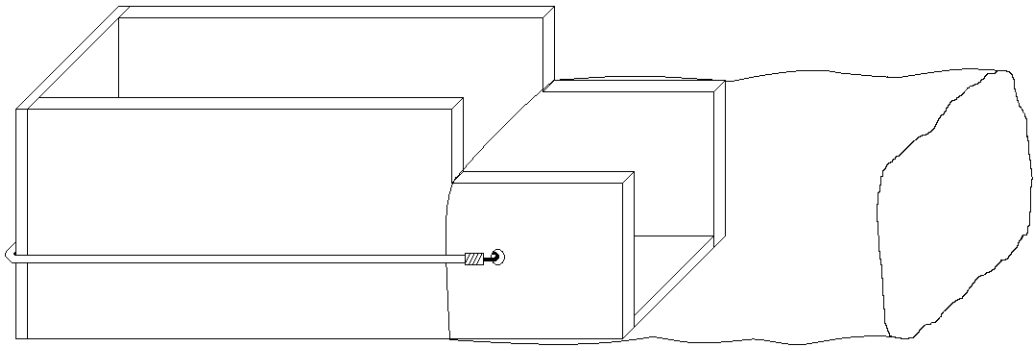


Figure 3. Diagram and dimensions of substrate sampler.



14cm wide X 14cm long
5mm plexi glass

Figure 4. Minimum and maximum temperature (°C) for sampling dates from Honey Creek, OK, February 2003-April 2004.

Figure 5. *C. variabilis* developmental stage preference for habitats found in Honey Creek, OK from February 2003-April 2004.

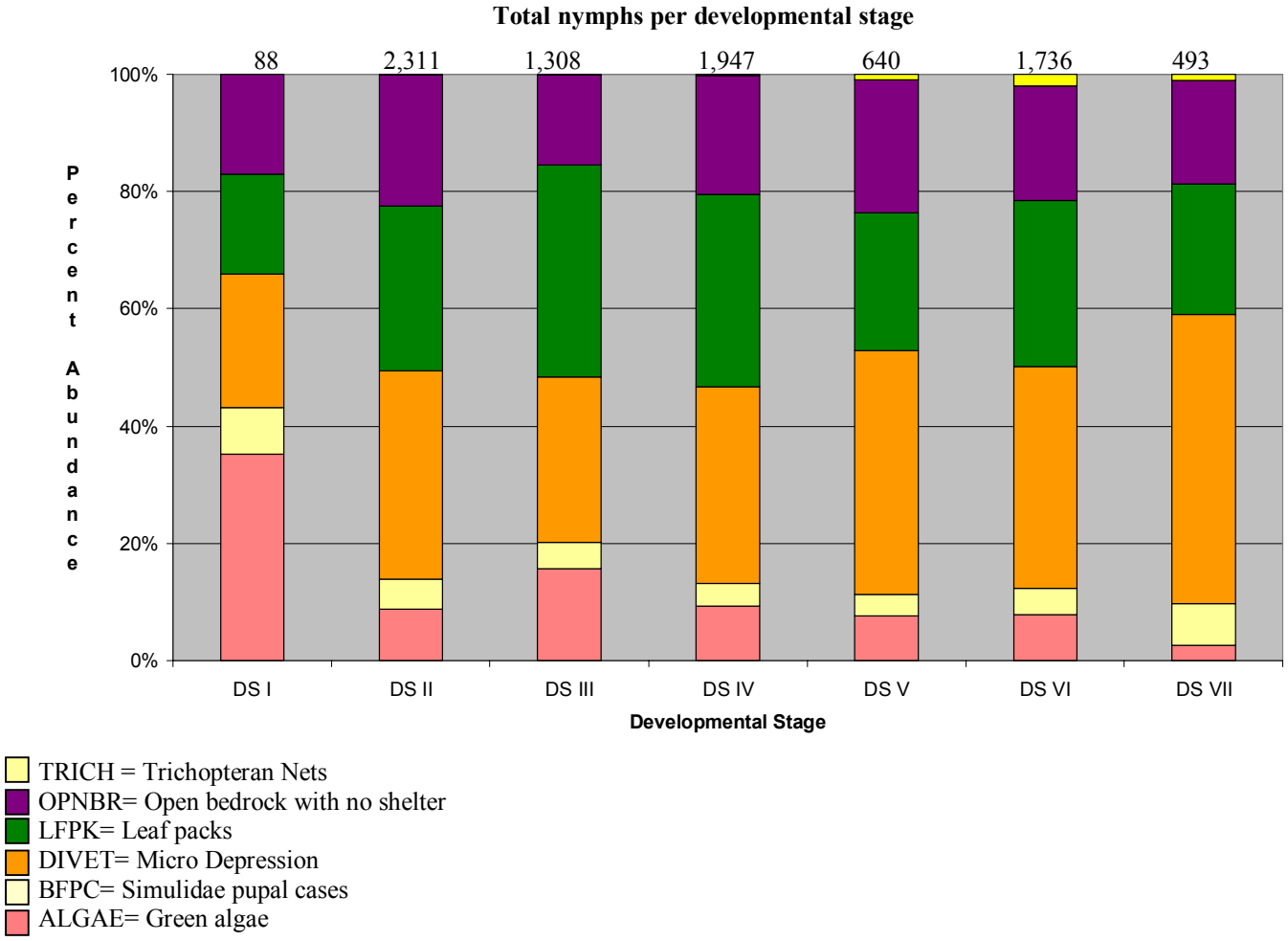


Figure 6. Velocity (cm/s) preference of *C. variabilis* from Honey Creek, Murray Co., OK from February 2003-April 2004.

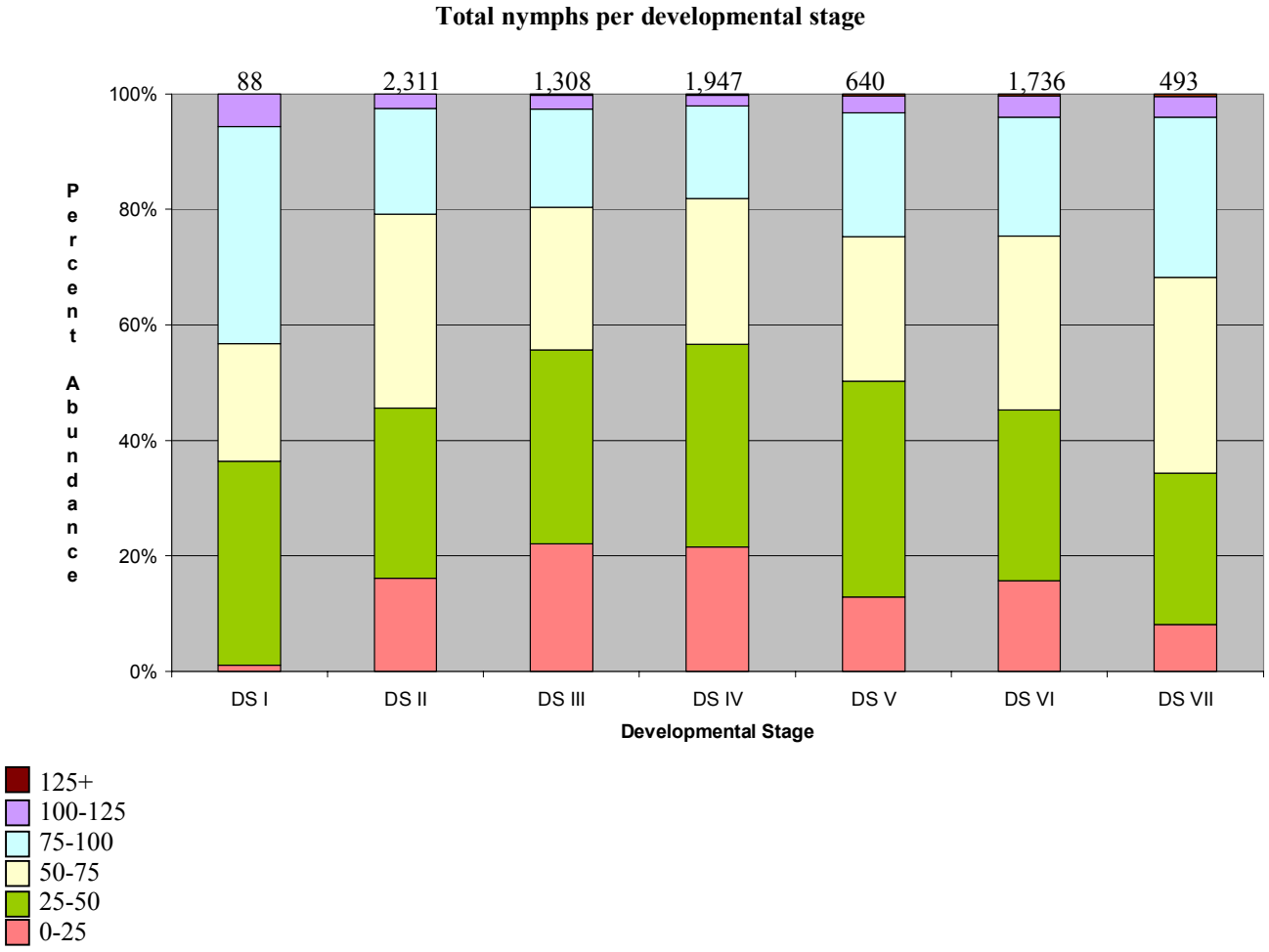


Figure 7. *C. variabilis* winter microhabitat distribution preference by developmental stage.

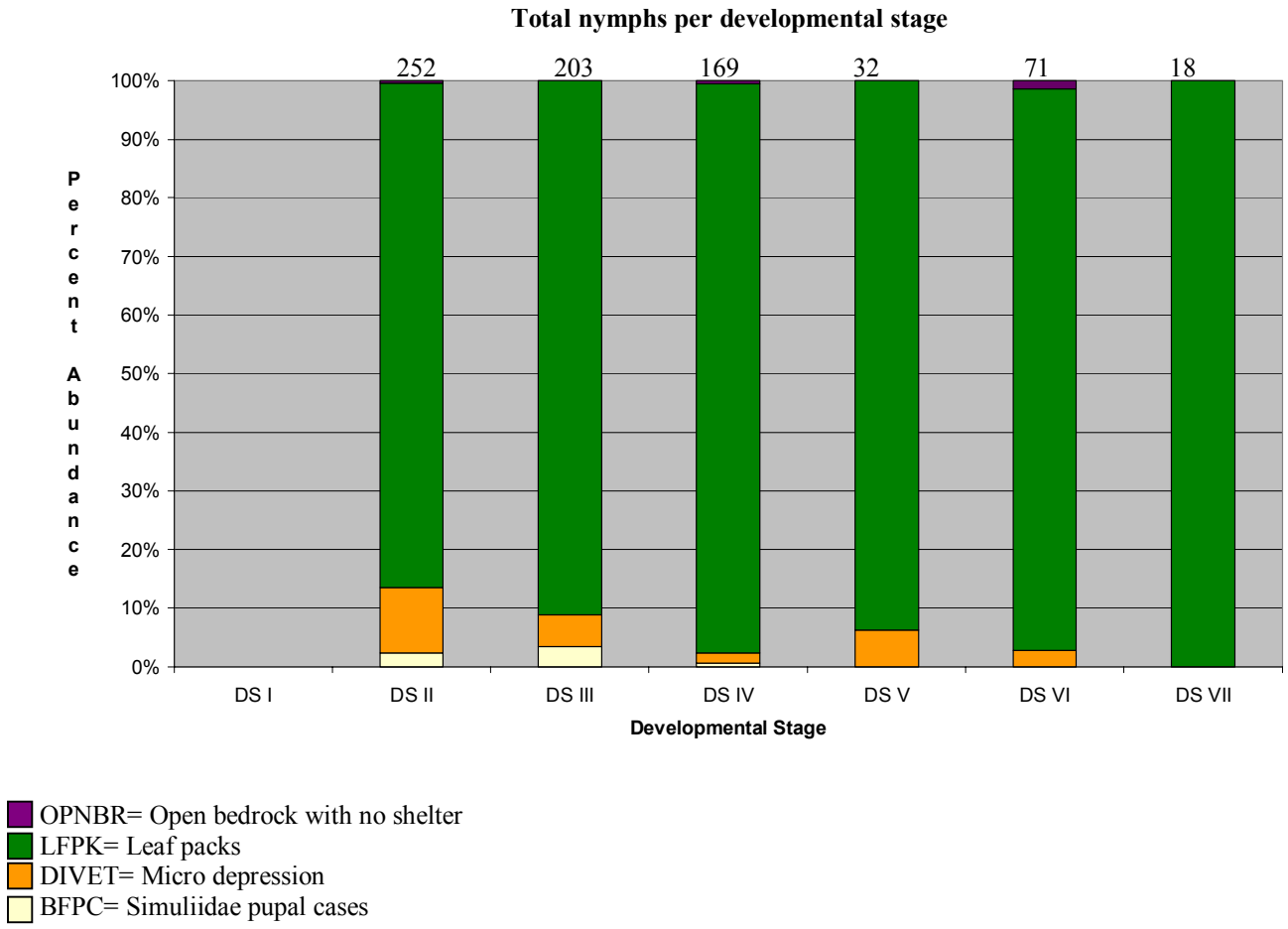


Figure 8. Winter velocity (cm/s) for each DS of *C. variabilis* from Honey Creek, Murray Co, OK, from February 2003 and December 2003-February 2004.

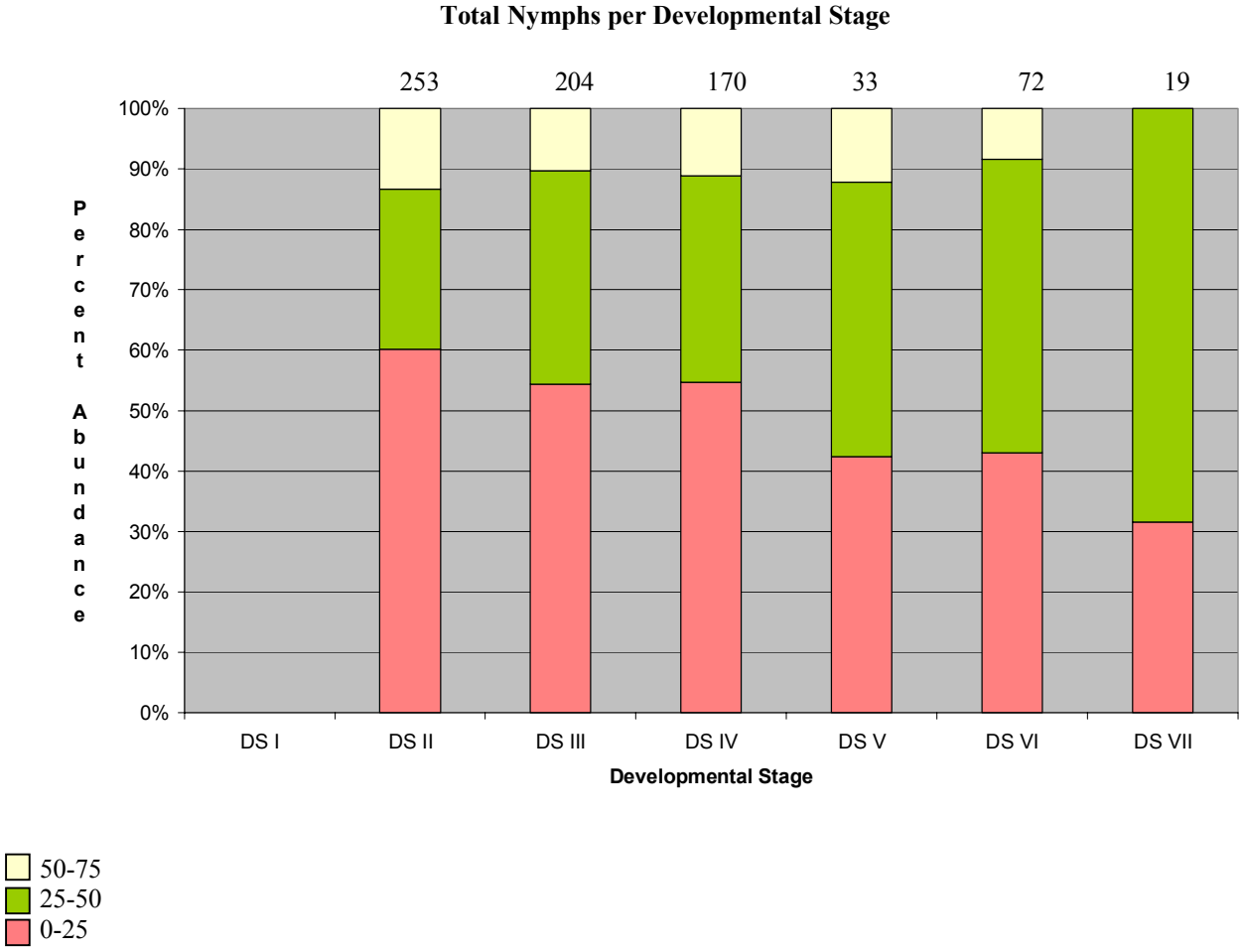
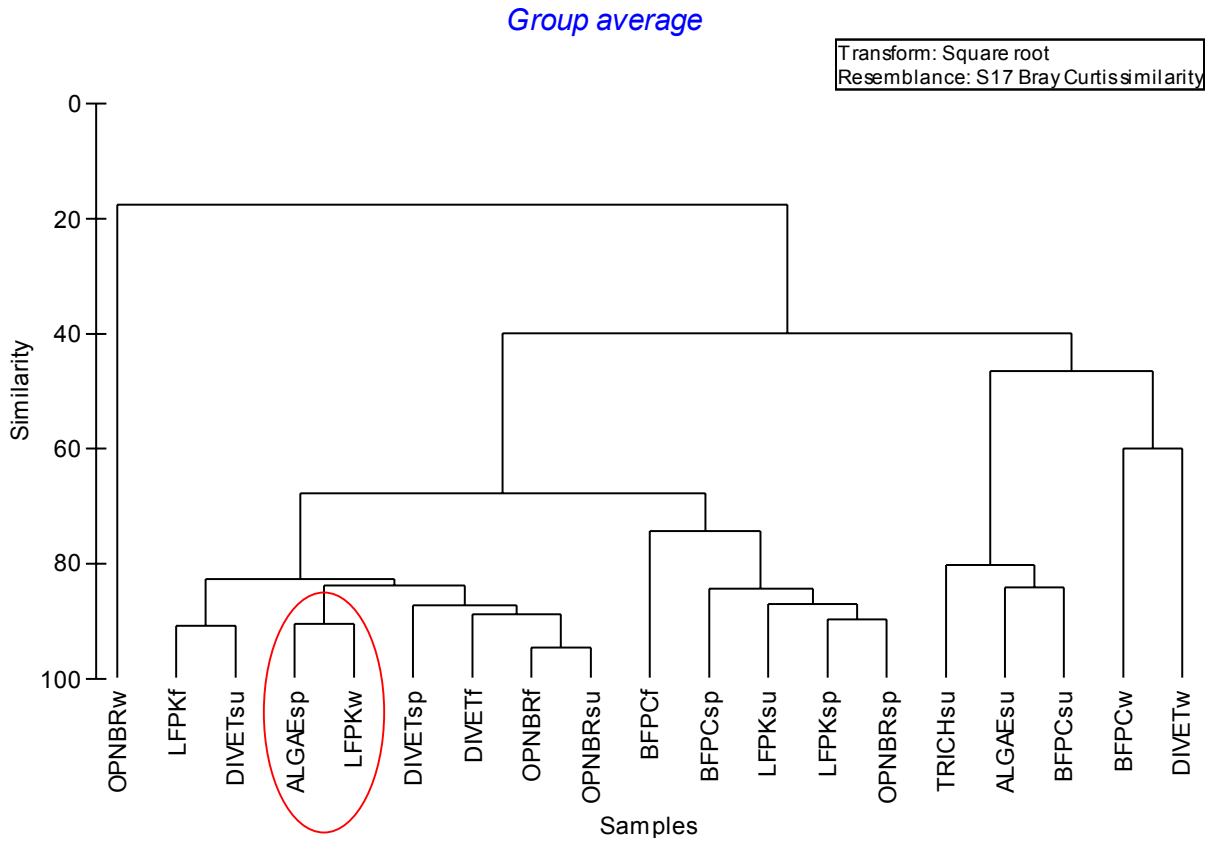


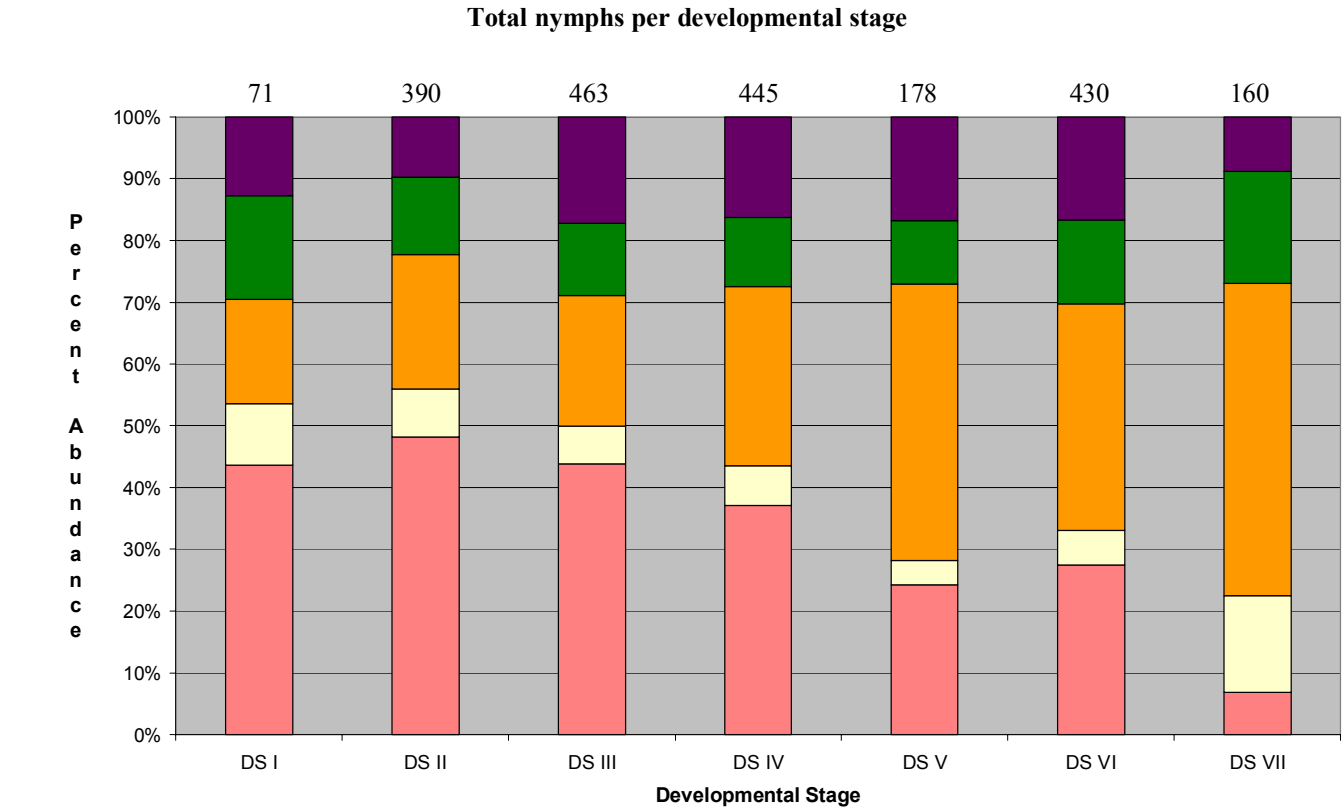
Figure 9. Cluster analysis by square root (Primer 6) of *C. variabilis* nymphal distributions by seasonal microhabitat relatedness (similarity).



OPNBR – open bedrock
LFPK – leaf pack
DIVET – micro depression
ALGAE – green filamentous algae
BFPC – Simuliidae pupal cases
TRICH – Hydropsychidae nets

w – winter
f – fall
sp – spring
su - summer

Figure 10. *C. variabilis* spring microhabitat distribution preference by developmental stage.



OPNBR= Open bedrock with no shelter
LFPK= Leaf packs
DIVET= Micro depression
BFPC= Simuliidae pupal cases
ALGAE= Green algae

Figure 11. Spring velocity (cm/s) for each DS of *C. variabilis* from Honey Creek, Murray Co., OK, from March-May 2003 and March-April 2004.

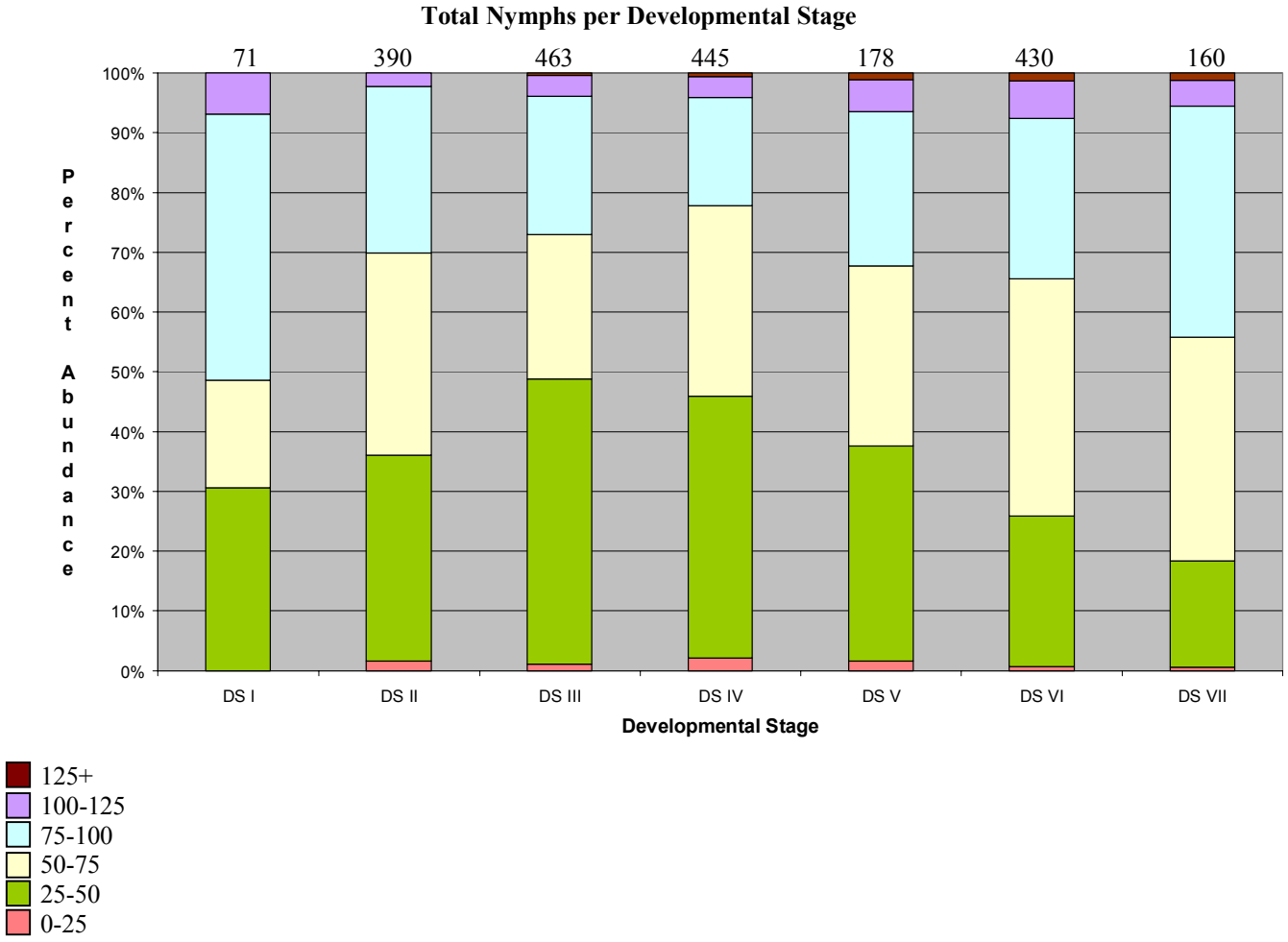


Figure 12. *C. variabilis* summer microhabitat distribution preference by developmental stage.

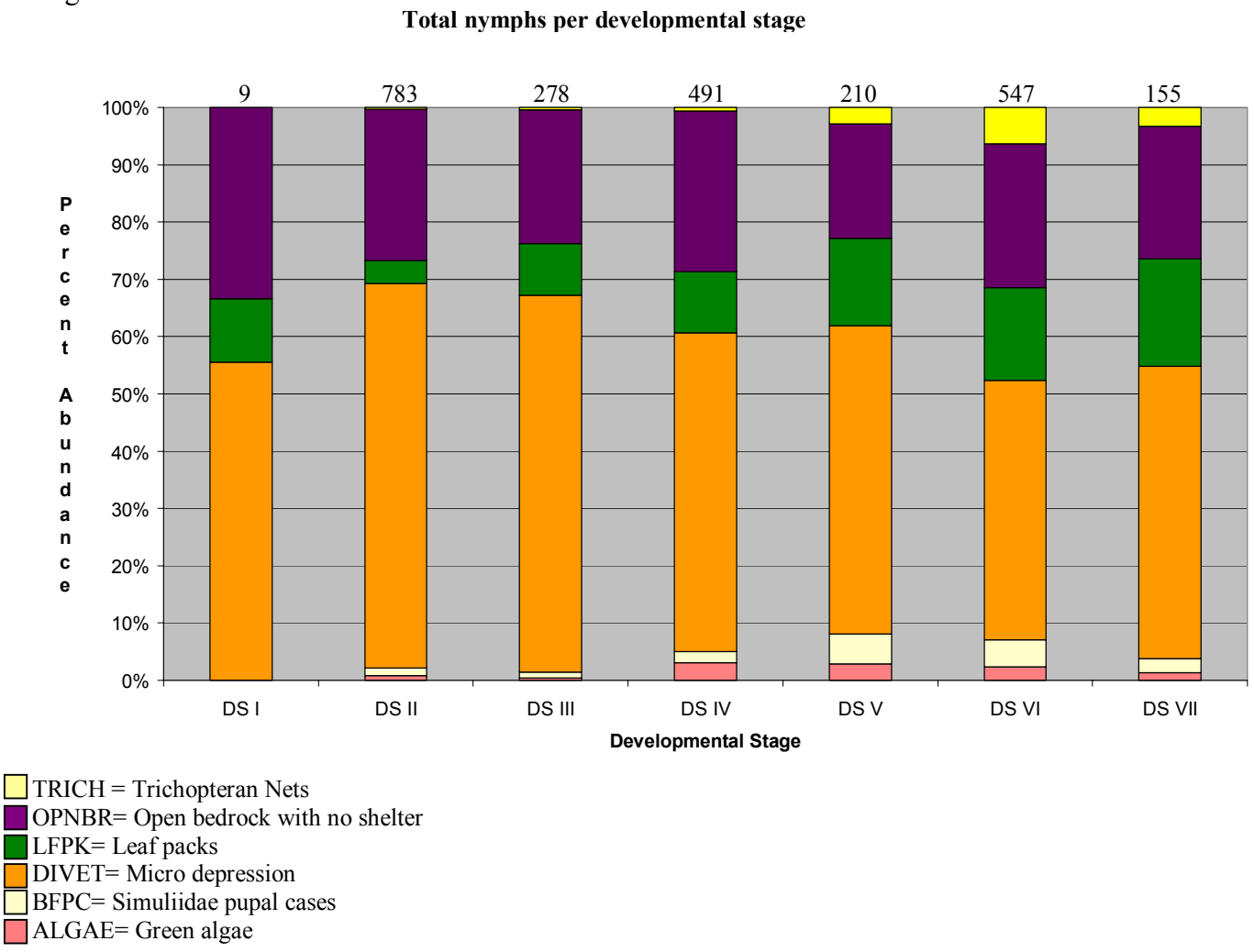


Figure 13. Summer velocity (cm/s) for each DS of *C. variabilis* from Honey Creek, Murray Co., OK, from June-August 2003.

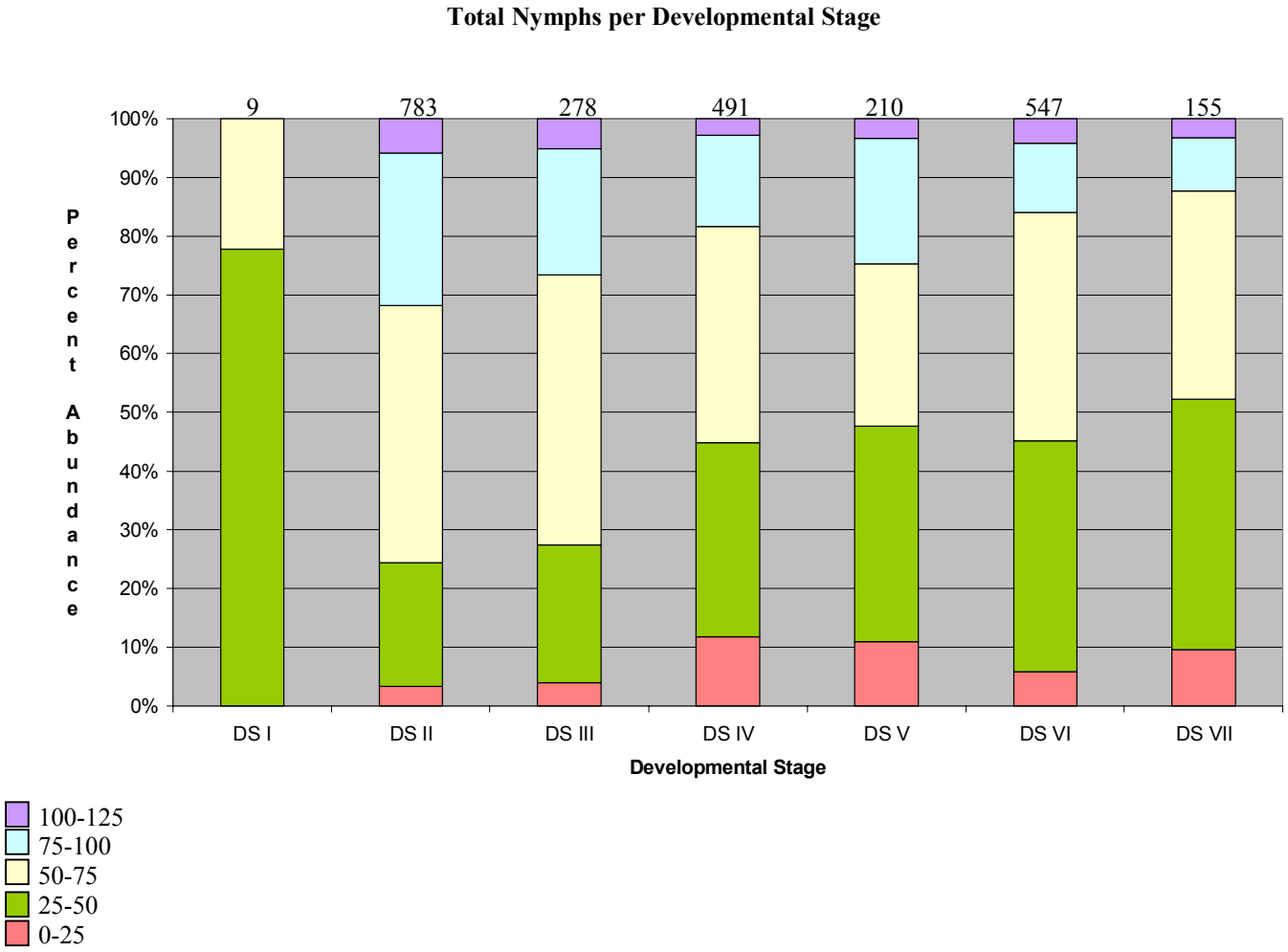


Figure 14. *C. variabilis* fall microhabitat distribution preference by developmental stage.

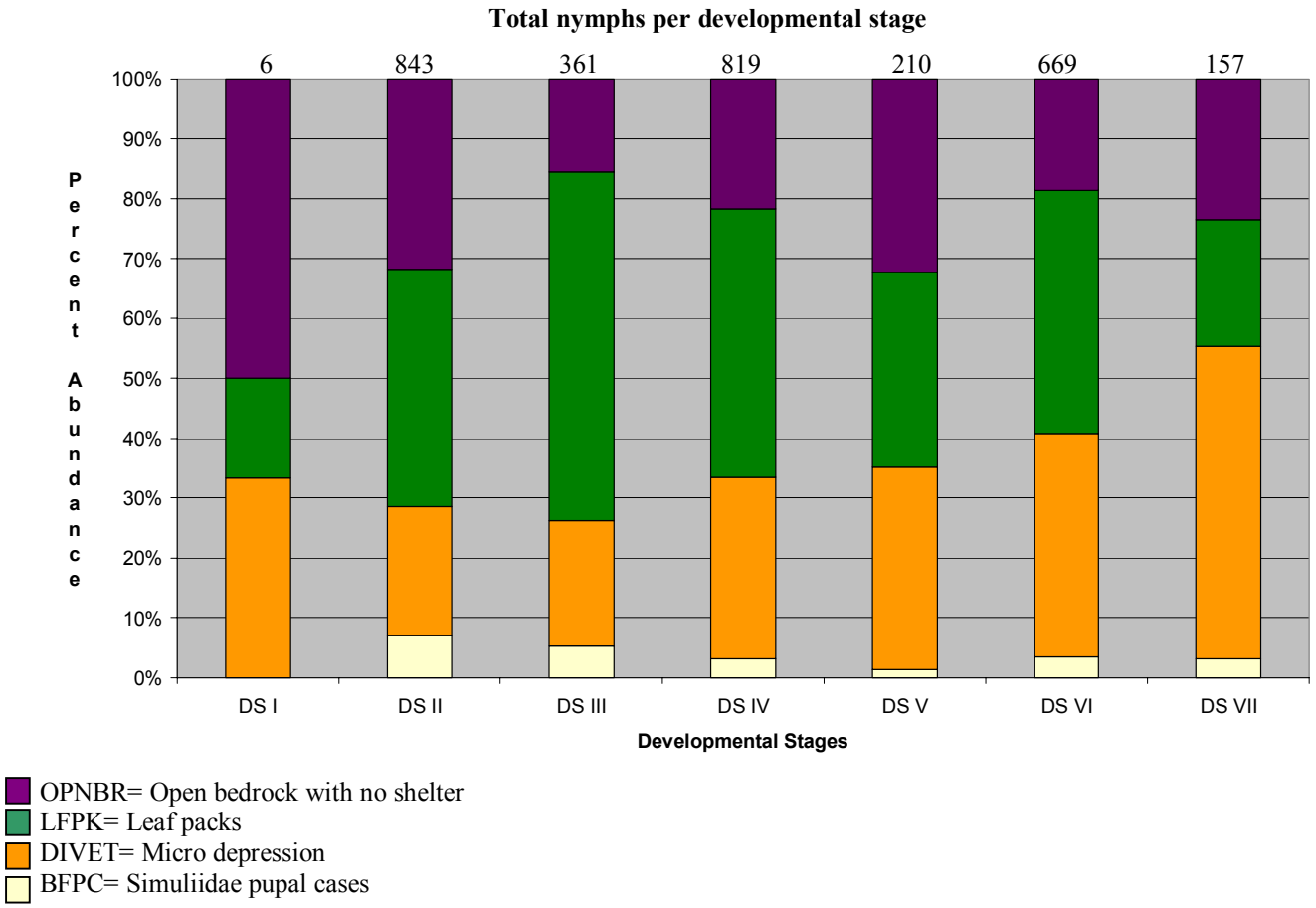
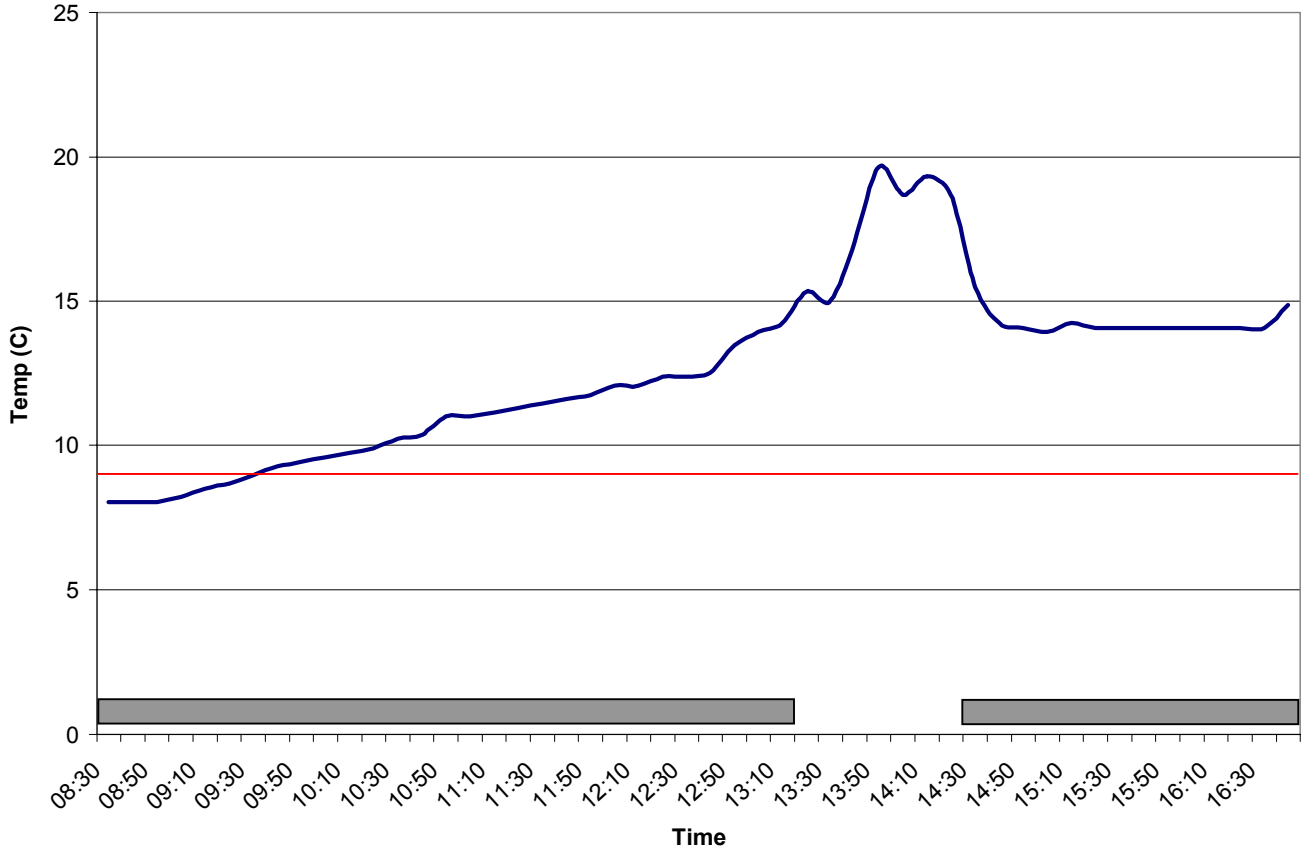


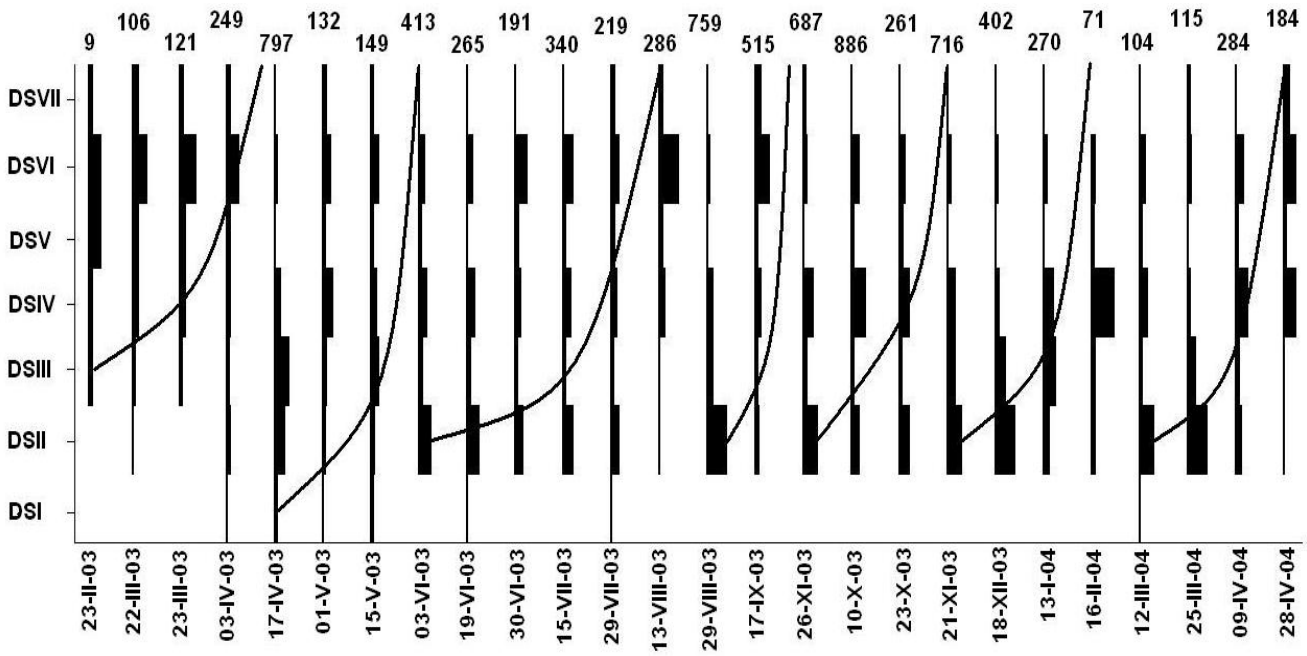
Figure 15. Fall velocity (cm/s) for each DS of *C. variabilis* from Honey Creek, Murray Co., OK, from September-November 2003.

Figure 16. Internal temperature (°C) of leaf pack placed in Honey Creek, January 2005. Shaded horizontal bars indicate leaf pack is shaded, break between bars indicates leaf pack exposure to sun.



Water Temperature
Internal Leaf pack Temperature
Shaded Leaf pack

Figure 17. Voltinism developmental frequency for *C. variabilis*.



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