Summer 7-2017

Biodiversity and Phenology of the Epibenthic Macroinvertebrate Fauna in a First Order Mississippi Stream

Jamaal Bankhead

BIODIVERSITY AND PHENOLOGY OF THE EPIBENTHIC

MACROINVERTEBRATES FAUNA IN A FIRST ORDER

MISSISSIPPI STREAM

by

Jamaal Lashwan Bankhead

A Thesis

Submitted to the Graduate School,
the College of Science and Technology,
and the Department of Biological Sciences
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

August 2017

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ABSTRACT

BIODIVERSITY AND PHENOLOGY OF THE EPIBENTHIC MACROINVERTEBRATES FAUNA IN A FIRST ORDER MISSISSIPPI STREAM

by Jamaal Lashwan Bankhead

August 2017

I used Hester-Dendy multiplate samplers and stick and leaf samples to collect the aquatic insects of Granny Creek, a first-order perennial stream in southern Mississippi, on twelve sampling dates from March 2014 to June 2015. The dominant insect/invertebrate group in Granny Creek was the dipteran family Chironomidae. Forty-two distinct taxa of chironomid larvae were collected over the duration of my study. Two of the dominant chironomid subfamilies in my samples, the Orthocladiinae and the Chironominae, displayed a phenological pattern in which the Orthocladiinae were more prevalent, both in terms of number of species and in abundance of individuals, in the cooler months of the year. Conversely, the Chironominae was the dominant subfamily in my collections in the summer months. Other aquatic insect groups were also present in my samples, including seven species of stoneflies, six species of mayflies, six species of caddisflies, eleven species of beetles, five species of dragonflies, one damselfly species and two species of megalopterans, including the hellgrammite, *Corydalus cornutus*. All of the stoneflies collected were members of the family Perlidae; as later instars perlids are predators on aquatic insects. The stonefly species *Perlesta placida*, displayed an extended

diapause, with nymphs present in the March and June 2014 collections, ensuring absence from samples from July 2014 through January 2015, and re-appearance in the February and March 2015 collections. The beetle family Elmidae was well represented in Granny Creek. Nine genera of elmids have been collected from Florida, seven of which had representatives in Granny Creek.

ACKNOWLEDGMENTS

I thank Dr. Beckett for showing me how to mount chironomids and helping with the many tireless days of identifications. I am grateful for Maylisa Smith for going out to Granny Creek and helping me collect samples through many hot and cold days. I also thank my committee members and the Department of Biological Sciences for their support throughout this project.

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CHAPTER I - INTRODUCTION

The measurement of biodiversity is a key attribute in assessing lotic freshwater macroinvertebrate communities (Ferrington 2008, Maltchik et. al 2012). For example, researchers have used diversity to assess the health of aquatic communities (Cereghino et. al 2003, Johsson and Malmqvist 2000). The macroinvertebrate fauna can give indications of disturbances from abiotic or biotic factors (Brooks 2000, Erman and Erman 1995, Johsson and Malmqvist 2000, Park et. al. 2003). Some orders of aquatic insects such as the Plecoptera, Ephemeroptera and Trichoptera are highly sensitive to pollutants (Arimoro and Ikomi 2009, Lenat 1983 and Wahizatul et. al 2011). Consequently, investigators have been especially interested in measuring the species richness of these three orders to provide information regarding the health of stream and river systems (Park et. al. 2003, Townsend et. al. 1997).

A key component of measuring biodiversity, in turn, is the identification of the species present. The identification of organisms to the lowest taxonomic level, preferably to genus or species, is essential in determining invertebrate diversity (Resh and Unzicker 1975, Binckley and Resetarits 2005, Lenat and Resh 200l). Unfortunately, often identification of aquatic insects to the genus level, and more specifically to species, can be a difficult task. Lenat and Resh (2001) make the case that most scientists use morphological characteristics to assign animals to certain species. Lenat and Resh (2001) argue that this means of categorizing freshwater macroinvertebrates can be difficult because scientists often collect immature stages, larvae or nymphs, of these animals. Additionally, the early instars may lack key features which are useful or even necessary for identification. Nevertheless, taxonomic keys (discussed later) are available for the taxa collected in my study.

Beckett (1992) studied the Chironomidae (order Diptera) of the Ohio River over a complete calendar year. His phenological study revealed that seasonal cycles in temperature can dramatically effect species composition (phenology = the study of animal and plant life cycles and how they are influenced by seasonal variations). The dramatic change in temperature over the seasons in the Ohio River in 1979 (low = -0.2 C on February 18 and 19 to a high of 28.9 C on August 12) resulted in striking differences in species dominance. Some members of the chironomid subfamily Orthocladiinae such as *Hydrobaenus pilipes* and *Orthocladius* spp. dominated invertebrate collections in the Ohio River in the colder periods from December to April, and species such as *Polypedilum convictum* were much more prevalent in the warmer months of July and August. Also, in warmer summer temperatures, species like *Hydrobaenus pilipes*, *Orthocladius obumbratus*, *Orthocladius oliveri*, and members of the *Eukiefferiella brevicalcar* group were completely absent from the Ohio River invertebrate collections.

Similar phenological changes in stonefly (order Plecoptera) composition were also observed in the Ohio River (Beckett 1987). The presence of “winter stoneflies,” which emerge in winter and early spring, has been well documented for a long period of time (see publications by Frison 1929 and 1935). Beckett (1987) found that nymphs of winter stoneflies colonized his samplers in the Ohio River over the winter months but were absent during the summer months. These dramatic seasonal changes in macroinvertebrate communities show how abiotic factors such as temperature can heavily influence the species composition in freshwater communities (Beckett 1987, Beckett 1992, Binckley and Resetarits 2005). Heino (2002) described changes in species richness across multiple organisms from insects, fish and some macrophytes, in Denmark, Norway, Sweden and Finland. His data showed that seasonal changes affected a wide range of organisms. Different species moved in and out during warm and cold months.

My study involved the collection of epibenthic invertebrates, rather than embenthic (= infaunal) animals. The epibenthic fauna occurs on substrates, but does not occur in or penetrate them. Epibenthic freshwater invertebrates therefore live on submerged logs, sticks, leaves, rocks, plants, surfaces of animals, and on human-made objects. Although I dealt with all the freshwater invertebrate taxa I collected, the major emphasis of my study concerned insects in the family Chironomidae. Individuals of this dipteran family outnumbered all the individuals of the other taxa I collected. This is not surprising. Epler (2001) stated that "the Chironomidae are usually the most abundant macroinvertebrate group, in number of species and individuals encountered in the majority of freshwater aquatic habitats." Coffman (1978) stated that chironomid species often make up at least 50% of the total number of macroinvertebrate species present in freshwater studies.

My study had two major goals. The first was to document the composition of the aquatic insect community in a first-order Mississippi stream over the span of one year. The second was to determine if phenological changes took place in my study site over the calendar year and, if so, were they similar to those observed by Beckett (1987, 1992) in the Ohio River.

Study Site and Study Hypothesis

I selected my study stream on the basis of three attributes: 1) since I wanted to do a phenological study the stream needed to flow year-around (a "perennial" stream), 2) I needed to have easy access to the stream and be able to sample each month (a higher-order stream nearby to Granny Creek selected earlier for my study flooded for long durations during the rainy portions of the year, making it difficult to sample); and 3) the stream needed to be relatively undisturbed. Granny Creek fulfilled all three of these criteria. Granny Creek is located in Forrest County, Mississippi (31.06°N, -89.24°W) and lies entirely within the DeSoto District of DeSoto National Forest. Granny Creek is a first order perennial stream with a sandy and silt bottom and is approximately 47 meters above sea level. Granny Creek drains into Black Creek shortly after crossing Rockhill-Brooklyn Road. My sampling area was accessed from a bridge located on Rockhill-Brooklyn Road near its intersection with West Thompson Road (Figure 1).

Because one of my major goals was to document the composition of the freshwater invertebrate community of a Mississippi stream I wanted to select a stream which was relatively undisturbed anthropogenically. The area along Granny Creek may have been logged in the early 1900s. However, at present there are no nearby roads (other than Rockhill-Brooklyn Road which crosses the creek) and views along the stream from Google Earth show a "carpet" of trees along the entire length of the stream, and no residences. Thus, the results of my study could be used as a standard by which the invertebrate composition of other small streams (both disturbed and undisturbed) in the East Gulf Coastal Plain ecoregion could be compared.

The following are the hypotheses I evaluated in my study: 1) that the epibenthic chironomid community would be relatively diverse; 2) that as an undisturbed stream Granny Creek would contain a relatively diverse EPT (Ephemeroptera-Plecoptera-Trichoptera) community; 3) that a shift in the chironomid subfamilies Orthocladiinae and Chironominae would occur as a consequence of seasonal changes in temperature; and 4) that individual species among the Chironomidae and/or the Plecoptera would exhibit life cycle patterns which would include extended periods of non-activity (diapause), as shown by their absence from the invertebrate collections over extended periods.

Figure 1.
Location of the Granny Creek study site. Arrow shows where the sampling site was entered. The larger stream at top of the figure is Black Creek. The road to the right of Granny Creek is West Thompson Road.

CHAPTER II - METHODS

Sampling Methods

For each sampling period **s**ix Hester-Dendy multiplate samplers were placed within a 60 meter section of Granny Creek near the bridge crossing. The Hester-Dendy multiplates (Hester and Dendy 1962) were each constructed of eight Masonite plates secured on a 5/16 inch x 5 inches eyebolt and held in place with a wing nut. Each of the plates was separated by washers to establish varying spaces between the plates (see Beckett 1992). Each of the plates was 70 mm x 70 mm and 3 mm thick. Also, four additional samples were taken on each sampling trip, two from leaf debris and two from submerged wood (twigs or snags). The leaf debris and submerged wood (stick/leaf samples) were gathered from the stream and their surfaces and brushed with a toothbrush into a pan. Invertebrates brushed off these substrates were then collected on a U.S. Standard No. 60 sieve with openings equaling 250 micrometers and placed into sample containers.

The multiplate samplers were tied with rope onto structures in the stream in areas with a mean average current speed of 36.7 cm/sec (minimum to maximum current speed = 13.4 cm/sec-52.2 cm/sec) and were suspended 2-3 cm above the stream bottom so invertebrates could colonize them (either directly from the water surface or via drift). The Hester-Dendy samplers were placed at multiple locations in case flooding occurred and washed them onto the stream bank. Generally multiplate samplers were collected once per month with the first day of collections starting on June 18, 2014 (from samplers placed in the creek on March 31, 2014). Multiplate samplers were recovered on each sampling date with the exceptions of March 31, 2014 when they were first placed in the stream, and in February 2015 when the samplers were washed out of the stream following an extended and heavy period of rain. On the dates when invertebrates were collected from the multiplate samplers a new set of samplers was placed in the stream for colonization. Leaf and wood (stick/leaf) invertebrate samples were collected on the same dates as the multiplate collections. After the multiplate samplers were collected, the plates were removed from the eyebolt, and invertebrates brushed off the plates and the spacers with a toothbrush. Invertebrates were subsequently collected using a U.S. standard No. 60 sieve (with a 250 micrometer mesh). The invertebrates from a multiplate sampler were placed into its individual container and were preserved in 70% ethyl alcohol for later identification. The stream temperature was determined on each collection date.

In the laboratory invertebrates were identified to the lowest possible taxonomic level. All invertebrates, with the exception of individuals in the families Chironomidae and Simuliidae (of the order Diptera), were identified using a Wild M3Z Heerbrugg dissecting microscope. The Chironomidae and Simuliidae were mounted on slides using the method of Beckett and Lewis (1982) and identified using a Wild Heerbrugg compound microscope.

Table 1

Temperatures and dates on which samples were collected from Granny Creek.

Collection Dates	Temperature (°C)
March 31, 2014	20.1
June 18, 2014	25.7
July 27, 2014	28.2
August 25, 2014	24.6
September 27, 2014	28.9
October 29, 2014	25.8
November 29,2014	18.3
January 19,2015	9.5
February 20, 2015	7.2
March 20, 2015	20.4
April 26, 2015	21.0
June 17, 2015	25.5

Data Analysis

For the purpose of analysis the information (the number of individuals per species) from the four stick/leaf samples, per sampling date, was combined. I analyzed the data collected from the multiplates separately from the stick/leaf samples. However, the data from the multiplates, per month, were also combined. After identifying chironomid larvae to genus or species (and analyzing those data) I also summarized that information at the subfamily level as Beckett (1992) had observed striking variations in the abundance of species and individuals in the subfamilies Orthocladiinae and Chironominae in comparing winter and summer communities in the Ohio River. Invertebrate abundance was recorded by totaling the number of individuals collected for each month (see Appendices A and B).

I used two measures to compare relatedness between two samples (or stations or communities), coefficient of community (CC) and percentage similarity (PS) (Whittaker 1975). Both CC and PS are often described as similarity indices. Coefficient of community is a presence-absence index and is calculated using the following formula:

$$CC = 2S_{ab}/(S_a + S_b)$$

where S_a= the number of species in sample A; S_b = the number of species in sample B; and S_{ab} = the number of species found in sample A which are also found in sample B (i.e. the number of species common to both samples or sites) (Whittaker 1975). Coefficient of community has been widely used in comparing aquatic macroinvertebrate communities (Beckett 1978, 1992, Beckett et al. 1992, Rogers 1998).

The second similarity index I used, PS, takes into account the quantitative representation of the species involved in a comparison of two samples, sites, or communities (Whittaker 1975). Percentage similarity is determined using the following formula:

$$PS= \Sigma \min (p_a \text{ or } p_b)$$

where p_a is the decimal proportion of a given species in sample A and p_b is the decimal proportion for that same species in sample B; min= minimum with this procedure being carried out for every species in the two samples and then summed (Whittaker 1975, Beckett 1992).

I performed all the possible pair-wise comparisons for the chironomid collections present on my multiplates over the various sampling dates, producing a similarity matrix from the multiplate data based on CC (Table 2). I was also interested in seeing how similar my multiplate samples were to my stick/leaf samples. Subsequently I used both CC and PS to compare the two collections (multiplates invertebrate collections versus stick/leaf invertebrate collections) on a month by month basis (see Table 3).

Similarity matrices allow for data interpretation but can also allow for additional analysis. I further analyzed the larval chironomid communities collected over the sampling dates using polar ordination based on the CC similarity matrix I had generated. Polar ordination was introduced by Bray and Curtis (1957) and, in my study, provided a graphical summary of the chironomid communities. Polar ordination uses endpoints of two stations and the other stations are ordinated from those two endpoints based on their similarity (Gauch et. al. 1977 and Giraudel 2001). Generally samples that are similar biotically should be located near each other in the ordination whereas samples that are biotically dissimilar should be widely separated. The procedure used for the ordination is described in Cottam et al. 1973).

Taxonomic Literature and Notes

I used the aquatic insect key of Merritt et al. (2008) as a general key in my invertebrate identifications. In addition I used a variety of keys for the identification of specific taxa. The principal key I used for the identification of chironomid larvae was authored by Epler (2001). I also used keys produced by Simpson and Bode (1979) and Wiederholm (1983) for the identification of chironomid larvae. Some species of chironomids cannot be identified to the species level as larvae but can be designated to a "species group." Also, genera such as *Cricotopus* and *Orthocladius* contain some species that are almost identical in the larval form and thus cannot be assigned definitively to one of the two genera, and are designated in my thesis as *Cricotopus/Orthocladius* sp. Epler (2001) suggests identifying species identified as larvae in the genus *Parametriocnemus* as *Parametriocnemus* sp. The larvae I identified as belonging to this species look to be identical to those illustrated by Simpson and Bode (1979) and Wiederholm (1983) as *Parametriocnemus lundbecki*. Consequently I have identified these specimens as *Parametriocnemus* nr. *lundbecki* (nr.= near).

I used Pescador and Richard's key (2004) to the mayflies of Florida for the identification of the Ephemeroptera as well as the *Stenonema* key of Lewis (1978). The primary key I used for the order Plecoptera was the guide to the stoneflies of Florida (Pescador et al. 2000). In addition, I also used Stewart and Stark's (1993, 2002) keys to the stonefly nymphs of North America. The nymphs I have identified as *Acroneutia arenosa* could be either *A. arenosa* or *Acroneuria evoluta* since the two species cannot be distinguished from each other as nymphs. However *A. arenosa* is much more common in

Florida than *A. evoluta* (Pescador et al. 2000) and I have therefore used *A. arenosa* as the identification for nymphs that fit the description of *A. arenosa/evoluta*.

I used Wiggins (1977) treatment of North American caddisflies and the key of Pescador et al. (2004) to the caddisfly larvae of Florida for the identification of individuals in the order Trichoptera. Pescador et al. (2004) list two species in the genus *Neureclipsis* from Florida, *N*. *crepuscularis* and *N*. *melco*. The difference between the two species as larvae is based on pigmentation; unfortunately all the larvae I collected were small and lightly colored. Hence, I identified all the *Neureclipsis* I collected to just the generic level (*Neureclipsis* sp.).

Epler (1996 and 2010) were the major keys used to identify individuals in the order Coleoptera (beetles). Soltesz (1996) was used as an additional reference (to Merritt et al. 2008) for identification of members of the suborder Anispotera (dragonflies). The keys of Huggins and Brigham (1982) were used as additional references (along with Merritt et al. 2008) for the identification of damselfly and dragonfly nymphs.

CHAPTER III – RESULTS

Measurements of stream temperatures on my sampling dates showed that water temperatures exceeded 20˚C on all of the dates from March 2014 through late October 2014, fell below 10˚C on the January and February 2015 sampling dates, and then returned to temperatures exceeding 20˚C by the following March (2015) (Table 1). I collected a total of 42 distinct chironomid taxa during my study (this sum includes all the chironomid taxa collected via the Hester-Dendy multiplates and the stick/leaf collections, see Appendix A). All the chironomid larvae collected were members of three subfamilies: the Tanypodinae, the Chironominae, and the Orthocladiinae.

The Hester-Dendy multiplate data (Figure 3, Table 4) showed dominance by the subfamily Chironominae in terms of chironomid species present in the warm days of summer. The three tribes (Chironomini, Tanytarsini, and the Pseudochironomini) which constitute the subfamily Chironominae accounted for at least 50% of chironomid species richness on the Hester-Dendy multiplates in June, July, and August of 2014 and again in June of 2015 (Figure 3). This pattern was not as clearly demonstrated by the stick/leaf samples (Figure 4). By species, the Chironominae were especially dominant in June of 2014 and June of 2015. Inspection of the ranking of the monthly multiplate collections by percent Chironominae species (in comparison to total chironomid species), showed that the two June months exhibited the highest ranks (Table 4). Two spring months, March and April 2015, showed the lowest ranking of percent Chironominae (Table 4).

Analysis of dominance by the Chironominae by abundance (number of individuals) rather than number of species did not demonstrate the clear patterns exhibited in Figure 2 and the rankings by species richness (shown in Table 4). The causation of this difference was the very large number of individuals in the subfamily Tanypodinae collected in some months. For example, in June 2015, a total of 67 chironomids were collected on the multiplates; 41 of them (61%) were members of the *Thienemannimyia* sp. gr. (a genus within the subfamily Tanypodinae).

Another dominant chironomid subfamily in my samples, both by species and by number of individuals collected, was the Orthocladiinae. The breakdown of chironomid larvae by number of species belonging to particular subfamilies collected on the multiplates showed dominance by the Orthocladiinae in the relatively colder months of October, November, January, and March, with markedly lower representation in June and July of 2014 and June 2015 (Figure 3). A ranking of the percent Orthocladiinae by species showed the highest values in the spring months of March and April 2015, with the lowest rankings in July 2014, June 2014, and June 2015, respectively (Table 5).

The representation of chironomids collected on the multiplates in the various subfamilies by individuals echoed the pattern demonstrated by the breakdown by number of species (see Figure 5). Members of the Orthocladiinae dominated collections from November, January, March, and April, but were low in the June 2014, July2014, and June 2015 collections. This pattern was also reflected in the rankings of the percent abundance of the Orthocladiinae; the highest rankings were in January and March of 2015, and the lowest rankings were in the summer months of July 2014, June 2015, and June 2014, respectively (Table 5). This was less obvious in the chironomid data from the stick/leaf collections although the representation by members of the Orthocladiinae was relatively high in February, March and April of 2015 (Figure 5).

Beckett (1992) showed that several species within the Orthocladiinae were present (often abundant) on his multiplate and rock-basket samplers in the Ohio River in the colder months of the year, but were completely absent from collections in the warner months. Therefore, I was interested in whether any species in my Granny Creek multiplate collections showed a similar pattern. Only two species that were collected in sizeable numbers showed such a pattern: *Parametriocnemus* nr. *lundbecki* and *Thienemanniella lobapoderma. Parametriocnemus* nr. *lundbecki* was present on the multiplates in September, October, and November of 2014, and in March and April of 2015 (Appendix A). It was not present on the multiplates in either of the June collections or the July or August collections. Similarly, *Thienemanniella lobapodema* was not collected in the warmer months of June 2014, July 2014 or June 2015 in either the multiplate or the stick/leaf collections, but was present either in one or the other collection types, or both, on all the other months (Appendix A).

Some chironomid species persisted throughout the year on both the Hester-Dendy multiplate samplers and stick/leaf samples. Species like *Rheocricotopus robacki*, *Cricotopus bicinctus* and *Polypedilum convictum* were recorded, for the most part, throughout the year in both types of samples (Appendix A).

As stated above, a high percentage (at least 40%) of the summer chironomid species richness for both sampling techniques came from the subfamily Chironominae; for some months a substantial contribution of this species richness was contributed by the tribe Tanytarsini (Figures 3 and 4). One taxon, *Tanytarsus* sp. accounted for the highest number of individuals within this subfamily (Appendix A). Members of this genus were abundant throughout the year on the multiplates and in the stick/leaf collections and contributed markedly to the individuals represented by the Tanytarsini on both sampling types (see Appendix A and Figures 5 and 6).

The subfamily Tanypodinae was represented by only six taxa collected over my sampling year. The Tanypodinae did not show a clear seasonal pattern. Members of the *Thienemannimyia* gr. were the most abundant of the Tanypodinae species collected, and were found throughout the year on both the multiplates and stick/leaf samples (Appendix A). Two *Ablabesmyia* species were collected. *Ablabesmyia mallochi* and *A. rhamphe* were present over most of the year on both the stick/leaf and multiplate samples. *Procladius bellus* was collected only on the stick/leaf samples.

The similarity matrix based on the coefficient of community showed a wide dissimilarity between the chironomid community collected on the multiplates in July 2014 and the chironomid community present in January 2015 (Table 2). Hence the collections on those two dates were selected as the endpoints for the polar ordination. The polar ordination shows the chironomid collections in the warmer months were closer to the July endpoint than the January endpoint. During the autumn months of September, October, and November the chironomid communities on those dates shifted their affinities from the warmer months of July 2014, August 2014, and June 2015 toward the January endpoint (Figure 2). The ordination also showed that the chironomid communities of the spring months of March and April 2015 were quite similar to each other, and relatively similar in composition to those of the autumn months.

Three specimens of *Xylotopus par*, a member of the Orthocladiinae, were collected during my study, all on stick/leaf samples. A comparison of the chironomid community collected on the multiplate samplers with the chironomid community collected in the sticks and leaves per month showed, somewhat surprisingly, considerable variation in values, with some values reflecting high relatedness (e.g., PS = 0.89 in July 2014 and CC = 0.70 in June 2014) and some relatively low similarities (PS = 0.39 in March 2015 and CC = 0.33 in January 2015) (see Table 3).

Table 2

Similarity matrix, based on coefficient of community (CC), showing results of comparison of chironomids collected on Hester-Dendy multiplate samplers. 14 = 2014, 15 =2015.

	18-Jun-14	27-Jul-14	25-Aug-14	27-Sep-14	29-Oct-14
18-Jun-14		0.56	0.48	0.46	0.61
27-Jul-14	0.56		0.59	0.45	0.39
25-Aug-14	0.48	0.59		0.47	0.73
27-Sep-14	0.46	0.45	0.47		0.61
29-Oct-14	0.61	0.39	0.73	0.61	
29-Nov-14	0.55	0.48	0.55	0.57	0.53
19-Jan-15	0.42	0.08	0.33	0.38	0.50
20-Mar-15	0.52	0.43	0.46	0.70	0.64
26-Apr-15	0.60	0.43	0.62	0.65	0.71
17-Jun-15	0.50	0.50	0.35	0.65	0.48

	29-Nov-14	19-Jan-15	20-Mar-15	26-Apr-15	17-Jun-15
18-Jun-14	0.55	0.42	0.52	0.60	0.50
27-Jul-14	0.48	0.08	0.43	0.43	0.50
25-Aug-14	0.55	0.33	0.46	0.62	0.35
27-Sep-14	0.57	0.38	0.70	0.65	0.65
29-Oct-14	0.53	0.50	0.64	0.71	0.48
29-Nov-14		0.38	0.65	0.47	0.39
19-Jan-15	0.38		0.42	0.42	0.29
20-Mar-15	0.65	0.42		0.75	0.55
26-Apr-15	0.47	0.42	0.75		0.62
17-Jun-15	0.39	0.29	0.55	0.62	

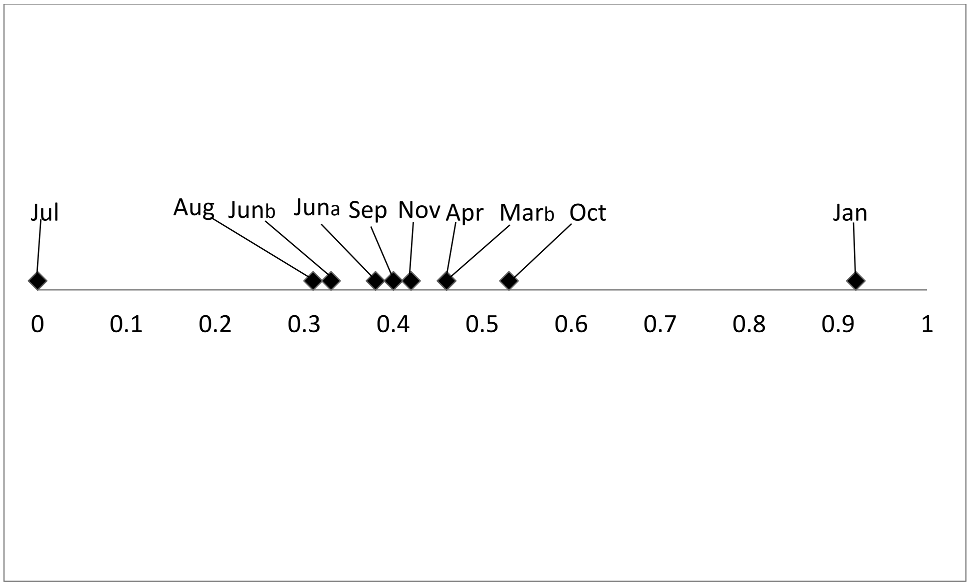

Figure 2.
Polar ordination of the Granny Creek chironomid community collected on Hester-Dendy multiplates samples on 10 dates, 2014 - 2015. Sampling collection dates: Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015.

Plecoptera

Stoneflies are an order of insects (Plecoptera) that are adapted to clean, flowing water. Some can be found in large oligotrophic lakes, but most are restricted to lotic habitats (Merritt et al. 2008). I collected seven species as nymphs from Granny Creek: *Acroneuria abnormis*, *A. arenosa*, *Agnetina annulipes*, *Eccoptura xanthans*, *Neoperla carlsoni*, *Pargnetina fumosa*, and *Perlesta placida* complex (Appendix B). All of the stoneflies I collected from Granny Creek are members of the stonefly family Perlidae. The most common stonefly species in my collections was *A. arenosa*. This species was collected every month of my sampling dates with the exception of February, March, and June of 2015. I was particularly interested in determining the phenological pattern in the development of *Perlesta placida* in Granny Creek, as Beckett (1987) found this species (or species complex, see Stark 1989) in the Ohio River only in May and June, suggesting emergence of this species from the Ohio River as adults in summer. Similarly, in Granny Creek I collected this species in March and June 2014; it was then absent from all of my collections until February, March, and April of 2015 (Appendix B).

Ephemeroptera

Mayflies inhabit a wide array of lentic and lotic environments. Most nymphs are either collectors (feeding on fine particulate organic matter) or scrapers (feeding on algae off of surfaces) with only a few species being carnivorous (Merritt et al. 2008). I collected six distinct mayfly species representing five families from Granny Creek (Appendix B). Two of the six species were members of the family Heptageniidae: *Maccaffertium exiguum* and *Maccaffertium smithae.* I assigned *Maccaffertium* sp. to the specimens that were too small to identify to species. *Maccaffertium* was once a subgenus within the genus *Stenonema* but it (*Maccaffertium)* was elevated to the generic level by Wang and McCafferty (2004). The family Isonychiidae was represented by the genus, *Isonychia*; this mayfly was relatively uncommon in my samples and was only collected in three months of the year (March and June 2014 on the stick/leaf samples and October 2014 on the multiplates).The families Leptohyphidae and Leptophlebiidae were each represented by one species in my collections. *Tricorythodes albilineatus* (family Leptohyphidae) and *Paraleptophlebia volitans* (family Leptophlebiidae) were both relatively common in my collections and were present throughout the year (Appendix B). *Centroptilum triangulifer,* a member of the family Baetidae, is geographically widespread and was also common in my samples (Appendix B).

Trichoptera

Caddisflies (order Trichoptera) are closely related to the order Lepidoptera and are known for their many species which build cases from environmental materials (Merritt et al. 2004). Six species belonging to four families were collected from Granny Creek. The genus *Chimarra* sp. (family Philoptamidae) is commonly found in clear, sand-bottom streams (Wiggins 1977). This genus was collected in only one month (March 2014) in Granny Creek and only on the stick/leaf samples (Appendix B). The family Hydroptilidae, the microcaddisflies, was represented in Granny Creek by two species (*Hydroptila* sp. and *Oxytheira* sp.) that feed on filamentous algae, diatoms, and other algae (Wiggins 1977). I collected two species from the family Polycentropodidae in Granny Creek. They were *Cyrnellus fraternus*, which is the only species within the genus *Cyrnellus*, and a species of *Neureclipsis*. The Hydropsychidae, a very diverse trichopteran family (Pescador et al. 2004), was represented by the genus *Cheumatopsyche* in my samples from Granny Creek. At present individuals in this genus cannot be identified beyond the generic level (Pescador et al. 2004).

Coleoptera, Odonata, and Megaloptera

Beetles are among the largest order of insects in regard to number of insect species (Merritt et al. 2004). There were eleven species from five families present in my samples. The family Elmidae was well represented with seven species collected: *Ancyronyx variegatus*, *Dubiraphia vittata*, *Gonielmis dietrichi*, *Macronychus glabratus*, *Microcylloepus pusillus*, *Promoresia* sp., and *Stenelmis* species (Appendix B). *Stenelmis*, often described as riffle beetles, were especially common in my samples. Representatives of four other families of beetles were also present in my samples: Gyrinidae (whirligig beetles – *Dineutus* sp.), Psephenidae (water pennies – *Ectopria thoracica*), Haliplidae (*Haliplus* sp.), and Hydrochidae (*Hydrochus minumus*) (Appendix B).

Dragonflies (order Odonata, suborder Anisoptera) were occasionally present in my samples with five species represented: *Boyeria venosa*, *Cordulegaster maculatum*, *Progomphus obscurus*, *Perithemis* sp., and *Maromia illinoiensis*. Only one damselfly (order Odonata, suborder Zygoptera) species was present in my samples: *Argia fumipennis*. This species was relatively common in my warm weather samples but was absent from my November 2014, January 2015, February 2015, March 2015, and April 2015 samples. Two species in the order Megaloptera were present in my invertebrate collections. The predatory dobsonfly (hellgrammite) *Corydalus cornutus*, was fairly common in my samples. A single alderfly larva, *Sialis* sp., was collected.

Table 3

Percent similarity (PS) and Coefficient of Community (CC) for Hester-Dendy multiplates and stick/leaf samples compared to each other, by month. Sampling collection dates: Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015.

CHIRONOMIDAE

	Jun a	Jul	Aug	Sep	Oct	Nov	Jan	Mar b	Apr	Jun b
PS	0.78	0.89	0.42	0.45	0.57	0.53	0.41	0.39	0.74	0.49
CC	0.70	0.52	0.38	0.70	0.55	0.48	0.33	0.37	0.55	0.51

AQUATIC INSECTS

	Jun a	Jul	Aug	Sep	Oct	Nov	Jan	Mar b	Apr	Jun b
PS	0.28	0.25	0.04	0.54	0.20	0.49	0.52	0.74	0.29	0.19
CC	0.56	0.48	0.08	0.69	0.23	0.57	0.48	0.24	0.38	0.40

Table 4

Percent of Chironominae composition compared to total chironomid composition by species and by abundance (total number of Chironominae/total number of Chironomidae), with accompanying ranks (lowest to highest) in Granny Creek by month.

Month	% of Species	Rank by % Species	% of Abundance	Rank by % Abundance
June '14	73.2	10	71.2	10
July '14	50.0	6	40.0	5
Aug. '14	50.0	6	39.3	4
Sept. '14	41.7	4	37.1	3
Oct. '14	36.3	3	52.6	9
Nov. '14	47.1	5	45.0	6
Jan. '15	50.0	6	50.0	8
Mar. '15	26.7	1	27.0	1
Apr. '15	35.7	2	47.3	7

June ’15	54.6	9	29.9	2

Table 5

Percent of Orthocladiinae composition compared to total chironomid composition by species and by abundance (total number of Orthocladiinae/total number of Chironomidae), with accompanying ranks (lowest to highest) in Granny Creek by month.

Month	% of Species	Rank by % Species	% of Abundance	Rank by % Abundance
June ’14	18.2	2	8.5	3
July ’14	12.5	1	2.4	1
Aug. ’14	37.5	4	32.1	5
Sept. ’14	37.5	4	47.9	7
Oct. ’14	45.5	6	13.2	4
Nov. ’14	47.5	7	48.1	8
Jan. ’15	50.0	8	50.0	9
Mar. ’15	60.0	10	56.8	10

Apr. ’15	57.1	9	46.4	6
June ’15	27.3	3	7.5	2

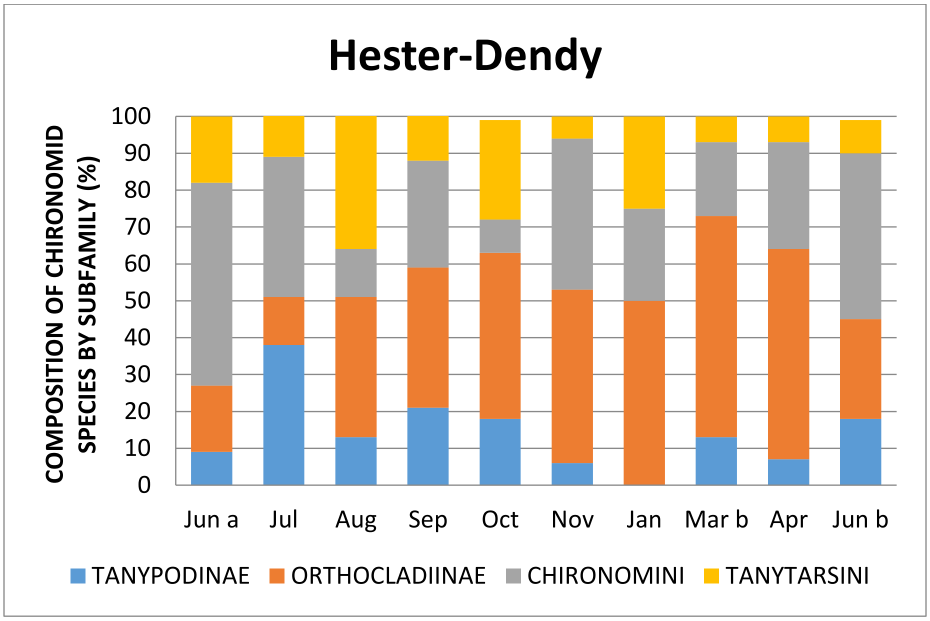

Figure 3.
Species richness composition (%) of the Chironomidae on Hester-Dendy multiplate samples from Granny Creek by subfamily (Chironominae = the Tribe Chironomini and Tribe Tanytarsini in this figure) for each sampling date. Sampling collection dates: Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015

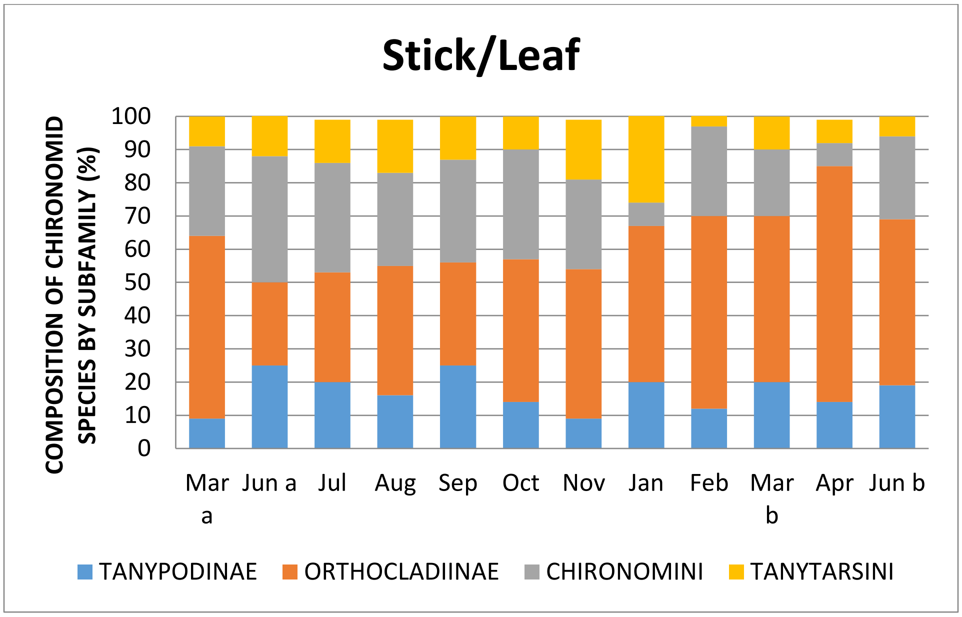

Figure 4.

Species richness composition (%) of the Chironomidae for the stick and leaf samples from Granny Creek by subfamily (Chironominae = the Tribe Chironomini and Tribe Tanytarsini in this figure) for each sampling date. Sampling collection dates: Mar a= March 31, 2014; Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Feb= February 20, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015.

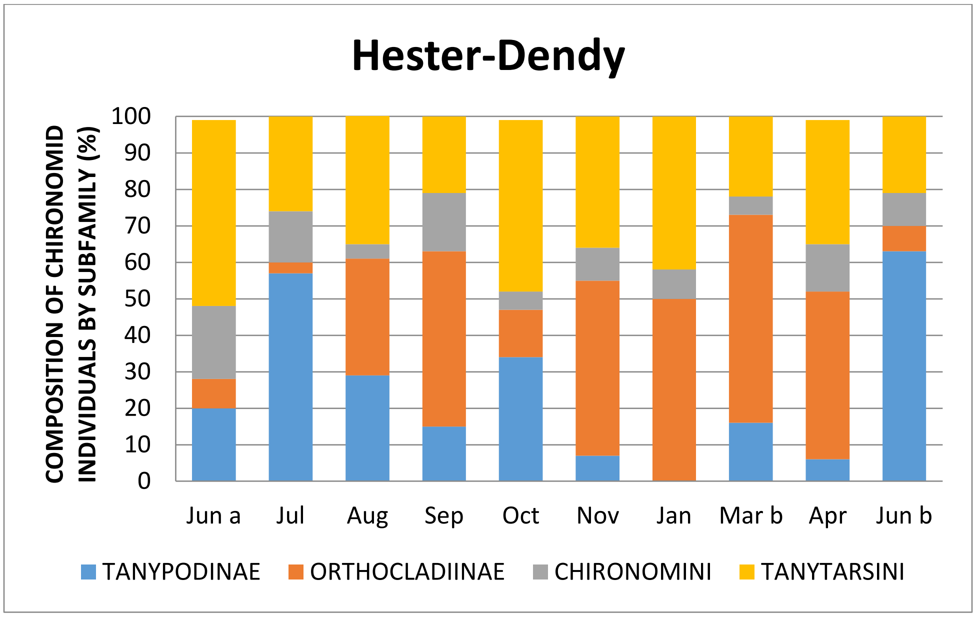

Figure 5.
Abundance composition (%) of the Chironomidae on Hester-Dendy multiplate samples from Granny Creek by subfamily (Chironominae = the Tribe Chironomini and Tribe Tanytarsini in this figure) for each sampling date. Sampling collection dates: Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015.

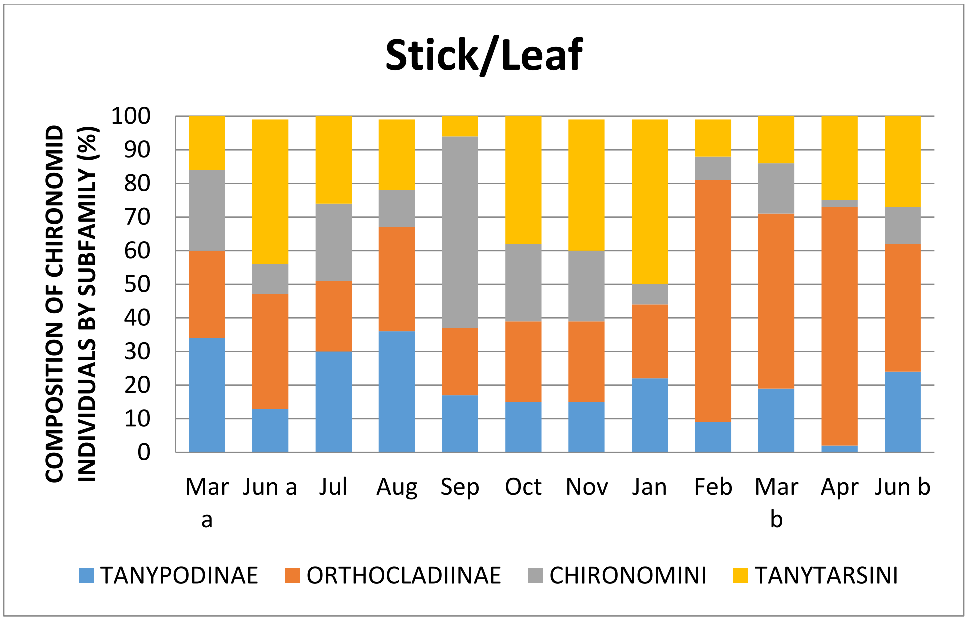

Figure 6.
Abundance composition (%) of the Chironomidae for the stick and leaf samples from Granny Creek by subfamily (Chironominae = the Tribe Chironomini and Tribe Tanytarsini for this figure) for each sampling date. Sampling collection dates: Mar a= March 31, 2014; Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Feb= February 20, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015.

CHAPTER IV – DISCUSSION

The range of water temperatures measured in Granny Creek over my year of sampling was 22.7˚C, somewhat less than the range of 29.1˚C in water temperatures reported from the Ohio River in 1979 by Beckett (1992). With its more northern latitude the Ohio River clearly demonstrates colder temperatures over the winter than a southern Mississippi stream. Of course there are obvious large differences in the two systems in term of size. The Ohio River is a very large river; it is the third largest river in North America by discharge (United States Geological Survey 1992) and has a stream order of nine. Granny Creek, in contrast, is a first-order stream.

Despite those differences, both systems possess diverse chironomid communities. Beckett's phenological survey of the chironomids of the Ohio River revealed 63 distinct taxa; my study showed 42 distinct chironomid taxa inhabit Granny Creek. It should be noted that both surveys (Ohio River and Granny Creek) focused on epibenthic invertebrates, i.e. embenthic (infaunal) collections would further increase the chironomid species richness in both systems. In a summary of stream order and chironomid species richness, Coffman (1989) proposed a mean species richness of 26 (species) and a range of 10 to 64 species for first order streams. It is apparent from my epibenthic sampling that the chironomid species richness of Granny Creek would be near the upper end of Coffman's estimate.

Despite its location within southern Mississippi near 31˚N latitude, yearly temperature variations in Granny Creek were sufficient to produce a seasonal shift in dominance in the larval chironomids collected on the multiplate samplers between the chironomid subfamilies Orthocladiinae and Chironominae. This was evident in a breakdown of species composition by subfamilies (Figure 3) and of individuals by subfamilies (Figure 5) (see also Tables 4 and 5). Overall, species in the family Orthocladiinae were more abundant in Granny Creek in the colder months and species in the family Chironominae were relatively more abundant in the warmer months. Oliver (1971) described the Chironominae as being "very abundant in the warmer parts of the Holarctic" and stated that the Chironominae "decrease in numbers with increasing latitude, or its climatological equivalent." Additionally, Oliver described the Orthocladiinae as "primarily [a] cold-adapted subfamily" that "in contrast with the Chironominae and the Tanypodinae decrease in numbers in increasingly warmer regions." Similarly, Ashe et al. (1987) indicated that the Orthocladiinae dominate in the arctic, with increasing dominance by the Chironominae as the tropics are approached. Similar patterns altitudinally and latitudinally have also been demonstrated. Lindegaard-Petersen (1972) concluded that mountain brooks were dominated by members of the Orthocladiinae (with only a small proportion of the chironomids constituted by Chironominae), with a gradual shift and increasing dominance by the Chironominae as the waters flowed into lowland areas. Ward and Williams (1986) showed a similar longitudinal shift in the Chironomidae in a river in Canada, with the Orthocladiinae dominating the cooler headwaters whereas species in the Chironominae became dominant as the river neared its mouth. These geographic, altitudinal, and longitudinal shifts in subfamily dominance are mirrored in single locations (Granny Creek or the Ohio River) as a consequence of changing larval chironomid composition as a function of season. As concluded by Beckett (1992) (for the Ohio River chironomid community), the chironomids of Granny Creek have used the variation in temperature over the year, "with each species selecting the appropriate season and temperature for its development."

Perhaps because I did not collect as many chironomid larvae per sampling occasion in Granny Creek as Beckett did in his Ohio River study, or perhaps because the cold weather conditions in Granny Creek were not as marked or extended as in the Ohio River, I did not observe as many clear patterns where individual Orthocladiinae species dominated my colder weather collections on multiplate samplers and then were completely absent from the collections in the warm months. An exception was *Parametriocnemus* nr. *lundbecki* which was present on my multiplate samplers in September, October, and November of 2014 and March and April of 2015, but absent from those samplers in the summer months of June, July, and August 2014, and June 2015. Similarly, Beckett (1992) found the same (or a very similar species) on his Ohio River samplers in 1979 in January through May; they were absent from the June, July, and August samples, and then were present in his October, November, and December collections. Such a pattern suggests a period of inactivity as larvae in the summer months. Hudson et al. (1990) commented that larval *P. lundbecki* "may be the most common chironomid found in clear streams of the piedmont and mountains." The piedmont abuts the eastern side of the Appalachian Mountains and this reference and evidence from Granny Creek and the Ohio River clearly indicate that this species is active as a larva in cooler conditions. Hudson (1971) found that another Orthocladiinae species (*Hydrobaenus pilipes*) estivates as a second instar larva after forming a canopy-like structure around themselves. Kondo (1996) found that in Japan another species of *Hydrobaenus* (*Hyrobanus kondoi*) builds a cocoon and estivates as second instar larvae during the warm summer months. From my data from Granny Creek and the similar pattern exhibited in the Ohio River it seems possible that other chironomid genera (other than *Hydrobaenus*) employ some life-history strategy whereby they are inactive for a portion of the year,even in permanent habitats such as perennial streams and rivers.

The chironomid species that were present on the multiplate samplers in Granny Creek over the entire year (collected nearly every month), *Rheocricotopus robacki*, *Cricotopus bicinctus*, and *Polypedilum convictum*, demonstrated the same pattern in the Ohio River (Beckett 1992). Also, in a European study Lindegaaard-Petersen (1972) showed that *P*. *convictum* was present in almost all the monthly samples from a Danish stream. Despite the size and latitudinal differences between Granny Creek and the Ohio River (and in the case of *P*. *convictum* that the data are from two continents), it is evident that larval members of these species are active throughout the year.

The collection of the stick/leaf samples added another dimension to my collection of the epibenthic fauna from Granny Creek. The chironomid species *Xylotopus par* was collected only in my stick/leaf samples (not on the multiplate samplers even though Masonite is a wood material). Epler (2001) has described *X*. *par* as a miner in submerged, partially decomposed wood, and hence sticks that had been in the water for a potentially long time were probably a more desirable substrate for these larvae than hardwood plate submerged for only a month. However, the primary dimension added by the stick/leaf samples had to do with the dimension of time rather than composition. Colonization of either the multiplate samplers or the stick/leaf samples could come from drifting invertebrates or by adult female insects laying eggs on the samples which eventually developed into the insect I collected. However, because the multiplate samplers were in place for generally only a month it would appear more likely that these samplers were colonized by active drifting insects. On the other hand, the stick/leaf samples may have been in place in Granny Creek for an extended period before I used them for collecting my invertebrate samples. Hence they may have been colonized by active movement such as drift, but may also contain insects in an inactive state. Even though Beckett (1992) did not collect several Orthocladiinae species as larvae from the Ohio River in the summer, he also pointed out that these species were still present in the river, but were probably estivating as eggs or early instar larvae. Because they were inactive they did not enter the drift and did not colonize his samplers. The chironomid species *P.* nr. *lundbecki* provided a good example of this concept. As pointed out earlier, *P*. nr. *lundbecki* was present on my multiplate samplers in September, October, and November of 2014 and March and April of 2015, but absent from those samplers in the summer months of June, July, and August 2014, and June 2015. Similarly, Beckett (1992) found the same species on his Ohio River samplers in January through May, they were absent from the June, July, and August samples, and then occurred again on his samplers as the river cooled. However, I did collect this species in my stick/leaf samples from Granny Creek in July 2014 and in June 2015, even though they were not on the multiplate samplers on the same dates. It seems likely that the *P.* nr. *lundbecki* I collected in the summer on my stick/leaf samples were larvae that were inactive at that time, but still present in the system. The difference in colonization "history" may explain why the monthly comparisons of my multiplate samples and stick/leaf samples showed such variability (see Table 3).

The "winter stoneflies" belong to the families Capniidae and Taeniopterygidae. Although Stewart and Stark (2002) list some winter stonefly species (such as

Taeniopteryx burksi and *Strophopteryx fasciata*) as occurring in Mississippi, all the stoneflies I collected from Granny Creek were members of the family Perlidae; therefore no winter stoneflies were present in my samples. The Perlidae is the most diverse family of stoneflies in Florida (Pescador et al. 2000) and all of the species I collected are also found in Florida. *Acroneuria arenosa* was the most abundant stonefly species in my samples; Pescador et al. (2000) remarked that *Acroneuria* species are commonly encountered in Florida. *Eccoptura xanthans*, which I also collected in Granny Creek, has an interesting distribution in terms of its habitat. It is quite common in headwater streams of the southern Appalachians, but is uncommon in Florida where it appears to be fairly specific for small seepage-fed streams which flow through mixed mesophytic forests (Pescador et al. 2000).

I was particularly interested in the collection of *Perlesta placida* from Granny Creek. Beckett (1987) collected this species as nymphs from the Ohio River only in May and June and suggested that the nymphs had a very rapid development over those two months, with probable emergence in June followed by a lengthy inactive period. In an intermittent stream in Texas Snellen and Stewart (1979) found that this species emerged mostly in June and produced eggs, which then went into an extended diapause of five to six months [note: in a 1989 paper Stark amended the identification of *P. placida* to *Perlesta decipiens* for the species studied by Snellen and Stewart in the intermittent Texas stream]. In contrast, Pescador et al. (2000) stated that these nymphs are collected throughout the year in Florida. In Granny Creek I collected *P. placida* in March and June of 2014, but they did not reoccur in my samples until the following spring (February, March, and April), indicating an extended period of inactivity as part of their life cycle in Granny Creek (see Appendix B).

Four of the six mayfly (= order Ephemeroptera) taxa present in my Granny Creek collections were relatively common in my samples: *C. triangulifer*, M. *smithate*, *T. albalineatus*, and *P. volitans.* The two species of *Maccaffertium* in my samples (*M. exiguum* and *M. smithae*) are commonly found in Florida (Pescador and Richard 2004).

The two most abundant caddisflies in my samples were *Cyrnellus fraternus* and *Neureclipsis* sp., both members of the family Polycentropodidae (Appendix B). Wiggins (1977) remarked that *C. fraternus* has a wide habitat tolerance, with a preference for large rivers, but stated that it also occurs in small streams and even in lakes and reservoirs. This species is common in the Ohio River (Beckett, personal communication), but as my data show, *C. fraternus* was also common throughout the year on my samples from Granny Creek. *Neureclipsis* sp. was also common in both my multiplate and stick/leaf collections. Species of this genus construct a silken trumpet-shaped net which the larvae use to filter food particles suspended in lotic systems.

The assemblage of beetle in Granny Creek was quite diverse, especially as represented by the family Elmidae. Nine genera of elmids have been collected from Florida (Epler 2010), seven of which had representatives in Granny Creek. Among those seven beetle taxa, the genus *Stenelmis* was particularly abundant and was present in every month of my collections.

All of the stonefly taxa I collected from Granny Creek are predators, feeding on benthic invertebrates (Stewart and Stark 2002), although early instars of *Perlesta* may feed largely on detritus (Snellen and Stewart (1979). Additional invertebrate predators in

Granny Creek include the five dragonfly taxa I collected, the damselfly *Argia fumipennis*, and the megalopterans *Sialis* sp. and *Corydalus cornutus*.

APPENDIX A -

. Chironomid numbers for the Hester-Dendy multiplate and stick/leaf samples in Granny Creek for 12 sampling dates from 2014 to 2015 (HD= Hester-Dendy multiplates samples and S/L= Stick and Leaf samples). Sampling collection dates: Mar a= March 31, 2014; Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Feb= February 20, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015

	Mara	Juna		Jul		Aug		Sep		Oct	
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
TANYPODINAE											
Ablabesmyia mallachi				5	10		3	4	6	4	3
A. rhamphe			1	1	1		1	1	1		
Nilotanypus sp.								8	1		1
Pentaneura inconspicua								1			
Procladius bellus											
Thienemannimyia sp. gr.	17	12	3	14	9	8	29	11	9	9	7
ORTHOCLADINAE											
Corynoneura celeripes					1		1	7			1
C. lobata	1	3	8					9		1	5
Cricotopus bicinctus	4	2	3				17	6	4	1	2
Cricotopus /Orthocladius sp.							1	2			

	Mara		Juna		Jul		Aug		Sep		Oct
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
Parametriocnemus nr. *lundbecki*	1				2			6	1	1	2
Psectrocladius psilopterus gr.							1		5		2
P. octomaculatus								8	1		
P. sordidellus gr.						2					1
Rheocricotopus robacki	4			1	2	3	1	10		1	3
Synorthocladius semivirens	1				8		6	31	8		3
Thienemanniella lobapodema						4		1		1	
T. Xena											
Tvetenia discoloripes	2						2				
Unniella multivirga											
Xylotopus par					1						1
CHIRONOMINAE											
Chironomini											
Crytochironomus sp.							2	3			
Crytotendipes sp.									1		
Dicrotendipes modestus		1	1		2						1
D. neomodestus		1					2	8	49		7
Microtendipes pedellus											1
M. ryclalensis	1	1		1							3
Paralauterborniella sp.							1				
Phaenopsectra puctipes gr.											
Polypedilum convictum	10	4	1	2	4	1	4	10	5	2	5

	Mara		Juna		Jul		Aug		Sep		Oct
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
P. fallax	1	3		2				2			
P. illinoense											
P. scalaenum		2	1		1		1	1			1
Pseudochironomous sp.											
Stenochironomus sp.					3			1	1		1
Tribelos jucundus					5			2	1		
T. Fuciorne											
Tanytarsini											
Cladotanytarsus diaviesi		2			1	3	1			7	
Rheotanytarsus sp.						3		12	1	3	14
Stempellinella sp.							8	2			
Tanytarsus sp. gr.	8	28	14	9	16	4	10	21	5	8	18
Total	50	59	32	35	66	28	91	167	100	38	84

	Nov		Jan		Feb	Marb		Apr		Junb	
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
TANYPODINAE											
Ablabesmyia mallachi					3						
A. rhamphe				2			1		1	1	1
Nilotanypus sp.					7	2					
Pentaneura inconspicua											
Procladius bellus				1					1		3
Thienemannimyia sp. gr.	9	5		10	12	10	4	7		41	20
ORTHOCLADINAE											
Corynoneura celeripes	1			1	6	2	2		1		1
C. lobata	7	4	1	5	11	4	9				
Cricotopus bicinctus			7	1	56	16		20	34	1	3
Cricotopus /Orthocladius sp.				1	1		1	6	7		2
Parakiefferiella					1						
Parametriocnemus nr. *lundbecki*	4			3	14	7		6	1		3
Psectrocladius psilopterus gr.		1			1	1		5	8	2	6
P. octomaculatus	8				2	4	1	3		2	
P. sordidellus gr.				1							1
Rheocricotopus robacki	7				18	1		2	1		4
Synorthocladius semivirens	25	1	1		36	6		9	11		10
Thienemanniella lobapodema	9	1	3		12		1	1	1		
T. Xena	1	1			9						

		Nov		Jan	Feb		Marb		Apr		Junb
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
Unniella multivirga				1	1				1		
Xylotopus par					1				1		
CHIRONOMINAE											
Chironomini											
Crytochironomus sp.										2	
Crytotendipes sp.	1										
Dicrotendipes modestus	1		1								1
D. neomodestus	2				3	2		10		1	4
Microtendipes pedellus			1								
M. ryclalensis	2				1						
Paralauterborniella sp.											
Phaenopsectra puctipes gr.	1										
Polypedilum convictum	1	3		4	7	1	3	2	2		5
P. fallax	3				4	1		2		1	
P. illinoense											1
P. scalaenum		3			2		1	1		1	
Pseudochironomous sp.					1						
Stenochironomus sp.											
Tribelos jucundus		1									
T. Fuciorne					1					1	
Tanytarsini											
Cladotanytarsus diaviesi				1							
Rheotanytarsus sp.		2	4	6							

		Nov		Jan	Feb		Marb		Apr		Junb
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
Stempellinella sp.				5							
Tanytarsus sp. gr.	47	11	6	17	25	16	4	38	23	14	28
Total	129	33	24	59	236	74	27	112	93	67	102

APPENDIX B –

Aquatic insect order numbers (excluding family Chironomidae) for the Hester-Dendy multiplate and stick/leaf samples in Granny Creek for 12 sampling dates from 2014 to 2015 (HD= Hester-Dendy multiplates samples and S/L= Stick and Leaf samples). Sampling collection dates: Mar a= March 31, 2014; Jun a= June 18; 2014; Jul= July 27; 2014; Aug= August 25, 2014; Sep= September 27, 2014; Oct= October 29, 2014; Nov= November 29, 2014; Jan= January 19, 2015; Feb= February 20, 2015; Mar b= March 20, 2015, Apr= April 26, 2015; Jun b= June 17, 2015

	Mara	Juna		Jul		Aug		Sep		Oct	
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
EPHEMEROPTERA											
Baetidae											
Centroptilum triangulifer		3			14	4		13	15	8	3
Heptagenniidae											
Maccaffertium exiguum	1	1		1		2					
M. smithae	1	9	2	9		3		9	15	8	
Maccaffertium sp.											
Leptohyphidae											
Tricorythodes albalineatus		1	1		6	4		3	19		1
Leptopllebiidae											
Paraleptophebia_volitans	3				1	4	5	16	3		1
Isonychidae											

	Mara		Juna		Jul		Aug		Sep		Oct
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
Isonychia sp.	1		1					1			
ODANATA											
Anisoptera											
Aeshindae											
Boyeria venosa					2	2					
Cordulegastridae											
Cordulegaster maculata							1				
Gomphidae											
Progomphus obscurus					1			1			
Libellulidae											
Perithemis sp.											
Macromiidae											
Macromia illinoiensis											1
Zygoptera											
Coenagrionidae											
Argia fumipennis		1		2		1		4	1		1
PLECOPTERA											
Perlidae											
Acroneuria abnormis		1				1			1	1	
A. Arenosa	1	1	3	2	1	2		5		2	1
Agnetina annulipes			1								
Eccoptura xanthans											
Neoperla carsolni	1	2			2	1					

	Mara		Juna		Jul		Aug		Sep		Oct
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
Paragnetina fumosa	6	1	1								
Perlesta placida	11	1	2								
TRICHOPTERA											
Hydropsychidae											
Cheumatopsche sp.	1					4		1	1		
Hydroptilidae											
Hydroptila sp.			1					4	5		1
Oxytheira sp.						1		2	7		2
Philoptamidae											
Chimarra sp.	4										
Polycentropodidae											
Cyrnellus fraternus		2		1	4	10		9		1	4
Neureclipsis sp.				7	5	15					2
MEGALOPTERA											
Corydalidae											
Corydalus sp.		1	3			3		6			
Sialidae											
Sialis sp.		1									
COLEOPTERA											
Elmidae											
Ancyronyx variegatus				5	2	1		3	5		
Dubiraphia vittata		2		1		10		3		1	
Gonielmis dietrichi			2	1		1			2		

	Mara		Juna		Jul		Aug		Sep		Oct
Species	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L	HD	S/L
Macronychus glabratus	1	4	4			1		3	2	1	
Microcylloepus pusillus			2		2	21			1		
Promoresia sp.	1										
Stenelmis sp.	3	22	1		7	7		15	11		5
Gyrinidae											
Dineutus sp.			1							2	
Psephenidae											
Ectopria thoracica									1		3
Haliplidae											
Haliplus sp.											
Hydrochidae											
Hydrochus minimus		1									
DIPTERA											
Ceratapogonidae											
Palpomyia sp.		4	1	1	1				3		
Simuliidae											
Simulium sp.	2	1							1		
Total	44	61	29	37	50	101	5	100	93	26	25

	Nov		Jan		Feb	Marb		Apr		Junb	
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
EPHEMEROPTERA											
Baetidae											
Centroptilum triangulifer	4	8	13	8	4			5	2		8
Heptagenniidae											
Maccaffertium exiguum			3								
M. smithae	19	7			1	5					
Maccaffertium sp.			3		2	2					1
Leptohyphidae											
Tricorythodes albalineatus			3	1	2	1			1		1
Leptopllebiidae											
Paraleptophebia volitans	2		6	1	1			4	2		1
Isonychidae											
Isonychia sp.											
ODANATA											
Anisoptera											
Aeshindae											
Boyeria venosa	2										
Cordulegastridae											
Cordulegaster maculate											

		Nov		Jan	Feb		Marb		Apr		Junb
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
Gomphidae											
Progomphus obscurus					1	1		1			
Libellulidae											
Perithemis sp.								1			
Macromiidae											
Macromia illinoiensis								2			
Zygoptera											
Coenagrionidae											
Argia fumipennis										1	
PLECOPTERA											
Perlidae											
Acroneuria abnormis			1			1					
A. Arenosa	3		3					2			
Agnetina annulipes								1			
Eccoptura xanthans			2			1		1			
Neoperla carsolni	1		1			2		2		2	1
Paragnetina fumosa	1		2								
Perlesta placida					1	2		1			
TRICHOPTERA											
Hydropsychidae											
Cheumatopsche sp.	1		3								1
Hydroptilidae											
Hydroptila sp.		2	5			1					3

		Nov		Jan	Feb		Marb		Apr		Junb
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
Oxytheira sp.	2	4	9								
Philoptamidae											
Chimarra sp.											
Polycentropodidae											
Cyrnellus fraternus	11	3	9	2	3	1		1	2	1	5
Neureclipsis sp.	3	7	4		3	1			2	5	1
MEGALOPTERA											
Corydalidae											
Corydalus sp.	4		2					2			
Sialidae											
Sialis sp.											
COLEOPTERA											
Elmidae											
Ancyronyx variegatus	4	2	8		1	1			1		2
Dubiraphia vittata				2				1	1		1
Gonielmis dietrichi		8	3						1		
Macronychus glabratus	1	2	3			1			1		
Microcylloepus pusillus									1		
Promoresia sp.											
Stenelmis sp.	3	6	15	2	4	7	2	6			4
Gyrinidae											
Dineutus sp.			1								1
Psephenidae											

Species		Nov		Jan	Feb		Marb		Apr		Junb
Species	HD	S/L	HD	S/L	S/L	HD	S/L	HD	S/L	HD	S/L
Ectopria thoracica		1	2		2						
Haliplidae											
Haliplus sp.		2									
Hydrochidae											
Hydrochus minimus											
Diptera											
Ceratapogonidae											
Palpomyia sp.			5	1				1	1	2	1
Simuliidae											
Simulium sp.				2	2	2	2				2
Total	66	52	106	21	28	29	4	31	15	11	33

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