# EVALUATING MACROINVERTEBRATE DIVERSITY IN POND COMMUNITIES: A COMPARISON OF TWO SAMPLING TECHNIQUES

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# Evaluating Macroinvertebrate Diversity in Pond Communities:

# A Comparison of Two Sampling Techniques

# A Thesis

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Master of Science

Laura Ashlie Farmer

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### DEDICATION

To my sweet little boy Cash, whose beautiful smiling face made all the long days and late nights worth it. May you forever marvel at the beauty of the natural world and always carry with you the curiosity and gentleness I see today. You can accomplish anything by questioning, listening, working hard, being kind, determined and never ever giving up on yourself! My every step in this world is to make it better for you. You are my greatest accomplishment. May the good Lord be with you down every road you roam.

To my husband Robert, whose love and support has never faltered. I am forever grateful to you for standing by me and allowing me to accomplish my dream. You are a wonderful father and a fine man. I love you dearly and intend on doing everything in my power to make you happy for the rest of our lives.

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### Abstract

LAURA ASHLIE FARMER. Evaluating Macroinvertebrate Diversity in Pond Communities: A Comparison of Two Sampling Techniques (under the direction of STEVEN W. HAMILTON).

Milan Army Ammunition Plant (MLAAP), located in Gibson and Carroll counties, Tennessee, is a munitions production and storage facility comprising 90.48 km<sup>2</sup> of upland hardwood forest, interspersed with agricultural fields. In 1987, MLAAP was placed on the Environmental Protection Agency's National Priority List for groundwater contamination. Due to this environmental status, MLAAP has established an Integrated Natural Resources Management Plan, coupling organismal research and land use practices to develop long-term sustainability of natural resources. Macroinvertebrates, often used in water quality monitoring, are among the taxa being studied. In June, 2009, a research project was begun to compare the efficiency of two sampling techniques in inventorying macroinvertebrate diversity within pond communities at MLAAP. Funneltrap and dip net sampling methods were employed in 10 ponds. Four funnel-traps were set in each pond for two consecutive 48-hour periods during June 18-22 and December 8-12, 2009 and April 18-22, 2010. Dip net samples were collected on June 29 and November 11, 2009 and April 2, 2010 with two collectors sampling simultaneously for 30 minutes in each pond. A total of 10,082 individuals comprising 146 unique taxa were identified. Statistical analysis comparing sampling methods showed significant differences in taxa richness within cattle ponds. The differences between sampling

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methods for Shannon-Weaver values were significant among all ponds. A significant difference between sampling methods for Shannon-Weaver Index values was also found within non-cattle ponds as well as for the summer sampling season. Jaccard's Similarity Coefficient values were generally low (mean = 0.2896, range = 0.125 - 0.576), signifying both methods collected very different sets of taxa. Differences between Jaccard's Similarity Coefficient values were significant among cattle and non-cattle ponds and for the winter sampling season. Evaluation of sampling methods regarding addition of new taxa indicated the dip net method more effectively added new taxa in the orders Coleoptera, Odonata and Hemiptera, while the funnel-traps were more successful adding to the order Diptera. These results can be attributed to the mobility of the dip nets, versus the funnel- traps, which are dependent on invertebrate movement. Taxa accumulation curves indicate a combination of sampling methods would be the best strategy for assessing the biodiversity of pond habitats. Due to sampling method constraints and habitat limitations to sampling effort, the choice of sampling technique should be based on habitat structure. Timed-effort sampling cannot be standardized if habitat complexity is not taken into consideration and passive sampling alone will not produce accurate community diversity data.

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### **CHAPTER I**

### INTRODUCTION

# **History of Pollution**

The idea of Earth having an infinite supply of fresh air and water is one most people have embraced throughout history. The Industrial Revolution marked the onset of major technological advances and industrial growth (Rosenberg and Resh, 1993; Glasby, 1988; Grove, 1975). By the turn of the 20<sup>th</sup> century, the population was expanding at an alarming rate and the modern conveniences of cars and electricity became widely available. Unfortunately, along with this growth and advancement came a heavy reliance on natural resources for electricity, transportation and fertilizer (Grove, 1979). Soon smokestack emissions and automobile exhausts clouded the atmosphere with excessive amounts of gases and chemicals. Non-point source pollution from farms and cities and point-source discharges from municipalities and industries were released into rivers, streams, lakes and ponds without concern. The notion of natural resources being limited and the potential disastrous environmental effects this pollution could cause was not recognized (Grove, 1975). Over time, pollutants have negatively impacted human health and the environment. Polluted or "low quality" air has been proven to be detrimental to body function and has led to chronic disease (Stead and McGauhey, 1968). Water pollution from various residential and commercial sources has severely restricted both essential and recreational water use (Luzio, 1967). It wasn't until the passage of major

environmental policies such as the Clean Air Act and Clean Water Act of the 1970's and 1980's, and the National Environmental Policy Act of 1969, that the United States began to make progress in addressing environmental concerns (Kotas, 1997).

Before the existence of the environmental policies mentioned, there were individuals aware of the delicate state of the environment and the need to protect natural resources. These early activists, realizing the importance of natural areas and recognizing potential environmental quandaries, greatly contributed to preservation efforts and knowledge. In the 1800's, John Muir successfully petitioned the U.S. Congress for the National Park Bill establishing both Yosemite and Sequoia National Parks (Fox, 1985). He also co-founded the Sierra Club which is now one of the leading conservation organizations in the United States (Fox, 1985). Theodore Roosevelt, the 26th President of the United States has been hailed as an "environmental hero" and "wilderness warrior" (Peterson, 1994; Brinkley, 2009). During his presidency he passed the Antiquities Act of 1906, created the United States Forestry Service and National Park Service and established various wildlife refuges (Brinkley, 2009). He was ultimately responsible for preserving 234 million acres of American wilderness for future generations (Brinkley, 2009). Rachel Carson, an ecologist and writer, actively spoke out on the apparent harmful effects of pollution on human health and the environment. One of her many books, "Silent Spring" published in 1962, described the dangers of the pesticide DDT (dichlorodiphenyltrichloroethane) and "deliberately challenged the wisdom of a government that allowed toxic chemicals to be put into the environment before knowing the long-term consequences of their use" (Carson et al., 2002). Carson (1962) asked, "Can anyone believe it is possible to lay down such a barrage of poisons on the surface of the earth without making it unfit for all life?" Thought provoking statements and challenging questions such as these enabled her to become one of the most influential advocates for environmental awareness and conservation.

The conservation and public awareness efforts of these individuals and many others paved the way for modern environmental and natural resource management practices and set in motion the environmental movement still alive today (Kuzmiak, 1991). However, even with the personal dedication and preservation efforts of the aforementioned people and others, there was still a need for a structured system which could monitor and correct negative environmental issues on a broader scale. This need led to one of the most important and influential environmental conservation events in history, the establishment of the Environmental Protection Agency (EPA). The EPA was founded in 1970 under the Nixon administration and was "established to consolidate in one agency, a variety of federal research, monitoring, standard-setting and enforcement activities to ensure environmental protection" (Curtis, 2005). The EPA, as a single government agency, was to be responsible for all possible pollution issues regarding air, water and land.

Several early environmental protection laws such as the Federal Water Pollution Control Act of 1948 and Air Pollution Control Act of 1955, including various amendments to both, were already in place prior to the establishment of the EPA (Stein et al, 1971; Schnelle and Brown, 2002). However, under the management of the EPA these laws were strengthened, enforced and revised to collectively become known as the Clean Water Act (CWA) and Clean Air Act (CAA) (Portney and Stavins, 2000). Revisions were designed to protect, monitor, and control various air and water pollutants through

the establishment of standards (Portney and Stavins, 2000). Another important environmental effort was the passing of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, commonly called the "Superfund Law." This law established a National Priority List for contaminated waste sites, financed the cleanup and held firms retroactively liable for past pollution (Hird, 1993). There are approximately 1300 Superfund sites still active in the United States today (Johnson, 1995). The contaminants of these areas affect multiple environmental elements such as soil, groundwater and air, and are problematic to the flora and fauna of the affected area. As science advanced and more sophisticated ecological applications were employed, scientists determined that the animal and plant life of an area could be used as indicators to monitor the current and long-term health of an environment (Niemi and McDonald, 2004).

# **Aquatic Habitats and the Importance of Ponds**

Anthropogenic influences have negatively impacted aquatic habitats, particularly small ponds and wetlands (Becerra-Jurado et al., 2008). These small aquatic habitats can provide habitat, food, breeding ground, and protection for a large array of aquatic life. Many faunal inhabitants such as amphibians, fish, and macroinvertebrates play a vital role in aquatic environments. Aquatic macroinvertebrates, for example, comprise a wide range of trophic levels and are integral in the cycling of nutrients and rate of decomposition within aquatic habitats (Merritt et al., 2008; Roni, 2005). These small organisms are ubiquitous within freshwater habitats and represent a primary source of

food for a variety of fish and other vertebrates (Rosenberg and Resh, 1993; Merritt et al., 2008). Aquatic macroinvertebrates, being in constant contact with water, are quite vulnerable to anthropogenic impacts such as pollutants, sedimentation, and riparian removal (Merritt et al., 2008). These living organisms can, therefore, provide information regarding changes in the environment, often caused by human impact.

Large freshwater bodies such as lakes and rivers are often given more attention with regards to protection, due to the vast commercial and recreational use by humans and government regulation of specific sites. Under the Clean Water Act, most aquatic habitats involved in interstate commerce are considered "waters of the state," therefore, public property of the state in which a particular water body flows through (40 CFR 230.3(s)). The State of Tennessee defines "waters of the state" as follows:

Any and all water, public or private, on or beneath the surface of the ground, which is contained within, flows through or borders on Tennessee or any portion thereof except those bodies of water confined to and retained within the limits of private property in single ownership which do not combine or effect a junction with natural surface or underground waters (TCA Section 69-3-103(33)).

Smaller freshwater habitats such as ponds, often stand alone and lack a direct connection to flowing waters; therefore, most states do not consider them "waters of the state" and do not offer the protection or regulatory monitoring afforded to other aquatic habitats (40 CFR 230.3(s)).

The term "pond" has been variously defined as permanent or seasonal, man-made and natural water bodies between 25 m² and 2 hectares in area with a depth of no more

than 8 m, which will allow plants to colonize the entire area (Oertli et al., 2000; Biggs et al., 2005). Ponds are quite numerous and are found throughout the world (Meester, 2005). The size range and ubiquitous nature of ponds imply that these aquatic habitats play a very important role in the global carbon cycle (Oertli et al., 2009). It has even been suggested that these small ecosystems may trap more carbon than the world's oceans (Downing et al., 2008). Ponds often provide habitat for rare or unique species and therefore contribute to overall biodiversity (Nicolet et al., 2004; Oertli et al., 2002; Williams et al., 2004).

Ponds are smaller in size with less water depth, which can allow for more plant growth or drying periods, and often ponds have more shading which allows more organic matter to enter the system (Declerck, et al., 2006). These conditions allow for a variety of research opportunities in an underrepresented ecosystem. Ponds can serve as models for hypothesis testing, be used as early warning systems for change on a global scale and their small size makes them easy to sample repeatedly offering great potential for field surveys (Oertli, 2008; Meester et al., 2005).

# **Biomonitoring: An Important Ecological Tool**

Aquatic macroinvertebrates are small invertebrate organisms that can be seen with the naked eye and live at least part of their lives in freshwater habitats (Rosenberg and Resh, 1993; Watson-Ferguson, 2006). Sampling macroinvertebrates and evaluating their community diversity can be used to measure water quality. Because macroinvertebrates

are long-lived, diverse and sedentary in nature, they are the target organisms for biological monitoring, or biomonitoring, in aquatic habitats (Rosenberg and Resh, 1993). Biomonitoring is defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program" (Mathews et al., 1982).

Most state and government agencies use macroinvertebrates as biomonitoring tools to assess the quality of water within lotic habitats only, specifically rivers and streams (Rosenberg and Resh, 1993). These habitat types are considered "waters of the state" and are, therefore, provided regulatory monitoring on a continuous basis (T.C.A. 69-3-101). Furthermore, sampling protocols and methods for data analysis are developed for use in lotic habitats (Rosenberg and Resh, 1993). Because ponds typically have characteristics that disqualify them as "waters of the state," very few biomonitoring studies are conducted within these environments.

While a great deal of information exists for lotic habitats, little environmental data exists for lentic habitats. The bias toward flowing waters is most certainly due to the vast commercial and recreational use of these habitats by humans, as well as the availability of funding for monitoring studies due to their inclusion as "waters of the state". Despite the generally small size of ponds, these habitats are biologically diverse and are in dire need of more research to assess their structure and ecological function. Recent studies, mostly within Europe, have begun to shed light on the ecological importance of pond habitats; as a result, ponds within some Mediterranean countries have been identified as conservation priorities, garnering preservation action (Cereghino et al., 2008).

Biomonitoring in ponds is crucial to understand and evaluate these valuable habitats. Standardizing sampling methods and data analysis to fit a lentic environment without relying on methods previously formulated for use in lotic habitats is necessary. Fortunately, organisms such as macroinvertebrates and fish used for biomonitoring within lotic habitats are also present in lentic environments and can be used to assess and evaluate overall pollution and physical impacts within pond habitats, as well as biodiversity (Oertli et al., 2005).

# Study Objective and Goals

The objective of this project was to conduct a comparison study of two macroinvertebrate sampling techniques and analyze the efficiency of each in assessing and inventorying the macroinvertebrate communities in ponds on the Milan Army Ammunition Plant (MLAAP). A goal of this project was to determine if there was a significant difference in the apparent macroinvertebrate diversity in ponds when utilizing the two sampling techniques. Thus, the null hypothesis of no significant difference in macroinvertebrate diversity based on data from the two sampling techniques was tested. For this study, the success of horizontal funnel traps was compared to timed-effort dipnet sampling.

Macroinvertebrate diversity studies within ponds could provide much needed information about the current water quality on MLAAP and provide data on an often overlooked ecosystem. Interest has grown in using various metrics to assess lentic habitat health, especially in wetlands (Oertli et al., 2005). This study will contribute baseline data

and provide insight into which commonly used sampling method is more effective and efficient when used to assess macroinvertebrate diversity within ponds. "Choice of sampling device is a critical aspect of study design and investigators must keep in mind that although cost, availability, and other logistical factors are important, sampler accuracy must be the primary consideration" (Rosenberg and Resh, 1993). In addition, data from this study could facilitate the development of lentic environment sampling protocols and contribute information regarding the condition and distribution of specific taxa. Furthermore, given the integration of research in decisions related to the management of natural resources on MLAAP, the findings of this study will help their managers improve or refine the existing Integrated Natural Resource Management Plan (Stephenson and Kennedy, 2008).

### **CHAPTER II**

### STUDY AREA

### **Location and Size**

The Milan Army Ammunition Plant is located in Western Tennessee within Gibson and Carroll counties (longitude 88" 50' W, latitude 35" 45' N; Fig. 1). It is a federally-owned, contractor-operated active munitions facility which produces, loads and stores containerized conventional ammunition (USEPA, 2000A). Including manufacturing facilities, the area comprises 90.48 km² (22,357 acres) of hardwood forest interspersed with agricultural crop and pasture fields and a small area of bottomland hardwood forest and wetlands (Fig. 2) (Malcolm Pirnie, Inc, 2010). In addition, extensive hunting and fishing also occur within the arsenal area. MLAAP's northwestern boundary is shared with the city of Milan and the University of Tennessee Agricultural Experiment Station. Most of the eastern boundary and a small portion of the northern, southern, and western boundaries are shared with the Tennessee National Guard (Stephenson and Kennedy, 2008).

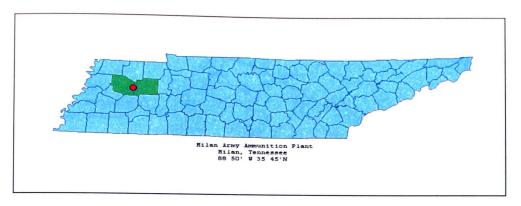


Figure 1. Tennessee county map highlighting Carroll and Gibson counties (in green) as well as location of the Milan Army Ammunition Plant (in red). (Map courtesy of Jerrod Manning, 2010).

## Physiogeography, Topography, Soils, and Geology

MLAAP lies within the Coastal Plain Physiographic Province of the Mississippi Embayment (Malcolm Pirnie, Inc, 2010; USEPA, 1998; USEPA, 2000A; USEPA, 2000B). This area is included in the Mississippi Valley Loess Plains ecoregion (Griffith et al., 1997). The general topography consists of slight rolling hills and intermittent streams with an elevation range of approximately 590 feet above mean sea level (ft-msl) at the southern boundary, to approximately 320 ft-msl at the northern boundary (USEPA, 1998; USEPA, 2000A; USEPA, 2000B).

The soils at MLAAP consist primarily of reddish-brown mottled clay which includes Memphis, Loring, Grenada, Calloway, Henry, Falaya, and Waverly soil types (USEPA, 1998; USEPA, 2000A; USEPA, 2000B). Sediments in the area ranging from the Cretaceous to the Anthropocene are distributed throughout MLAAP and consist of gravel, sand, clay, lignite, chalk, and limestone in units of varying thicknesses (USEPA, 1998; USEPA, 2000A; USEPA, 2000B).

# MAJOR SURFACE DRAINAGE AND PONDS

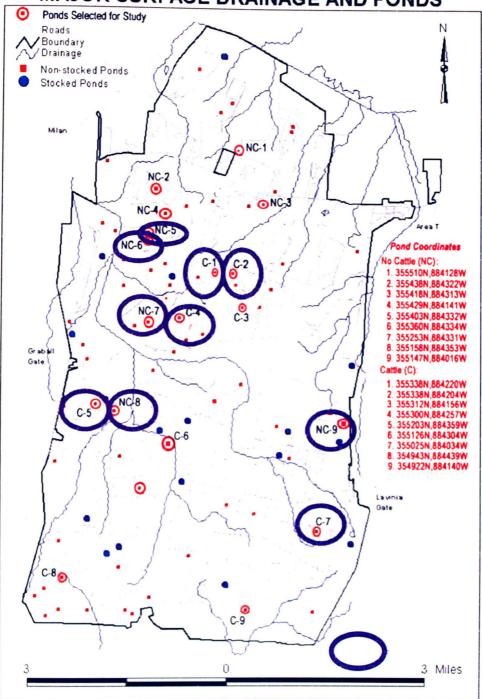


Figure 2. Map of the Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee, indicating major surface drainage and pond locations used for macroinvertebrate sampling (purple ellipses).

### History

Milan Army Ammunition Plant, constructed in 1941 during World War II, has been an active military arsenal since 1942 (Brew and Markol, 2001). The plant has the capability and facilities to load, assemble, pack and store various types of large and small caliber ammunitions. "The installation includes 10 ammunition load, assemble, and package (LAP) lines, one washout/rework line, one central x-ray facility, one test area, two shop maintenance areas, 12 magazine storage areas, a demolition and burning grounds area, an administration area, and a family housing area" (Beas, 2007).

In 1987, MLAAP was placed on the Environmental Protection Agency's National Priority List as a superfund site for groundwater contamination (USEPA, 1999). The water quality on MLAAP is of serious concern due to the large amount of nitroaromatic and nitramine explosives including 2,4,6-trinitrotoluene (TNT) 2,4-dinitrotoluene (DNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), which contaminated the soil and groundwater approximately fifty years ago (Best et al., 1999).

"Current levels of explosive compounds in groundwater pose unacceptable levels of human health risk if the groundwater were used as drinking water. Even though this is not occurring due to use of municipal water as drinking water by City of Milan residents, homeowners who live northwest of the city obtain their drinking water from private wells. These residents may in the future be exposed to elevated levels of explosive compounds as the area of contaminated groundwater migrates toward the northwest" (USEPA, 2000B).

### **CHAPTER III**

# MATERIALS AND METHODS

### **Pond Site Selection**

A total of 89 ponds are found on the MLAAP property, all included in MLAAP's Integrated Natural Resources Management Plan which couples organismal research and land use practices to develop long-term sustainability of natural resources (Stephenson and Kennedy, 2008). Macroinvertebrates were sampled from 10 non-stocked ponds located within MLAAP (Fig. 2). Five ponds had cattle access (Fig. 3a) and five ponds did not have cattle access (Fig. 3b). The ponds chosen for the macroinvertebrate research were randomly selected from among 18 ponds being used in ongoing herpetological studies with Austin Peay State University's (APSU) Center for Excellence in Field Biology (Beas, 2007). Pond numbers were previously assigned for the herpetological studies and were labeled NC (non-cattle) or C (cattle) based on use.





Figure 3. Photograph examples of (a) cattle access ponds (C-2) and (b) non-cattle access ponds (NC-5) at the Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. Letter codes (C-2, NC-5) refer to the specific ponds in photographs.

# **Collection Methods**

Macroinvertebrates were collected from ponds located on MLAAP during June and November/December 2009 and April 2010 using both funnel trap and dip net methods. These sampling events will be referred to as summer, winter and spring, respectively.

Dip-net samples were collected June 29 and November 11, 2009 and April 2, 2010. Sampling dates were proximate to dates of funnel trap sampling. Samples were collected using triangular dip-nets with 800/900 µm mesh size (Wildlife Supply Company, www.Wildco.com; Fig. 5). At each of the 10 ponds, a team of two collectors sampled multiple habitats for 30 minutes simultaneously providing one personhour/pond. Samples were placed in white plastic pans (46 cm x 36 cm; Fig. 6) and picked (i.e., specimens removed from debris) in the field using forceps. These pans provided a light background to the normally dark-colored sample often littered with organic debris, which allowed the collector to see the macroinvertebrates more easily. During picking, macroinvertebrate specimens were placed in 125 mL Nalgene bottles containing 70% isopropanol for preservation. Bottles were labeled with the pond number, date and persons collecting samples. Collectors were instructed to pick as many taxa as possible within the allotted time period, but no attempt was made to collect all macroinvertebrates in the samples. Prior to identification, the two sample bottles from each pond for each date were combined at the laboratory in the Center of Excellence for Field Biology at Austin Peay State University.



Figure 4. Photograph of the triangular dip nets used for timed-effort macroinvertebrate sampling.



Figure 5. Example of a dip net sample in white plastic pan prior to field picking.

Funnel-trap samples were collected June 18-22, December 8-12, 2009 and April 18-22, 2010. Funnel-trap samples were obtained using pre-assembled traps built with 30.4 cm long and 10 cm diameter clear PVC pipe with a 10 cm funnel at one end and a smoke test plug at the other (Fig. 7). The funnel was attached to the interior of the PVC pipe with silicone sealant. The PVC pipe and funnel were clear to eliminate visual obstruction to the macroinvertebrates. The smoke test cap allowed for easy removal of macroinvertebrates from the trap. Samples were collected by placing four submerged traps in shallow water at four different cardinal points in each of the 10 ponds and leaving them for two consecutive 48 hour periods. After each sampling period, each funnel trap was opened and all contents drained into a sieve. Contents were then transferred to a 750 mL Nalgene container using a large mouth plastic funnel and a 500 mL bent-neck squirt bottle containing 70% isopropanol (Fig. 8). The four funnel trap samples for each pond and sampling period were combined for identification at the laboratory in the Center of Excellence for Field Biology at Austin Peay State University.



Figure 6. Photograph of the clear PVC funnel traps attached to stake as it was used during macroinvertebrate sampling.



Figure 7. Example of sieve method used during collection of funnel trap samples.

## Macroinvertebrate Identification Procedures

Macroinvertebrate sorting and identifying was conducted in the aquatic laboratory of the Center of Excellence for Field Biology at Austin Peay State University with assistance from undergraduate and graduate laboratory assistants. Macroinvertebrates were separated by order then identified to family and, in most cases, further identified to genus. Specimens of Nematoda were not identified further. Only males of crayfish were identified to genus, all other specimens were identified to family Cambaridae. Leeches and aquatic oligochaetes were not identified below classes Hirudinea and Oligochaeta, respectively. Aquatic mites were identified to suborder Hydracarina. Snails and bivalves were identified to families Physidae, Lymnaeidae, Ancyclidae, Planorbidae and Sphaeriidae. Specimens in poor physical condition or too immature were not identified below family or order level. All macroinvertebrates contained in each sample, except non-biting midges (Diptera: Chironomidae), were identified using the following identification keys: Thorp and Covich (1991), Epler (1996), Voshell (2002) and Merritt et al. (2008). Chironomid larvae were initially separated by sub-family then mounted with CMC-10 (Masters Company, Inc., Wood Dale, IL) on glass microscope slides for generic identification using the following keys: Wiederholm (1983), Epler (2001) and Ferrington et al. (2008). Meijei MZS and Olympus SZH and G10X stereo-zoom microscopes with magnification ranges of 7-64X were used for most identification. Slide mounted chironomids were identified to genus using Olympus BH2 and CH30 compound microscopes with a magnification range of 40-1000X. Identified taxa were enumerated and entered into a Microsoft Excel spreadsheet.

## Statistical Analysis of Taxonomic Data

Macroinvertebrate diversity and sampling method efficiency was evaluated using both taxa richness and taxa abundance values, the Shannon-Weaver Index, Jaccard's Similarity Coefficient, taxa accumulation curves and linear regression models. All resulting data was displayed using pie charts, line charts or bar graphs created using Microsoft Excel. Statistical comparisons of metrics for the two sampling methods overall, for seasons and for the two types of ponds were made in Excel using T-test analyses. Due to the large number of sampling sites, the resulting values for most of the metrics were displayed in two sets of graphs based on cattle access although this was not originally an element of this study.

Taxa richness is simply the number of taxa present in a sample, community, or taxonomic group (Cole, 1983; Ludwig and Reynolds, 1988). This metric was calculated to determine the overall number of taxa collected from individual ponds utilizing each sampling method. The Shannon-Weaver Index, which combines taxa richness and equitability or evenness (Cole, 1983), was used to assess community heterogeneity under each method. According to Mackie (2008), Jaccard's Similarity Coefficient "measures the degree of similarity in taxonomic composition between two or more stations in terms of taxon presence or absence." This metric was calculated to compare taxa similarity in each pond based on the two sampling methods tested. The five most abundant taxa collected with both sampling methods were recorded and displayed using pie charts. Taxa accumulation curves were created to determine the number of additional new taxa added after each sampling session. Curves were displayed on line charts and evaluated two

ways, as a comparison of taxa added for each period within each sampling method independently and in terms of taxa added for each sampling period for both methods as a combined sampling effort. Linear regression models were created to evaluate number of samples and average seasonal taxa accumulation rate of each method. A T-test was run to determine if there was a significant difference in average seasonal taxa accumulation rate and sampling methods between pond types. Taxa added during each sampling event were identified to class or order level and evaluated seasonally and overall using bar graphs.

#### **Abiotic Data Collection and Analysis**

Water chemistry readings including temperature (°C), dissolved oxygen (% saturation and mg/l), specific conductivity (mS/cm), total dissolved solids (mg/l) and pH (SU) were taken on the first day of each sampling period using a YSI 600QS multiparameter meter. Before each sampling period the YSI was calibrated following the manufacturer's instructions. In addition, turbidity (NTU) levels of each pond were measured using a LaMotte model 2020e nephlometer. Prior to use, the instrument was calibrated following manufacturer's instructions. Notes regarding any abnormal environmental or physical conditions of ponds that may have affected readings were recorded on a field data sheet (see Appendix B for an example). Appendix C includes bar graph comparisons of all abiotic data.

## **Microbial Decontamination Protocol**

A microbial decontamination protocol was developed in response to possible cross-contamination concerns for amphibian-infecting pathogens. This protocol was employed during the spring sampling season. Three gallon sprayers were filled with aged tap water and two gallon sprayers with a 5% bleach solution. After exiting each pond, any equipment (waders, nets, boots, etc.) that came in contact with pond water or the surrounding ground, was first sprayed with the 5% bleach solution and allowed to sit for approximately one minute and then rinsed with the aged water for approximately one minute. The process was repeated for each pond in an attempt to prevent any unnecessary cross-contamination or outbreak of amphibian infectious diseases such as chytridiomycosis or *Ranavirus*.

#### **CHAPTER IV**

#### RESULTS

## Sample Collection and Evaluation

Using the two sampling methods, a total of 10,083 individual macroinvertebrates comprising 146 unique taxa were collected throughout the duration of this project. Funnel traps and dip nets collected a total of 6,529 and 3,554 individuals, respectively.

#### Statistical Analysis of Taxonomic Data

#### Taxa Richness

Richness values ranged from a high of 38 taxa collected in non-cattle pond 5 during the month of June (NC-5 June) using the funnel trap method, to a low of 8 taxa collected in non-cattle pond 7 during the month of December (NC-7 Nov/Dec) using the funnel trap method (Figs. 8-9). Comparison of taxa richness for all ponds by sampling method revealed that the dip nets collected the highest macroinvertebrate diversity overall, although taxa richness values were not significantly different for the two methods (p > 0.05, n.s.). Comparisons between ponds with and without cattle access showed that the dip nets collected the highest diversity within the majority of cattle ponds while the funnel traps collected the highest diversity within the majority of non-cattle ponds. Although no significant difference between sampling methods and taxa richness within

non-cattle ponds was indicated (p > 0.05, n.s.), a significant difference in taxa richness was found for sampling methods within cattle ponds (p < 0.05, s.).

Sampling method comparisons and taxa richness results were also analyzed by seasonal sampling period. Richness values for the summer samples (Fig. 10) ranged from a high of 38 taxa collected in non-cattle pond 5 (NC-5 June) using the funnel trap method to a low of 10 taxa collected in non-cattle pond 8 (NC-8 June) using the dip net method. For summer samples, the funnel trap method generally collected a greater number of taxa although the differences in richness values between methods were not significant (p > 0.05, n.s.).

Richness values for the winter samples (Fig. 11) ranged from a high of 34 taxa collected in cattle pond 7 (C-7 Nov/Dec) using the dip net method to a low of 8 taxa collected in non-cattle pond (NC-7 Nov/Dec) using the funnel trap method. For winter samples, the dip net method appeared to collect the highest macroinvertebrate taxa although the results were not significantly different (p > 0.05, n.s.) from the dip net sampling.

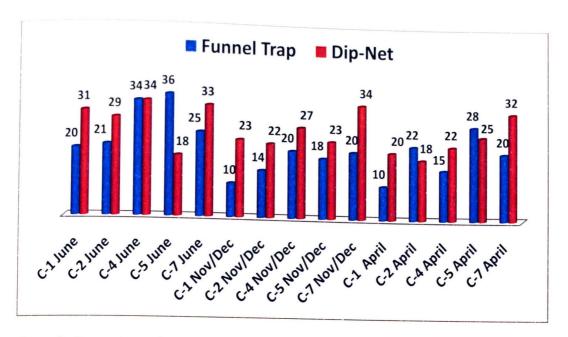


Figure 8. Comparison of sample methods and taxa richness of ponds with cattle access on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

Numbers above bars indicate total taxa collected at each cattle pond during all sampling seasons.

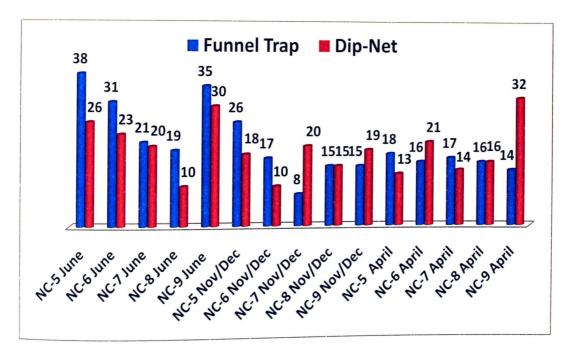


Figure 9. Comparison of sample methods and taxa richness of ponds without cattle access on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

Numbers above bars indicate total taxa collected at each non-cattle pond during all sampling seasons.

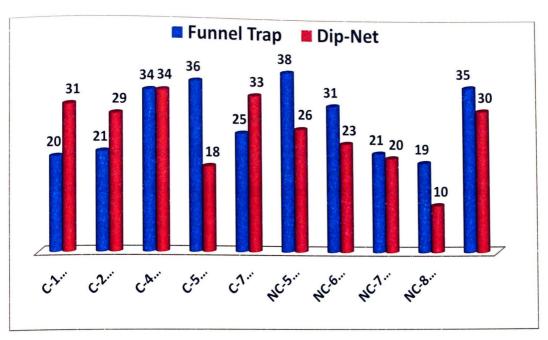


Figure 10. Comparison of sampling methods and taxa richness for June 2009 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. Numbers above bars indicate total taxa collected at each pond during summer sampling season.

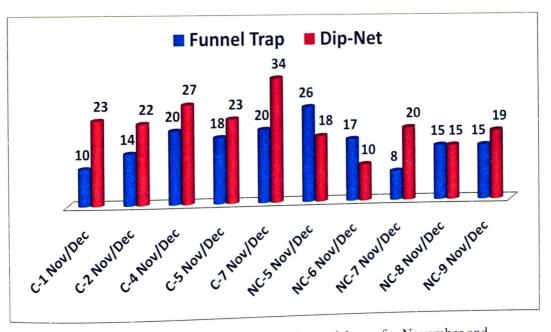


Figure 11. Comparison of sampling methods and taxa richness for November and December 2009 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. Numbers above bars indicate total taxa collected at each pond during winter sampling season.

Richness values for the spring samples (Fig. 12) ranged from a high of 32 taxa collected in both cattle pond 7 (C-7 April) and non-cattle pond 9 (NC-9 April) using the dip net method to a low of 10 taxa collected in cattle pond 1 (C-1 April) using the funnel trap method. For spring samples, dip nets typically collected the greatest macroinvertebrate taxa richness, but no significant difference was found between the sampling methods (p > 0.05, n.s.).

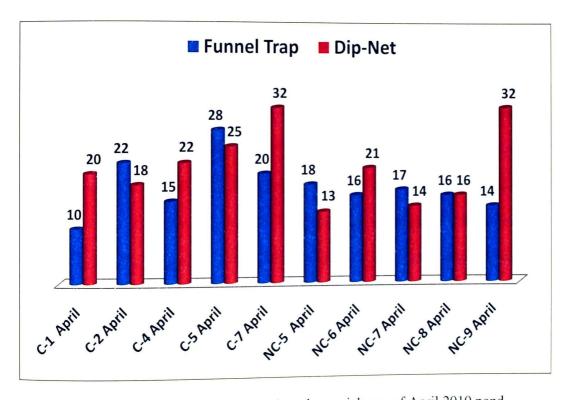


Figure 12. Comparison of sampling methods and taxa richness of April 2010 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. Numbers above bars indicate total taxa collected at each pond during spring sampling season.

#### Shannon-Weaver Index

Shannon-Weaver Index values for both sampling methods, all ponds and all seasons ranged from a low of 0.140 in non-cattle pond 8 in summer (NC-8) using a dip net (Fig. 14) to a high of 0.384 collected with funnel traps during summer in cattle pond 5 (C-5; Fig. 13). Funnel traps averaged 1.82 overall, which was higher than the dip nets, averaging 1.53 overall. The differences between sampling methods for Shannon-Weaver values were significant overall (p < 0.05, s.).

Shannon-Weaver Index results and sampling methods were compared by both cattle and non-cattle pond types. Shannon-Weaver Index values within cattle ponds ranged from a low of 1.18 in cattle pond 1 in winter (C-1) using a funnel trap to a high of 2.59 collected with funnel traps in cattle pond 5 (C-5) during June. Funnel traps and dip nets within cattle ponds averaged 1.67 and 1.61, respectively. No significant difference was found between sampling methods for Shannon-Weaver Index values in cattle ponds (p > 0.05, n.s.). Shannon-Weaver Index values within non-cattle ponds ranged from a low of 1.14 in non-cattle pond 8 in summer (C-1) using a dip net to a high of 3.84 collected during summer in non-cattle pond 5 (NC-5) with a funnel traps. Funnel traps and dip nets within non-cattle ponds averaged 1.97 and 1.45, respectively. A significant difference between sampling methods for Shannon-Weaver Index values within non-cattle ponds was found (p < 0.05, s.).

A comparison of Shannon-Weaver Index results for both sampling methods was also analyzed by seasonal sampling period. Shannon-Weaver values for summer samples (Fig. 15) ranged from a high of 3.84 collected in non-cattle pond 5 (NC-5) using the funnel trap method to a low of 1.14 collected in non-cattle pond 8 (NC-8) using the dip

net method. Summer Shannon-Weaver Index values for funnel traps and dip nets averaged 2.39 and 1.63, respectively. For summer, a significant difference in Shannon-Weaver Index values was observed between the two sampling methods (p < 0.05, s.).

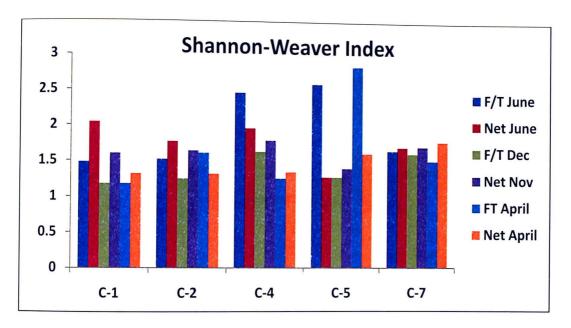


Figure 13. Comparison of sampling methods and Shannon-Weaver Index values for cattle pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. (F/T=Funnel Trap).

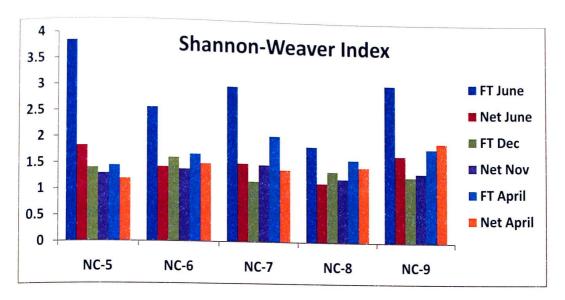


Figure 14. Comparison of sampling methods and Shannon-Weaver Index values for non-cattle pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee. (F/T=Funnel Trap).

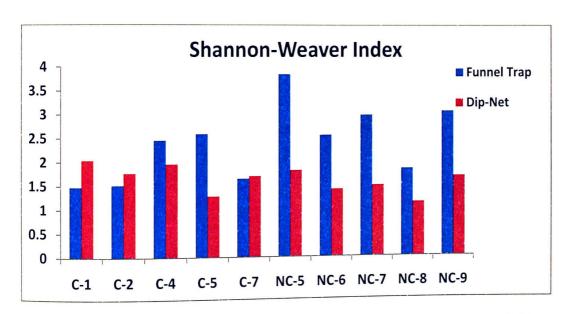


Figure 15. Comparison of sampling methods and Shannon Weaver values of June 2009 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

Shannon-Weaver Index values for the winter samples (Fig. 16) ranged from a high of 1.79 collected in cattle pond 4 (C-4) using the dip net method to a low of 1.16 collected in non-cattle pond 7 (NC-7) using the funnel trap method. Winter Shannon-Weaver Index values for funnel traps and dip nets averaged 1.37 and 1.48, respectively. There was no significant difference (p > 0.05, n.s.) between the sampling methods with regard to Shannon-Weaver Index values in the winter sampling season.

Shannon-Weaver values for the spring samples (Fig. 17) ranged from a high of 2.84 collected in cattle pond 5 (C-5) to a low of 1.18 collected in cattle pond 1 (C-1), in both cases using the funnel trap method. Spring Shannon-Weaver Index values for funnel traps and dip nets averaged 1.69 and 1.48, respectively. There was no significant difference (p > 0.05, n.s.) between sampling methods and Shannon-Weaver Index values for the spring sampling season.

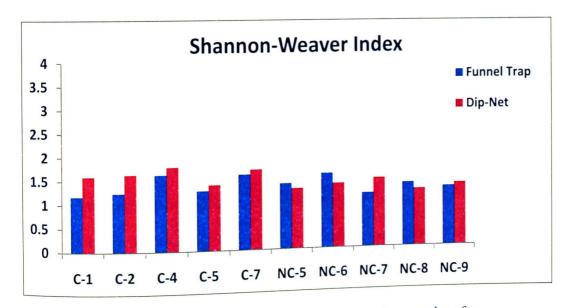


Figure 16. Comparison of sampling methods and Shannon Weaver values for November/December 2009 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

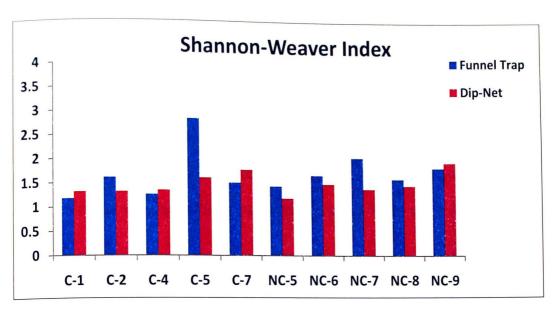


Figure 17. Comparison of sampling methods and Shannon Weaver values of April 2010 pond samples on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

### Jaccard's Similarity Coefficient

Jaccard's Similarity Coefficient values, which were used to compare taxa similarity between sampling methods for each sample date and pond, ranged from a low of 0.125 in non-cattle pond 6 in winter (NC-6 Nov/Dec; Fig. 19) to a high of 0.576 collected in cattle pond 5 in winter (C-5 Nov/Dec; Fig. 18). Jaccard's Similarity Coefficient values for non-cattle ponds averaged 0.256 overall, lower than the 0.322 average for cattle ponds. Differences between Jaccard's Similarity Coefficient values between cattle and non-cattle ponds were significant ( $p \le 0.05$ , s.)

Jaccard's Similarity Coefficient values for summer samples (Fig. 20) ranged from a high of 0.352 collected in non-cattle pond 7 (NC-7) to a low of 0.177 collected in cattle pond 2 (C-2) and averaged 0.265. There was no significant difference between

pond types with regard to Jaccard's Similarity Coefficient values for the summer sampling season (p > 0.05, n.s.).

Jaccard's Similarity Coefficient values for winter samples (Fig. 21) ranged from a high of 0.576 collected in cattle pond 5 (C-5) to a low of 0.125 collected in non-cattle pond 6 (NC-6) and averaged 0.295. There was a significant difference between pond types with regard to Jaccard's Similarity Coefficient values for the winter sampling season (p < 0.05, s.).

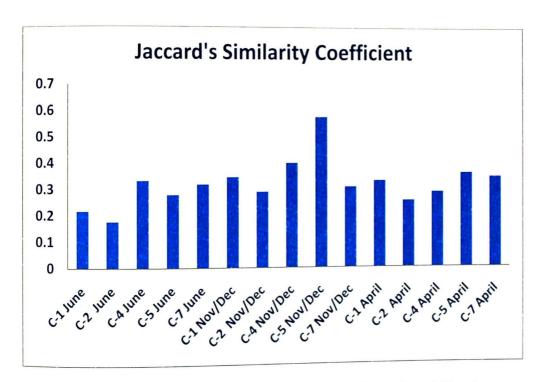


Figure 18. Jaccard's Similarity Coefficient values for cattle ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

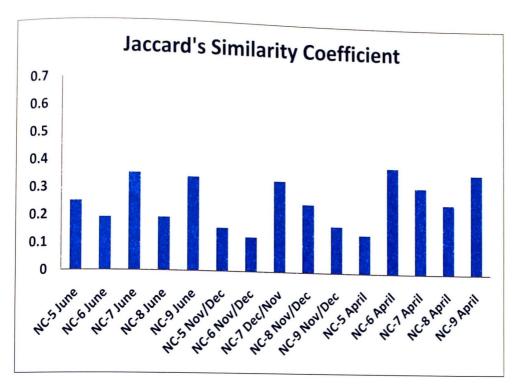


Figure 19. Jaccard's Similarity Coefficient values for non-cattle ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

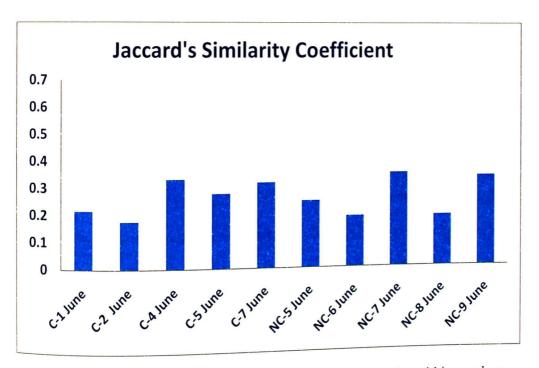


Figure 20. Jaccard's Similarity Coefficient values for June samples within ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

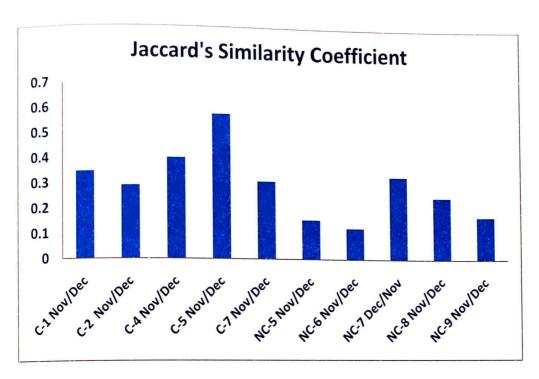


Figure 21. Jaccard's Similarity Coefficient values for June samples within ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

Jaccard's Similarity Coefficient values for spring samples (Fig. 22) ranged from a high of 0.392 collected in non-cattle pond 6 (NC-6) to a low of 0.142 collected in non-cattle pond 5 (NC-5). Spring Jaccard's Similarity Coefficient values averaged 0.307. There was no significant difference between the pond types with regard to Jaccard's Similarity Coefficient values for the spring sampling season (p > 0.05, n.s.)

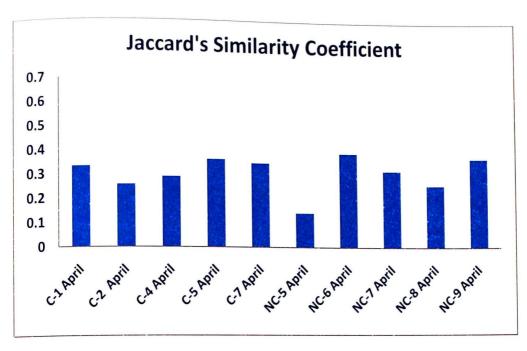


Figure 22. Jaccard's Similarity Coefficient values for June samples within ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

#### **Dominant Taxa**

The five most abundant taxa collected with funnel traps (Fig. 23) included Oligochaeta (1097 individuals), Hydracarina (812), *Chaoborus* (483), *Neoplea* (367) and Physidae (253). These five taxonomic groups totaling 3,012 individuals made up 46% of the 6,528 total individuals collected utilizing funnel traps. The five most abundant taxa collected for dip nets (Fig. 24 individuals) included *Laccophilus* (211), *Plathemis* (208), *Ischnura* (163), Oligochaeta (162) and *Neoporus* (159). These five taxonomic groups totaled 903 individuals and made up 25% of the 3,554 total individuals collected with dip nets.

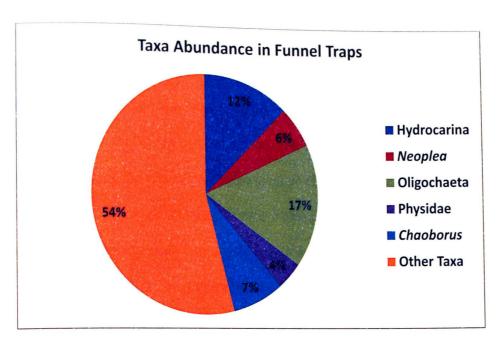


Figure 23. Total percentage of five most abundant taxa collected with funnel traps on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

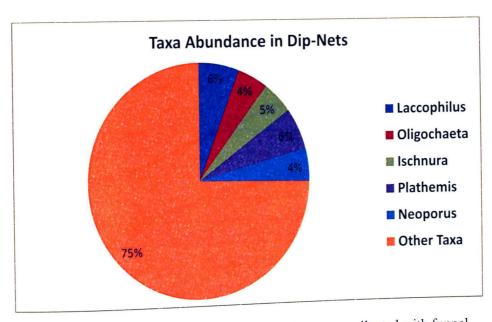


Figure 24. Total percentage of five most abundant taxa collected with funnel traps on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

### Taxa Accumulation by Method of Sampling

Taxa accumulation curves for each pond with sampling methods displayed independently on separate trend lines were graphed (Fig. 25-34). Dip nets collected the most taxa overall within four of the five cattle ponds and one of the five non-cattle ponds. Total taxa collected using dip nets ranged from a high of 61 taxa collected within cattle pond 7 (C-7; Fig. 29) to a low of 29 taxa collected within non-cattle pond 8 (NC-8; Fig. 33). Funnel traps collected the most taxa overall within four of the five non-cattle ponds and one of the five cattle ponds. Total taxa collected from ponds using funnel traps ranged from a high of 61 collected within non-cattle pond 5 (NC-5; Fig. 30) to a low of 31 taxa collected in cattle pond 1 (C-1; Fig. 25).

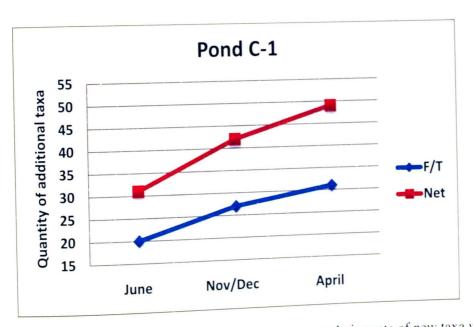


Figure 25. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for cattle pond 1 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

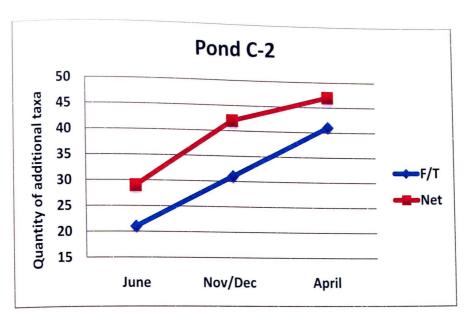


Figure 26. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for cattle pond 2 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

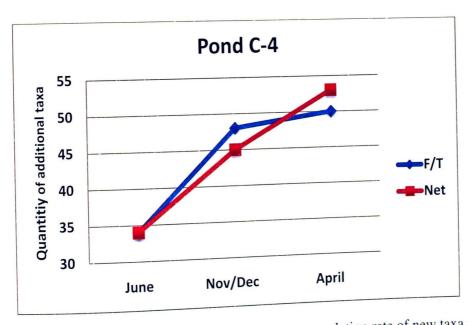


Figure 27. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for cattle pond 4 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

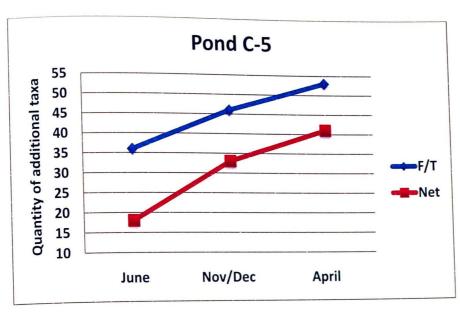


Figure 28. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for cattle pond 5 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

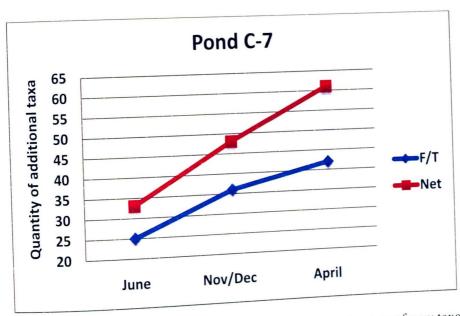


Figure 29. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for cattle pond 7 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

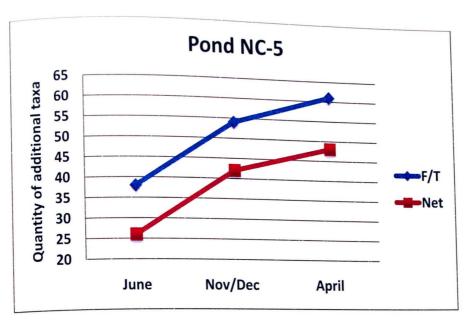


Figure 30. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for non-cattle pond 5 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

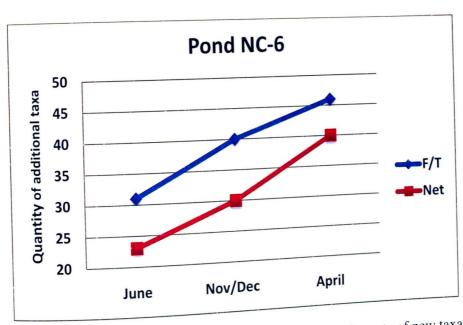


Figure 31. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for non-cattle pond 6 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

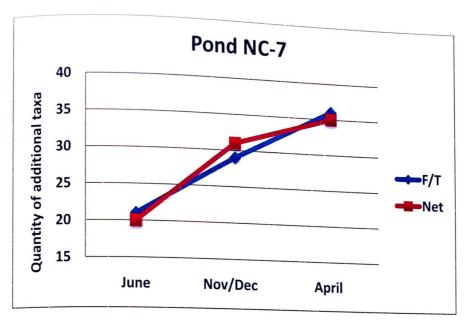


Figure 32. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for non-cattle pond 7 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

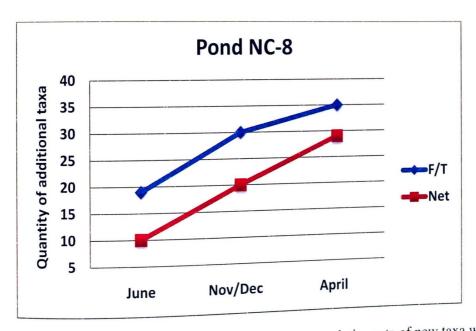


Figure 33. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for non-cattle pond 8 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

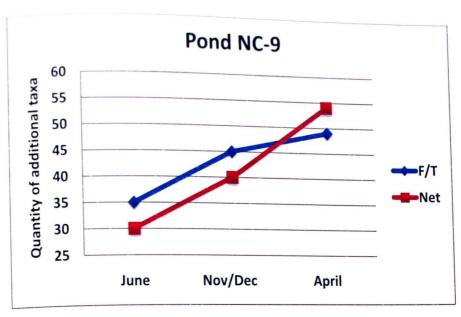


Figure 34. Taxa accumulation curve showing accumulation rate of new taxa with sampling methods represented independently for non-cattle pond 9 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

## **Linear Regression Models**

The linear regression models depict the same results as accumulation curves with dip nets appearing to collect more taxa at a faster rate within cattle ponds (Fig. 35) and funnel traps collecting more taxa at a faster rate within non-cattle ponds (Fig. 36). No significant difference was found for rates of taxa accumulation using the two methods for all ponds (p > 0.05, n.s.) or within cattle ponds only (p > 0.05, n.s.). However, within non-cattle ponds a significant difference between the rates of taxa accumulation was found for the two sampling methods (p < 0.05, s.). For both cattle and non-cattle ponds, high R-squared values indicate rate of taxa accumulation is highly correlated to the number of samples collected over as single year.

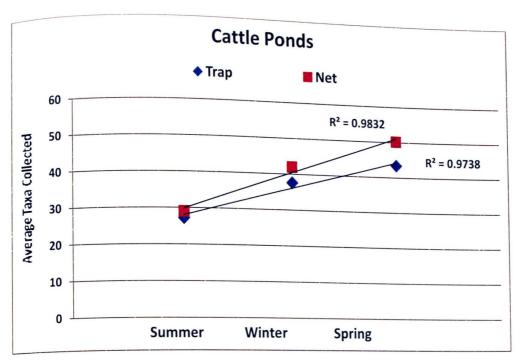


Figure 35. Taxa accumulation regression model showing average accumulation rate of new taxa with sampling methods represented independently for cattle ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

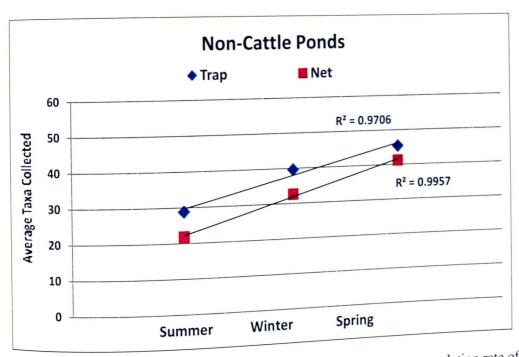


Figure 36. Taxa accumulation regression model showing average accumulation rate of new taxa with sampling methods represented independently for non-cattle ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

# Taxa Accumulation with Combined Sampling Methods

Total taxa collected in each pond for each sampling event (i.e., each seasonal funnel trap and dip net collection being a sampling event) are graphed on taxa accumulation curves (Fig. 37-46). Total taxa collected within all ponds using a combination of sampling methods ranged from a high of 76 taxa collected within non-cattle pond 5 (NC-5; Fig. 42) to a low of 47 taxa collected within non-cattle pond 8 (NC-8; Fig. 45). Total taxa collection within both cattle and non-cattle ponds averaged 65 and 60, respectively.

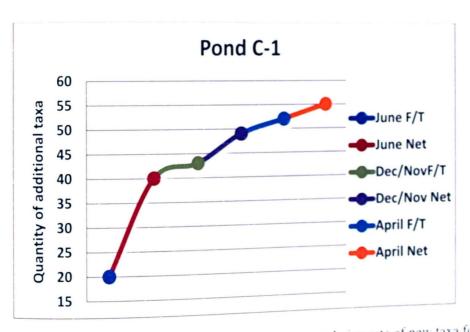


Figure 37. Taxa accumulation curve showing accumulation rate of new taxa for cattle pond 1 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

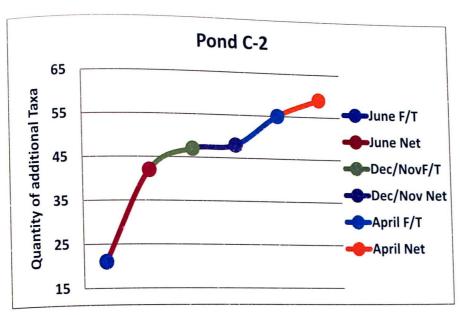


Figure 38. Taxa accumulation curve showing accumulation rate of new taxa for cattle pond 2 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

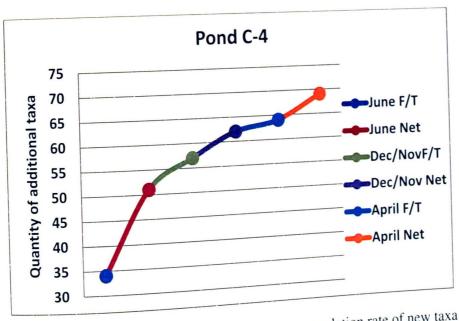


Figure 39. Taxa accumulation curve showing accumulation rate of new taxa for cattle pond 4 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

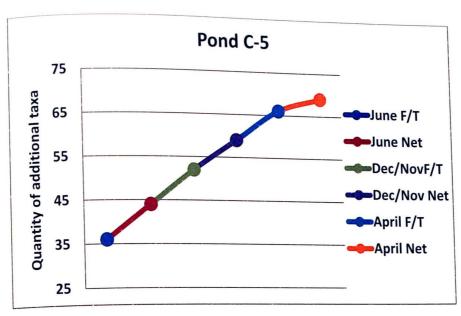


Figure 40. Taxa accumulation curve showing accumulation rate of new taxa for cattle pond 5 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

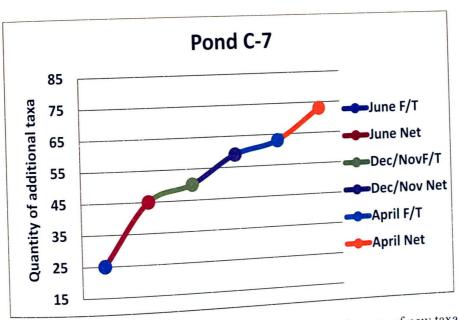


Figure 41. Taxa accumulation curve showing accumulation rate of new taxa for cattle pond 7 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

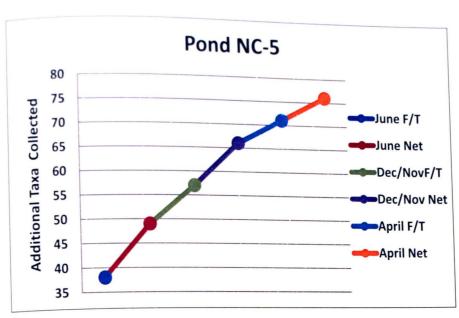


Figure 42. Taxa accumulation curve showing accumulation rate of new taxa for non-cattle pond 5 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

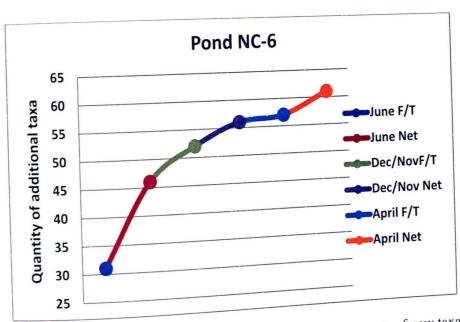


Figure 43. Taxa accumulation curve showing accumulation rate of new taxa for non-cattle pond 6 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

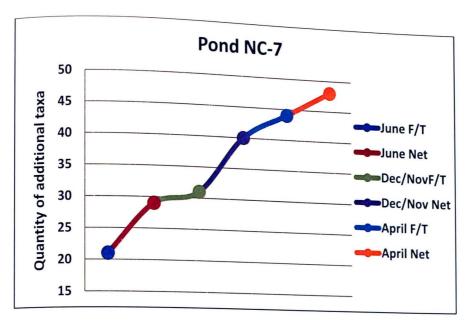


Figure 44. Taxa accumulation curve showing accumulation rate of new taxa for non-cattle pond 7 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

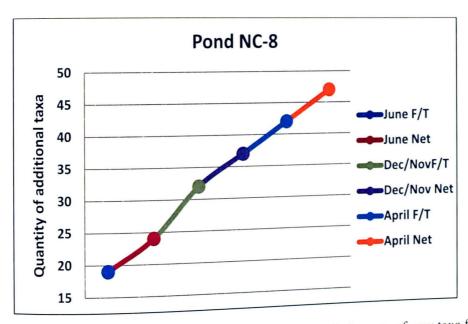


Figure 45. Taxa accumulation curve showing accumulation rate of new taxa for non-cattle pond 8 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

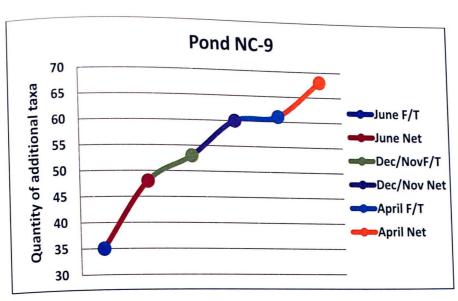


Figure 46. Taxa accumulation curve showing accumulation rate of new taxa for non-cattle pond 9 on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

## Identification of Added Taxa for Individual Sampling Methods

Identification of newly collected taxa accumulated seasonally utilizing both sampling methods were graphed (Fig. 47-49). A total of 37 additional taxa within five insect orders and the class Gastropoda were collected using funnel traps during the summer season (Fig. 47). The majority of these added taxa belonged to orders Diptera, Coleoptera and Odonata. Dip nets accumulated a total of 17 additional taxa within five insect orders during the summer season (Fig. 47). The majority of these taxa belonged to the order Coleoptera. Winter funnel trap samples accumulated a total of 17 additional taxa within four insect orders and the gastropods (Fig. 48). The majority of these taxa belonged to orders Diptera and Coleoptera. A total of 47 taxa within seven insect orders and the gastropods were collected with dip nets during the winter season (Fig. 48). The majority of additional taxa collected using dip nets belonged to orders Diptera,

Coleoptera, Odonata and Hemiptera. Spring funnel traps accumulated 26 additional taxa within five insect orders plus classes Gastropoda and Decapoda (Fig. 49). The majority of added taxa collected in the spring by funnel trapping are the orders Diptera and Coleoptera. Dip nets added 37 new taxa within seven insect orders plus classes Gastropoda, Decapoda and the phylum Nematoda (Fig. 49). The majority of the added taxa collected during the spring using dip nets belonged to the insect orders Coleoptera, Odonata and Hemiptera.

Identification of added taxa accumulated overall utilizing both sampling methods was graphed (Fig. 50). Funnel traps accumulated a total of 18 additional taxa within the three insect orders, Diptera, Coleoptera and Odonata. Dip nets added a total of 26 additional taxa within the five insect orders, Diptera, Coleoptera, Odonata, Hemiptera and Ephemeroptera.

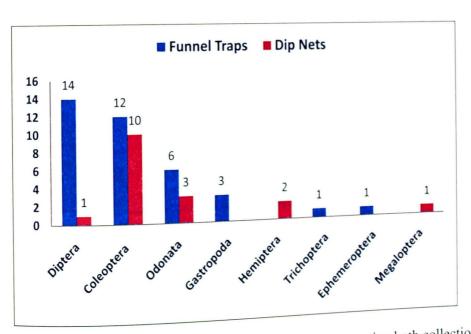


Figure 47. Addition and identification of new taxa representing both collection methods for the June sampling season within sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

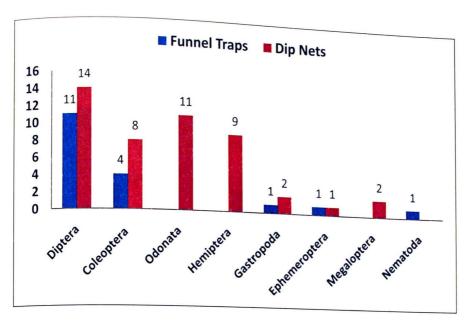


Figure 48. Addition and identification of new taxa representing both collection methods for the November/December sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

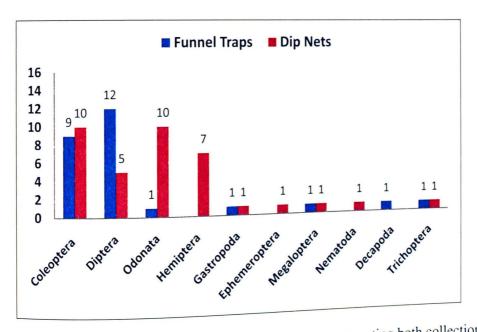


Figure 49. Addition and identification of new taxa representing both collection methods during the April 2010 sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

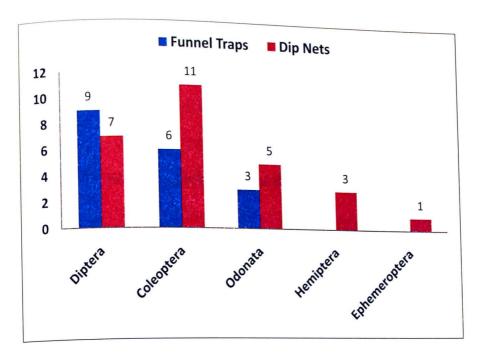


Figure 50. Addition and identification of new taxa representing both collection methods with sampling seasons combined for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

## CHAPTER V

### DISCUSSION

# Sampling Technique and Habitat Structure

Biomonitoring has been a tool used to measure water quality since the early twentieth century (Rosenberg and Resh, 1993). Lotic habitats are the focus of various aquatic biomonitoring programs throughout state and federal agencies. Although much research regarding methods of collection and appropriate metrics for analysis has been performed, there is still a lack of uniformity among programs regarding biomonitoring methodologies (Herbst and Silldorff, 2006). Choice of sampling device, where to sample and methodologies for sample analysis have been a long and heavily debated topic (Carter and Resh, 2001). When assessing macroinvertebrate diversity within any aquatic habitat, the most important concern is ensuring the intended group or groups is collected in a consistent manner (Rosenberg and Resh, 1993). The need for sampler consistency coupled with the variety of habitat types within aquatic environments has led to the development of numerous sampling devices including sweep-nets, rock-bags, activity traps, corers and Hester-Dendy substrates (Muzaffar and Colbo, 2002; Henke and Batzer, 2005; Beccerra Jurado et al., 2008). Lentic environments such as ponds have not been the focus of biomonitoring studies, and although efforts have been made to develop macroinvertebrate assessment methods within these habitats, no standardized method has

been designed (Muzaffar and Colbo, 2002; Conner et al., 2004; Becerra Jurado et al., 2008).

The objective of this study was to compare two commonly used sampling devices, funnel traps and dip nets, to determine which is more efficient when assessing macroinvertebrate diversity within pond communities. As in any aquatic environment, habitat structure will directly influence the success of any one sampling device (Garcia-Criado and Trigal, 2005). The two types of pond habitats selected for sampling, those with cattle access and those without, were structurally different. Cattle ponds had high turbidity, little or no aquatic macrophytes, sparse surrounding vegetation with a limited canopy and firmer mud-bottom substrates with little accumulated allochthonous organic matter. Non-cattle ponds had lower turbidity, aquatic macrophytes, surrounding canopies of shrubby and woody vegetation and a softer mucky bottom substrate that contained an abundance of fine and coarse allochthonous organic matter. These disparate qualities allowed for a true comparison of sampling methods by comparing sampling method efficiency across different habitats and under variable conditions.

### Macroinvertebrate and Sampling Technique Analysis

### Taxa Richness

Taxa richness calculations were performed to accurately determine the macroinvertebrate composition of ponds on MLAAP. Taxa richness values comparing sampling methods among ponds were variable. When examining both cattle and non-cattle ponds in all seasons, dip nets collected more taxa than funnel traps more frequently,

but the advantage was slight. On three occasions the two sampling techniques collected the same number of taxa. A T-test comparison of this data revealed no significant difference in the two sampling approaches despite the appearance of an advantage to the dip net approach.

When these same data for all three seasons were examined by pond type, a general trend that suggested dip nets were more successful in collecting greater numbers of taxa in cattle ponds while funnel traps were more successful in non-cattle ponds was observed. A significant difference in taxa richness between sampling methods existed within cattle ponds but there was no significant difference for non-cattle ponds. These results could be due to differences in the habitat structure of specific ponds. Specifically, the organic detritus clogging the non-cattle ponds may have interfered with persons collecting dip net samples under the 30-minute time constraint.

Richness values were also evaluated by seasonal sampling period. No significant difference in taxa richness between sampling methods was observed for any season. The funnel-trap method collected the most macroinvertebrate richness overall for summer samples. This result could be attributed to the highest taxonomic richness for order Diptera occurring in the summer and the fact that members of the order Diptera were consistently sampled most efficiently utilizing funnel-traps. The increased efficiency of funnel traps in sampling dipteran taxa relative to dip net samples was likely the result of chironomid larvae colonizing the surfaces of the funnel traps and the fact that the smaller, less active and more cryptic dipterans such as chironomids may have been missed during field-picking of dip net samples. During the winter samples, funnel traps deviated in two cases from the general trend of being more successful in non-cattle ponds. The traps

collected the least amount of taxa within non-cattle ponds 7 and 9 (NC-7; NC-9). The dip net method collected the greatest macroinvertebrate taxa richness overall for winter samples. These results may be attributed to the cooler temperatures during the winter season which will decrease macroinvertebrate activity and, therefore, decrease the success of the activity-dependant funnel traps. Dip nets, however, being a more mobile method, will collect macroinvertebrates whether active or not. During spring sampling, only one dip net sample collected the greatest richness within a non-cattle pond, which is uncommon due to structural limitations to dip net sampling efforts. Although both methods deviated somewhat from the general trend during the winter and spring season, the richness values for individual seasons were not significantly different.

#### **Shannon-Weaver Index**

When analyzing Shannon-Weaver Index (SWI) values for all ponds, differences between sampling methods were significantly different. Shannon-Weaver Index values were also evaluated by season and pond type with only non-cattle ponds for the summer sampling significantly different with regard to the SWI diversity.

The SWI is a widely used measure of community diversity that combines community richness and evenness of individuals (Rosenberg and Resh, 1994). Typically, values range from > 0 to about 4. Given an even number of individuals in samples, higher SWI values indicate a more even number of individuals distributed among taxa present and numbers closer to 0 indicate a less even distribution of individuals among taxa present. Alternatively, higher values can indicate more taxa and lower values can indicate fewer taxa. The SWI compounds these two measures of community diversity

and "is essentially a measure of randomness" (Vlach, et al., 2010). This commonly used diversity measurement requires a precise determination of taxa abundance to properly calculate SWI values. In order for this statistic to be truly useful, only two or more quantitative samples should be compared. In the case of this study, the dip net method is qualitative in that no effort is made to collect all individuals gathered in the net during the allotted time. The differences in richness and individual abundances of macroinvertebrates collected by the timed-effort dip net samples may not accurately reflect differences in these values, but instead reflect biases of this non-quantitative sampling approach. The SWI also excludes other factors that could influence results, such as organismal body size, habitat structure, time of year, duration of sampling and other factors (Rosenberg and Resh, 1994). This diversity metric was originally calculated for assessment of the preliminary results. As such, a decision was made to report the final results while noting that results may not accurately measure sampling method efficiency. Nonetheless, except for a few notably high SWI results, primarily with funnel trap samples, the SWI values were remarkably close across the majority of samples, averaging about 1.67 for all ponds.

### Jaccard's Similarity Coefficient

Jaccard's Similarity Coefficient (JSC) values were calculated for individual ponds to evaluate the shared taxa collected by the two sampling methods. This metric evaluates presence or absence of taxa in paired comparisons, but does not take into account abundances of taxa and, therefore, can be used appropriately to compare the quantitative

funnel trap samples to the qualitative dip net samples. Values can range from 0.0 when no taxa are shared to 1.0 when all taxa are shared in both populations.

Visual evaluation of the cattle ponds suggests that the sampling methods collected more similar samples overall in non-cattle ponds. However, only one of 30 JSC comparisons of sampling methods indicated a high JSC value, that is, greater than 0.50. Low JSC values indicate the two methods were collecting different sets of taxa across all ponds. A significant difference in JSC values between cattle and non-cattle ponds among all seasons was found. Ponds were also evaluated by differences in pond type for each collection season; only the results from the winter sampling season were significantly different from other seasons. The significant differences of the winter samples could be due to differing habitat complexities and the challenges associated with sampling in noncattle ponds. In cattle ponds where collecting with a dip net was easier, more of the same taxa was collected in funnel traps and dip netting. Conversely, dip net collecting was more difficult and time-consuming in the detritus-laden non-cattle ponds and thus fewer of the same taxa were found.

#### **Dominant Taxa**

Individual abundance of all taxa was calculated to determine the most abundant taxa collected by each sampling method during the study. Funnel traps are activity traps designed to capture organisms that swim or crawl into the device. Thus, as expected the traps collected water mites (Hydracarina), *Chaoborus* (phantom midges), *Neoplea* (pygmy backswimmers), physid snails and various diving beetles and other macroinvertebrates that actively swim or crawl about in the ponds. The traps also

collected smaller, quicker colonizing macroinvertebrates such as chironomids and oligochaetes. The dip nets, being more mobile sampling devices, were particularly effective at collecting odonates and coleopterans, which are large and active in pans when field picking.

The five most prominent taxa collected with funnel traps made up almost half of the total individuals collected with this method. Generally, dominance of a few taxa within a habitat is considered an indicator of poor water quality (Jackson et al., 2009). However, this analysis is evaluating funnel trap collection efficiency overall and not considering individual pond types or the water quality. In this case, the results indicate that funnel traps were more successful in collecting large numbers of a few taxa not because they are actually the dominant taxa in the ponds, but because of the way in which the traps preferentially collect active or colonizing macroinvertebrates.

Based on richness results, dip nets performed predictably, collecting larger, more mobile taxa such as the dragonfly larva *Plathemis* and the predacious diving beetles *Laccophilus* and *Neoporus*. The five most dominant taxa sampled with the timed-effort dip net sampling method made up only 25% of the total individuals collected. This suggests that dip nets have the ability to collect more taxa diversity than funnel traps. However, it is important to note that persons collecting timed-effort dip net samples were not collecting all individuals captured, but were collecting specifically for richness. Thus, the dominance results for dip nets do not accurately reflect the dominance of these taxa within the community.

Both methods collected oligochaetes as a dominant taxon. This could be due to the ubiquitous nature of aquatic worms and the occurrence of large and active species

(Smith, 1985). Funnel traps will collect oligochaetes because they are quick colonizers (Riley and DeRoja, 1989). Dip nets would be expected to collect the larger and often active specimens which are in turn more visible and easily picked from pans by collectors.

### Taxa Accumulation by Method of Sampling

Taxa accumulation curves were created to compare collection methods and evaluate the accumulation of previously uncollected (new) taxa with each additional sample. Funnel trap and dip net methods both collected the majority of new taxa overall in five of the ten total ponds throughout the study, although the rate of accumulation varied between ponds. In some cases the collection method that ultimately collected the most macroinvertebrates over the three sampling seasons was not the most successful after the first, and sometimes second sampling season. The dip nets added taxa at a faster rate within cattle ponds while funnel traps collected more new taxa, but at a slower rate within non-cattle ponds. This result provides further support for the general observation that dip nets were more efficient in sampling taxa richness in cattle ponds while funnel traps were more efficient in non-cattle ponds. However, Jaccard's Similarity Coefficient values indicated the two methods collected different sets of taxa. Therefore, even if one method is collecting additional taxa at a faster rate, there is a high likelihood that some taxa present in a pond would not be sampled using only the dip net or only the funnel trap. These results further support the idea that habitat structure plays a significant role in the success of sampling technique. Additionally, many of the resulting graphs show that after three seasons trend lines were not approaching an asymptote. The approach of an

asymptote would theoretically indicate that one has come close to collecting all taxa present within a particular pond.

### Taxa Accumulation with Combined Sampling Methods

Another set of taxa accumulation curves were created with the goal of evaluating the total accumulation of taxa within each pond following each additional sampling event. In all cases, results indicated a combination of sampling methods would greatly increase the number of macroinvertebrate taxa collected from a community. The total taxa collected using a combination of methods was higher in every pond than total taxa collected using a single method. In all but one pond a combination of sampling methods collected an equal or greater number of taxa after two sample seasons than after three seasons when using either method individually. Thus, a combination of methods would allow the theoretical asymptote to be reached more quickly. For many ponds, the trend line representing a combination of sampling techniques appears to be rising, indicating nearly all taxa have not been sampled after three seasons. These results indicate that a combination of sampling methods and a slightly longer sampling period is necessary to accurately determine macroinvertebrate richness in the ponds on MLAAP. This analysis also corroborates results from the Jaccard's Coefficient of Similarity analyses indicating the two methods collect different taxa. Previous studies by Hilsenhoff (1991), Muzaffar and Colbo (2002), Becerra-Jurado et al. (2008) and Vlach et al. (2010) comparing various collection methods produced similar results with all concluding a combination of methods would more accurately represent invertebrate diversity within an aquatic community.

## Identification of Added Taxa for Individual Sampling Methods

The accumulation and identification of additional taxa was evaluated by season for both collection methods. The majority of taxa added during the summer season using funnel traps belonged to orders Diptera, Coleoptera and Odonata. The dip nets collected the majority of additional taxa within the order Coleoptera. All orders mentioned above were in their highest abundance during this season.

The majority of added taxa collected during the winter season using funnel traps belonged to orders Diptera, which were abundant during the winter, and Coleoptera. The funnel traps were consistently the most successful method for collecting dipterans, particularly the family Chironomidae. The majority of added taxa collected during winter using dip nets belonged to the orders Diptera, Coleoptera, Odonata and Hemiptera. Dip nets have been shown by other metrics to be successful collecting these orders. The order Hemiptera was also more commonly sampled with dip nets due to their preference of various microhabitats which were more efficiently sampled using this collection method (Merritt and Cummins, 2008). Although funnel traps are generally more successful in collecting dipterans, dip nets collected a high number of dipterans during the winter months. The success of this method in capturing dipterans may have been the result of the large size of certain chironomid dipterans present in the ponds during the winter months. Genera of midges added during this season included Polypedilum, Kiefferulus and Endochironomus, all of which are relatively large members of this family and thus, are more visible to samplers picking through debris in the field.

The majority of additional taxa collected during the spring season using funnel traps were dipterans and coleopterans. These results may reflect the overall general

success of this method in collecting members of these orders and the relatively high abundance of dipterans encountered in spring compared to other seasons. Most of the added taxa collected during the spring using dip nets belonged to the orders Diptera, Coleoptera, Odonata and Hemiptera. Dip nets also have been shown to be quite successful in collecting members of these orders. These results likely reflect the relatively high abundance of hemipterans and dipterans in the ponds during this season. The relatively large chironomid *Kiefferulus* was the most abundantly collected midge during this season. The dipteran genus *Chaoborus* was also commonly collected. This planktonic dipteran larva is relatively large, and although nearly transparent, it is very mobile and active when placed in a pan for field picking.

Added taxa identified and evaluated previously by single season were compiled and compared again by sampling method across all seasons and evaluated at genus level in most cases. This removed taxa included as an additional taxa when sampled within a single season if, after comparison of all sampling periods, is found to be present in multiple seasons. This allowed for a determination of which taxa were truly sampled by only a single method throughout the study. Funnel traps accumulated additional taxa within orders Diptera, Coleoptera and Odonata. Several genera within the family Chironomidae were sampled utilizing only funnel traps. These included Labrundinia, Paratanytarsus and Corynoneura. One chironomid, called "Chironomini Genus 1" herein, was collected during a very early developmental stage. Hundreds of these individuals were collected by funnel traps only during the summer and spring sampling periods. Several chironomid experts were consulted in an effort to identify this taxon. Unfortunately, due to the level of immaturity and very small size, identification below

subfamily was not possible. Many individuals of another chironomid genus, Gluttipelopia, were collected utilizing funnel traps, while only one specimen was collected with a dip net. Other dipteran taxa collected only with funnel traps included the biting midge genera (family Ceratopogonidae) Ceratopogon, Alloudomyia, Serromyia and Stillobezzia and the mosquito genus (family Culicidae) Mansonia. These results indicate that funnel traps are very efficient collectors of small macroinvertebrates, especially Chironomidae and the thread-like Ceratopogonidae. A variety of coleopteran genera were also only collected using funnel traps. These included predacious diving beetle genera (family Dytiscidae) Heterosternuta, Copelatus, Pachydrus, Rhantus, and Illybius and the crawling water beetle genus (family Haliplidae) Haliplus. Funnel traps alone also added a few genera of Odonata, including the corduliid dragonfly genus Somatochlora, the gomphid dragonfly genus Arigomphus and the coenagrionid damselfly genus Amphiagrion. None of the genera mentioned were sampled in large numbers, with the exception of the chironomid genera, indicating the majority may have been uncommon taxa within ponds. Alternatively, these taxa may simply be ones less likely to move into or colonize the funnel traps.

Dip nets alone accumulated additional taxa within orders Diptera, Coleoptera, Odonata, Hemiptera and Ephemeroptera. These results could be partially due to the mobility of the dip net and thus the ability to collect from multiple habitats when using this method. Coleoptera taken only using dip nets included an undetermined Staphylinidae (rove beetles), the water scavenger beetle genera (family Hydrophilidae) Cymbiodyta, Derallus, Enochrus, Hydrobiomorpha, Hydrophilus Hydrochus, Hygrotus, Laccobius and Paracymus and the marsh beetle genus (family Scirtidae) Scirtes. Several

dipteran taxa were also collected using only dip nets; however, none were typically ubiquitous chironomids. These included mosquito genera *Aedes* and *Culista*, the deer fly genera (family Tabanidae) *Chrysops*, *Diachlorus* and *Haematopota*, the crane fly genus (family Tipulidae) *Erioptera*, as well as an undetermined genus of the family Syrphidae (hover flies). Odonata collected using only dip nets included the coenagrioinid damselfly genus *Argia*, the corduliid dragonfly genera *Epicordulia*, *Epitheca* and *Neurocordulia*, and the aeshnid dragonfly genus *Boyeria*. *Callibaetis* was the single genus of Ephemeroptera to be sampled with a dip net alone. Finally, three neustonic true bug genera (order Hemiptera) including the velvet water bug genus (family Hebridae) *Merragata*, the water strider (family Gerridae), *Gerris* and water measurer (family Hydrometridae), *Hydrometra* were collected using only dip nets. The surface-dwelling hemipteran genera would never be collected in a submerged funnel trap.

Certain chironomid genera such as *Omisus*, *Labrundia* and *Guttipelopia* were only collected within non-cattle ponds. The chironomid *Zavreliella* was also mostly found within non-cattle ponds. These four chironomid taxa are quite tolerant to low oxygen environments, with *Zavreliella* and *Labrundia* preferring a eutrophic, highly herbaceous and vegetation-filled habitat (Epler, 2001), which describes the non-cattle ponds sampled in this study. Other chironomids such as *Goeldichironomus* and *Tanytarsus* are also very tolerant of organically-enriched habitats, which would explain their occurrence only in cattle ponds (Epler, 2001) where cattle pollute the ponds with urine and feces

These results further demonstrate that the two sampling methods employed have the ability to sample different taxa more efficiently at certain times of the year and in

certain habitat types. Furthermore, after three sampling seasons, many taxa were only sampled with one method or the other, but not both. Thus, a combination of sampling methods would better assess actual community diversity within ponds.

## Habitat Variability among Cattle and Non-Cattle Ponds

Cattle ponds were often located in or adjacent to larger open fields or close to an access road. These ponds had limited canopies of woody vegetation due to constant cattle disturbance of banks and the mostly open pasture surrounding them, but the pond bottom substrate while muddy, was typically firm and silty. A firm bottom substrate with little structure allowed collectors performing dip net sampling to move easily and sample more of the available microhabitats.

In comparison, non-cattle ponds were often surrounded by vegetation including well-developed canopies of woody shrubs and trees. Because of this vegetation, these ponds contained abundant course and fine particulate organic matter. The organic material coupled with high levels of decomposition resulted in mucky, debris-filled ponds with very soft bottom substrate. This encumbered the movement of the samplers within the non-cattle ponds. Also, the large amounts of leaves and other organic material in each dip net sample made it difficult to pick through the sample quickly and thoroughly. The combination of the difficultly in moving in non-cattle ponds and the challenges of picking through large amounts of organic materials hindered the efficiency of the timedeffort sampling in the non-cattle ponds.

# Advantages and Disadvantages to Sampling Methods

Both sampling methods used during this study had advantages and disadvantages. The funnel traps were very time-consuming with regard to deployment time and personnel travel time because each sample was comprised of two consecutive 48-hour periods. Anytime traps were left unattended, there was potential for both cattle and human disturbance. While no traps were disturbed during this project, in previous years, samplers have been lost. Also, funnel trap sampling success is based on the macroinvertebrates swimming, crawling, or drifting into the traps, but at the same time, traps provided a substrate for quick colonization of certain taxa, often at levels that indicated dominance. However, because the traps were submerged they did not collect neuston. To their advantage, funnel traps were easily deployed and utilized in both pond types and allowed samples to be collected in a quantitative manner. Habitat disturbance was minimal with this method and funnel trap success is not dependent on field personnel experience or collecting efficiency.

In contrast, dip nets were highly dependent on experience of field personnel and, considering samples were field picked, this method can lead to sampler bias due to lack of familiarity with the variety and forms that could be encountered. Likewise, the collector's eye is drawn to larger and more active forms, and so, particularly for the less experience field personnel, this method may result in a bias toward these organism. Due to the sampling defined parameters of this approach, it did not result in collection of truly quantitative data. Dip nets were advantageous in regards to time efficiency and the ability

to sample from multiple habitats, although when time is limited as it was in this study, difficult habitats can reduce the sampler's collecting efficiency. Samples collected with dip nets were field picked and free of most debris making processing of the samples in the lab simpler. Additionally, this method was not dependant on the activity of macroinvertebrates, but rather the activity of personnel collecting samples.

### CHAPTER VI

### **CONCLUSIONS**

- 1. Analysis of data showed significant differences in both taxa richness and Shannon-Weaver values when comparing the two sampling techniques seasonally and by pond use. However, Shannon-Weaver results may not provide an accurate depiction of community structure because this method requires comparison of quantitative sampling methods and this study compared both a quantitative and qualitative method.
- Jaccard's Similarity Coefficient indicated collection of different sets of taxa using the two collection methods. Pond types were significantly different with regard to taxa similarity.
- 3. Dip net sampling was a more time-efficient method. It required fewer person-hours to complete, saving time and money.
- 4. However, dip net sampling was not quantitative, and often led to sampler bias specifically within smaller taxa such as Chironomidae, which were difficult to see when picking through debris in pans. These smaller taxa were collected more frequently using funnel traps.
- 5. Funnel trap samples were dependent upon activity of invertebrates and thus, were less likely to collect more sedentary or habitat specific organisms. Alternatively, dip nets allowed for multi-habitat sampling and performed better in collecting sedentary taxa or habitat specific taxa.

- 6. Funnel traps acted as artificial substrate for quick colonizing taxa like Chironomidae larvae and water mites and was the best method of capture for such groups.
- 7. These results and taxa accumulation curve comparisons indicate that a combination of the two approaches would be the best strategy for obtaining the most accurate representation of community diversity within ponds. However, if specific taxa are targeted, a single method may be more efficient. The sampler should consider habitat preference of targeted taxa when choosing a collection method.
- Observed sampling method constraints and habitat limitations to sampling effort strongly suggest choice of sampling technique should be based on habitat structure.
- Timed-effort sampling cannot be standardized if habitat complexity is not taken into consideration and passive sampling alone will not produce accurate community diversity data.
- 10. More studies are necessary to fully understand the function of macroinvertebrate communities within these habitats and their usefulness in assessing long-term health of the ponds on MLAAP.

LITERATURE CITED

- Beas, B. 2007. Herpetofaunal diversity and abundance at cattle-access and non-cattle access ponds at the Milan Army Ammunition Plant, Gibson and Carroll counties, Tennessee. Unpublished Master's Thesis, Austin Peay State University.
- Beccera Jurado, G., M. Masterson, R. Harrington and M. Kelly-Quinn. 2008. Evaluation of sampling methods for macroinvertebrate biodiversity estimation in heavily vegetated ponds. Hydrobiologia 597: 97-107.
- Best, E.P., S.L. Spreche, S.L. Larson, H.L. Fredrickson, and D.F. Bade. 1999.

  Environmental behavior of explosives in ground water from the Milan Army

  Ammunition Plant in aquatic and wetland plant treatments. Uptake and Fate of

  TNT and RDX in Plants. Chemosphere 39: 2057-2072.
- Biggs, J., P. Williams, P. Whitfield, P. Nicolet and A. Weatherby. 2005. 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation Aquatic Conservation. Marine and Freshwater Ecosystems 15: 693-714.
- Brew, P. and J. Markol, LTC. 2001. Installation Action Plan for Milan Army Ammunition Plant.
- Brinkley, D. 2009. The Wilderness Warrior: Theodore Roosevelt and the Crusade for America. Harper Collins Publishers, New York.
- Carson, R.L., E.O. Wilson, L. Lear. 2002. Silent Spring 40<sup>th</sup> Anniversary Edition.

  Mariner Books, Boston, Massachusetts.
- Carson, R.L. 1962. Silent Spring. Houghton Mifflin, Boston, Massachusetts.

- Carter, J.L. and V. H. Resh. 2001. After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. Journal of the North American Benthological Society 20: 658-682.
- Cereghino, R., J. Biggs, B. Oertli and S. Declerck. 2008. The ecology of European ponds: defining the characteristics of a neglected freshwater habitat. Hydrobiologia 597: 1-6.
- Cole, G.A. 1983. Textbook of Limnology. C.V. Mosby Company, St. Louis, Missouri.
- Conner, A.O., S. Bradish, T. Reed, J. Moran, E. Regan, M. Visser, M. Gormally and M.S. Skeffington. 2004. A comparison of the efficacy of pond-net and box sampling methods in Turloghs-Irish ephemeral aquatic systems. Hydrobiologia 524:133-144.
- Curtis, P. 2005. Guide to Food Laws and Regulations. Blackwell Publishing Ames, Iowa.
- Declerck, S., T. De Bie, D. Ercken, H. Hampel, S. Schrijvers, J. Van Wichelen, V. Gillard, R. Mandiki, B. Losson, D. Bauwens, S. Keijers, W. Vyverman, B. Goddeeris, L. De Meester, L. Brendonck and K. Martens. 2006. Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. Biological Conservation 131: 523-532.
- Di Luzio, F.C. 1967. Water pollution control: An American must. Journal (Water Pollution Control Federation) 39: 1-7.
- Downing, J.A., J.J. Cole, J.J Middelburg, R.G. Striegel, C. M. Duarte, P. Kortelainen, Y.T. Prairie and K.A. Laube. 2008. Sediment organic carbon burial in

- agriculturally eutrophic impoundments of the last century. Global Biogeochemical Cycles 22: GB1018.
- Epler, J.H. 1996. Identification Manual for the Water Beetles of Florida.

  <a href="http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/beetles96.pdf">http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/beetles96.pdf</a>
- Epler, J. H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. <a href="http://www.esb.enr.state.nc.us/BAUwww/Chironomid.htm">http://www.esb.enr.state.nc.us/BAUwww/Chironomid.htm</a>
- Ferrington, L. C., M.B. Berg and W.P. Coffman. 2008. Chironomidae. In Merritt, R.W., K.W. Cummins and M.B. Berg (editors). An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing, Dubudue, Iowa.
- Fox, S.R. 1985. The American Conservation Movement: John Muir and His Legacy.
  University of Wisconsin Press, Madison, Wisconsin.
- García-Criado, F. and C. Trigal. 2005. Comparison of several techniques for sampling macroinvertebrates in different habitats of a North Iberian pond. Hydrobiologia, 545: 103–115.
- Glasby, G.P. 1988. Entropy, pollution and environmental degradation. Ambio 17:330-335.
- Griffith, G.E., J.M. Omerick and S.H. Azevedo. 1997. Ecoregions of Tennessee. EPA 600R-97-022-516. Corvallis, OR: U.S. Environmental Protection Agency.
- Grove, E.W. 1979. Present and prehistoric problems of natural resources. American Journal of Agricultural Economics 61:612-619.
- Henke, J.A., and D. P. Batzer. 2005. Efficiency of four different sampling methods of wetland macroinvertebrates. Proceedings of the 2005 Georgia Water Resources Conference.

- Herbst, D. B. and E.L. Silldorff. 2006. Comparison of the performance of different bioassessment methods: similar evaluations of biotic integrity from separate programs and procedures. Journal of the North American Benthological Society 25:513-530.
- Hilsenhoff, W.L. 1991. Comparison of bottle traps with a d-frame net for collecting adults and larvae of Dytiscidae and Hydrophilidae (Coleoptera). The Coleopterists Bulletin 45:143-146.
- Hird, J.A. 1993. Environmental policy and equity: The case of superfund. Journal of Policy Analysis and Management 12:323-343.
- Jackson, J. K., J. M. Battle and B.W. Sweeney. 2009. Monitoring the health of large rivers with macroinvertebrates: Do Dominant taxa help or hinder the assessment? River Research and Applications 26: 931–947.
- Johnson, B.L. 1995. Nature, extent, and impact of superfund hazardous waste sites.

  Chemosphere 31: 2415-2428.
- Kotas, G. 1997. The future of pollution prevention. Chapter 8 of United States
  Environmental Protection Agency Pollution Prevention 1997: A National
  Progress Report.
- Kuzmiak, D.T. 1991. The American environmental movement. The Geographical Journal 157: 265-278.
- Ludwig, J.A., and J.F. Reynolds. 1988. Statistical Ecology, A Primer on Methods and Computing. John Wiley & Sons, New York.
- Mackie, G. 2008. Applied Aquatic Ecosystem Concepts. Kendall/Hunt Publishing Company, Dubuque, Iowa.

- Malcolm Pirnie, Inc. 2010. Milan Army Ammunition Plant (MLAAP) Environmental Assessment for the Implementation of actions to Relocate Production Operations from MLAAP to IAAAP.
- Mathews, R.A., A.L. Buikema, Jr., J. Cairns, Jr., and J.H. Rogers, Jr. 1982. Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. Water Research 16:129-139.
- Meester, L.D., S. Declerck, R. Stoks, G. Louette, F. Van De Meutter, T. De Bie, E.

  Michels and L. Brendonck. 2005. Ponds and pools as model systems in

  conservation biology, ecology and evolutionary biology. Aquatic Conservation:

  Freshwater Ecosystems 15: 715-725.
- Merritt, R.W., K.W. Cummins and M.B. Berg (editors). 2008. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing, Dubudue, Iowa.
- Muzaffar, S. B., and M. H. Colbo. 2002. The effects of sampling technique on the ecological characterization of shallow, benthic macroinvertebrate communities in two Newfoundland ponds. Hydrobiologia 477:31-39.
- Nicolet, P., J. Biggs, G. Fox, M.J. Hodson, C. Reynolds, M. Whitfield and P. Williams.

  2004. The wetland plant and macroinvertebrate assemblages of temporary ponds in England and Wales. Biological Conservation 120: 261-278.
- Niemi, G. J., and M.E. McDonald. 2004. Application of Ecological Indicators. Annual Review of Ecology, Evolution, and Systematics 35: 89-111.
- Oertli, B., J.D. Auderset, E. Castella, R. Juge and J.B. Lachavanne. 2000. Biological diversity and ecological typology of ponds and small lakes of Switzerland. Swiss

- Agency for the Environment, Forests and Landscape, Laboratory of Ecology and Aquatic Biology, University of Geneva, Geneva.
- Oertli, B., D.A. Joye, E. Castella, R. Juge, D. Cambin, J.B. Lachavanne. 2002. Does size matter? The relationship between pond area and biodiversity. Biological Conservation 104: 59-70.
- Oertli, B., J. Biggs, R. Céréghino, P. Grillas, P. Joly and J.B. Lachavanne (editors).

  2005. Special issue: Conservation and monitoring of pond biodiversity. Aquatic Conservation: Freshwater Ecosystem 15: 535-730.
- Oertli, B., N. Indermuehle, S. Angelibert, H. Hinden and A. Stoll. 2008.

  Macroinvertebrate assemblages in 25 high alpine ponds of the Swiss National

  Park (Cirque of Macum) and relation to environmental variables. Hydrobiologia

  597: 29-41.
- Oertli, B., R. Cereghino, A. Hull and R. Miracle. 2009. Pond conservation: from science to practice. Hydrobiologia 634: 1-9.
- Peterson, R.W. 1994. Teddy Roosevelt: The Conservation president. A Boys Life 28-29.
- Portney, P.R., and R. N. Stavins (editors). 2000. Public policies for Environmental Protection. Resources for the Future, Washington, D.C.
- Riley, T.Z. and D.M. DeRoja. 1989. Early decomposition and invertebrate colonization of nodding smartweed leaves. Wetlands 9: 219-225.
- Roni, P. (editor). 2005. Monitoring Stream and Watershed Restoration. American Fisheries Society, Bethesda, Maryland.
- Rosenberg, D.M., and V.H. Resh (editors). 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York, New York.

- Schnelle, K.B. Jr., and C.A. Brown. 2002. Air pollution Control Technology Handbook. CRC Press LLC, Boca Raton, Florida.
- Smith, M.E. 1985. Tubificid worms: Important organisms in aquatic ecosystems. The American Biology Teacher 47:412-415.
- Stead, F.M., and P. H. McGauhey. 1968. Air, water, land, and people. Journal (Water Pollution Control Federation) 40:233-240.
- Stein, M., T. C. McMahon, A. S. Lavin, F. M. Feldman, S. R. Gail and H. E
  Dunkelberger, Jr. 1971. Enforcement in water pollution control. Journal (Water
  Pollution Control Federation) 43: 179-199.
- Stephenson, S. and M.L. Kennedy. 2008. Conserving biodiversity on military lands: A guide for natural resources managers. Natureserve, Arlington, VA. <a href="http://www.dodbiodiversity.org/case\_studies/ch\_11\_1.html">http://www.dodbiodiversity.org/case\_studies/ch\_11\_1.html</a>>. Accessed 16 June, 2010.
- Thorp, J. H. and A. P. Covich. 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Inc., San Diego, California.
- USEPA (US Environmental Protection Agency). 1998. EPA Superfund Record of

  Decision: Milan Army Ammunition Plant. EPA/ROD/R04-98/020.

  <a href="http://www.epa.gov/superfund/sites/rods/fulltext/r0498020.pdf">http://www.epa.gov/superfund/sites/rods/fulltext/r0498020.pdf</a>>. Accessed 16

  June, 2010.
- USEPA (US Environmental Protection Agency). 1999. Technology Demonstration Plan Evaluation of Explosives Field Analytical Techniques. <a href="http://www.dtic.mil/cgibin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=ADA483431">http://www.dtic.mil/cgibin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=ADA483431</a>>.

Accessed 12 November 2010.

- USEPA (US Environmental Protection Agency). 2000a. Draft Final Five-Year Review Milan Army Ammunition Plant (MAAP) Milan, Tennessee. EPA3196301G. <a href="http://www.epa.gov/superfund/sites/fiveyear/f01-04005.pdf">http://www.epa.gov/superfund/sites/fiveyear/f01-04005.pdf</a>. Accessed 16 June 2010.
- USEPA (US Environmental Protection Agency). 2000b. EPA Superfund Record of Decision: Milan Army Ammunition Plant. EPA/ROD/R2000040001364. <a href="http://www.epa.gov/superfund/sites/rods/fulltext/r2000040001364.pdf">http://www.epa.gov/superfund/sites/rods/fulltext/r2000040001364.pdf</a>. Accessed 16 June 2010.
- U.S. Code of Federal Regulations, 1972. Federal Water Pollution Control Amendments of 1972. <a href="http://water.epa.gov/lawsregs/guidance/wetlands/CWAwaters.cfm">http://water.epa.gov/lawsregs/guidance/wetlands/CWAwaters.cfm</a>.

  Accessed 29 October, 2010.
- Vlach, J., P. Lambdin, C. Dilling, J. Grant, D. Paulsen and G. Wiggins. 2010. Diversity of the insect fauna within the unique sinking pond habitat in middle Tennessee.

  Journal of the Tennessee Academy of Science 85: 62-86.
- Voshell, J.R. 2002. A Guide to Common Freshwater Invertebrates of North America. The McDonald and Woodward Publishing Company, Blacksburg, Virginia.
- Watson-Ferguson, K. 2006. Guide to Aquatic Insects and Crustaceans. Stackpole Books Publishing, Mechanicsburg, Pennsylvania.
- Wiederholm, T. 1986. Chironomidae of the Holarctic Region. Entomologica Scandinavica Supply, Sandby, Sweden.
- Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet and D. Sear. 2004.

  Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115: 329-341.

#### APPENDIX A

**Individual Pond Photos** 



Figure A-1. Photo of pond C-1, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-2. Photo of pond C-2, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-3. Photo of pond C-4, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-4. Photo of pond C-5, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-5. Photo of pond C-7, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-6. Photo of pond NC-5, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-7. Photo of pond NC-6, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-8. Photo of pond NC-7, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-9. Photo of pond NC-8, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.



Figure A-10. Photo of pond NC-9, Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

#### APPENDIX B

Abiotic Field Data Collection Sheets

# MILAN ARMY AMMUNTHON PLANT - POND PROJECT

Weather conditions Date Temp. D.O. D.O. pH Spec. (°C) (mg/L) LOCATION (% SAT.) TDS Turbid. Cond. (g/L) (NTU) (mS/cm)

Notes and Comments:

### APPENDIX C

Abiotic Data

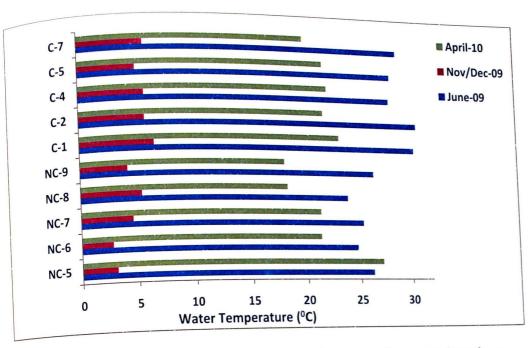


Figure C-11. Water temperatures during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

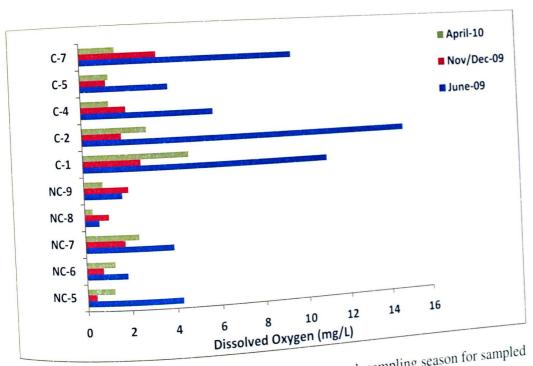


Figure C-12. Dissolved oxygen levels (mg/L) during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

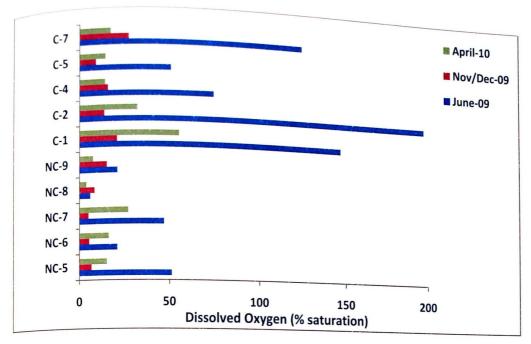


Figure C-13. Percent saturation of dissolved oxygen during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

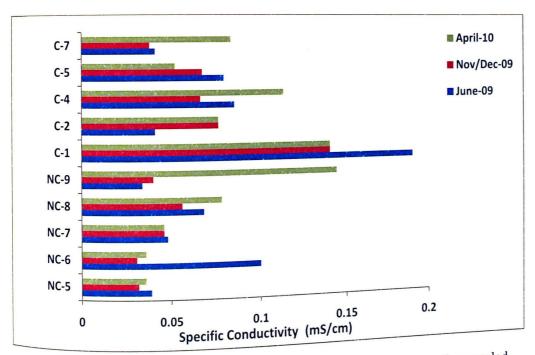


Figure C-14. Specific conductivity (mS/cm) during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

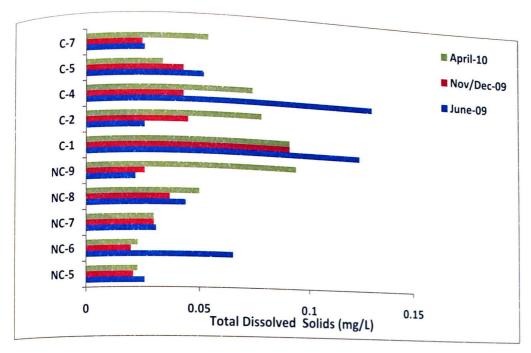


Figure C-15. Total dissolved solids (mg/L) during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

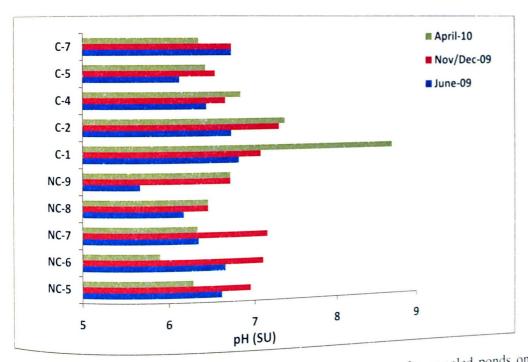


Figure C-16. The pH levels (SUs) during each sampling season for sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

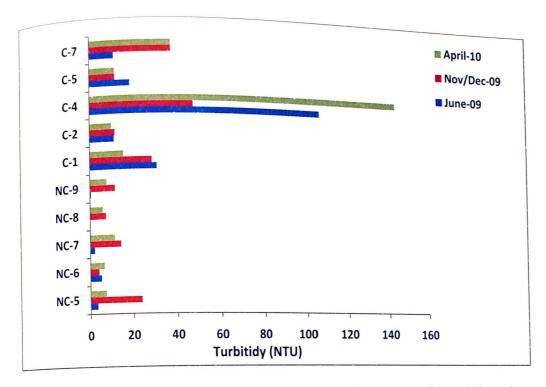


Figure C-17. Turbidity levels (NTUs) during each sampling season of sampled ponds on Milan Army Ammunition Plant, Carroll and Gibson counties, Tennessee.

## APPENDIX D

Taxonomic Data Sheets

	C-1 Trap June 2009	C-1 Net June 2009	C-2 Trap June 2009	C-2 Net June 2009	C-4 Trap June 2009	C-4 Net June 2009	C-5 Trap June 2009	C-5 Net June	C-7 Trap June	C-7 Net June
TAXA							2009	2009	2009	2009
BIVALVIA										
Sphaeriidae		12		8	2	12	2			
GASTROPODA									1	9
Ancylidae	-									
Limnaeidae					1				1	
Physidae		4			2	4	42			
Planorbidae		4				1	42	1	4	4
HIRUDINEA		32		24	2	5	14	1		
OLIGOCHAETA	3	5	2		160	8	163	4	4	2
NEMATODA		2		2		2	3	4	7	7
HYDRACARINA	509		45		2		5		67	1
DECAPODA										
Cambaridae										
Procambarus										
MEGALOPTERA										
Corydalidae										
Chauliodes										
Sialidae										
Sialis			1						19	14
EPHEMEROPTERA										
Baetidae	1				4					
Baetis										
Callibaetis						3				
Procloeon			2		1		1			1
Caenidae										2
Caenis		1		1	1	1				
ODONATA										
Anisoptera										
Aeshnidae										
Aeshna					4					

	C-1 Trap June 2009	C-1 Net June 2009	C-2 Trap June 2009	C-2 Net June 2009	C-4 Trap June 2009	C-4 Net June 2009	C-5 Trap June 2009	C-5 Net June	C-7 Trap June	C-7 Net June
Anax	2				3	3	2009	2009	2009	2009
Boyeria						3				2
Corduliidae										
Epitheca										
Neurocordulia										
Somatochlora					2		2			
vii -llulidae										
Libellulidae										
Erythemis						4				
Erythrodiplax										
Libellula				1			6			
Pachydiplax				1		4	2			
Perithemis										
Plathemis			6	32	1	14				2
Sympetrum					3					
Zygoptera										
Coenagrionidae			1			1			2	
Amphiagrion	1									
Chromagrion		1								
Enallagma/Coenagrio	2	2	1	24	6	6	2			
Ischnura		7				13		9		
Nehalenniae										
Telebasis					1			2		
Lestidae										
Lestes		3		3	1		1		1	
Unidentified	2				1					
TRICHOPTERA										
Hydroptilidae									1	
Hydroptila										
lanta								1		
Leptoceridae Oecetis								15		

	C-1 Trap June 2009	C-1 Net June 2009	C-2 Trap June 2009	C-2 Net June 2009	C-4 Trap June 2009	C-4 Net June 2009	C-5 Trap June	C-5 Net June	C-7 Trap June	C-7 Net June
Phryganeidae					,,,	2009	2009	2009	2009	2009
Ptilostomis										
HEMIPTERA										
Belostomatidae										
Belostoma	2	4	2	5	5	3	6	3		2
Notonectidae										
Buenoa		28		9		9	4	11		
Notonecta	1	2	9	4	36	6	7	7		11
Corixidae	2				139				1	
Hesperocorixa		29	1		4	1	10	2	2	15
Ramphocorixa								-	-	1.0
Trichorixa		1	1		23					
Gelastocoridae										
Gelastocoris		1								
Gerridae										
Gerris										. 3
Trepobates				1						
Hydrometridae										
Hydrometra										
Mesoveliidae										
Mesovelia		1								
Microveliidae										
Microvelia										
Nepidae							,	1		2
Ranatra				7	1		1			
Pleidae							185			
Neoplea				1	1		183			

	Trap June 2009	Net June 2009	C-2 Trap June 2009	C-2 Net June 2009	C-4 Trap June 2009	C-4 Net June 2009	C-5 Trap June	C-5 Net June	C-7 Trap June	C-7 Net June
COLEOPTERA						2009	2009	2009	2009	2009
Dytiscidae										
Larvae										
Acilius										
Agabetes								1		
Agabus sp 1 (Red)										
Agabus sp 2 (Striped)										
Agabus larvae										
Bidessonotus				2						
Coptotomus		23		3		9				
Cybister									2	
Desmopachria						22	4		2	
Dytiscus							,			
Heterostemuta							1			
Hvdroporus		1				1	1			
Hydrovatus		6								
Hygrotus										
Laccornis										
Laccophilus	24	74	75	22	23	32	3	2	1	
Neoporus	1	28	1	18	23				43	
Pachydrus							11			
Thermonectus						1	1			
Thermoneerus										
Gyrinidae										
Dineutus										
Haliplidae										
Haliplus										
Peltodytes				8		3			2	
,										
Hydrophilidae	1									
Berosus	1	4	3	3	1		2		1	
Derallus	1	1		3		1				
Dibolocelus		1								
Hydrobius				1						
Hydrochus				7		2				
Paracymus				1		11			2	
Tropisternus	2	8	5	15	12	32	4			

	Trap June	C-1 Net June	C-2 Trap June	C-2 Net June	C-4 Trap June	C-4 Net	C-5 Trap	C-5 Net	C-7 Trap	C-7
	2009	2009	2009	2009	2009	June 2009	June 2009	June 2009	June 2009	Net June 2009
Noteridae	-									
Hydrocanthus					17	10				
Suphisellus		2	1		8	19			1	
Scirtidae					0		2		1	
Cyphon										
Scirtes										
Staphylinidae		4								
0.14										
DIPTERA										
Larva										
Рира	1									-
Ceratopogonidae										
Alluaudomyia										
Bezzia	4		1				1	2		
Culicoides										
Mallochohelea										
Probezzia										
Serromyia										
Sphaeromias				2						_
Chaoboridae										
Chaoborus					1	2			5	-
Chironimidae										-
Chironominae										
Chironomini			1						1	
Chironomini Genus 1	18		1				1		7	3
Chironomus	1		5				38		1	3
Dicrotendipes				1					9	1.
Endochironomus	4		7		5		1	2	9	1
Glyptotendipes	1		2		5		4	3		
Goeldichironomus	1							7		
Kiefferulus	1	7		1	2	1	77	1		
Parachironomus	1	,					1			

	Trap June 2009	Net June 2009	Trap June 2009	C-2 Net June 2009	C-4 Trap June 2009	C-4 Net June 2009	C-5 Trap June 2009	C-5 Net June	C-7 Trap June	C-7 Net June
Polypedilum	3		1				6	2009	2009	2009
Zavreliella							0		5	
								2		
Tanytarsini										
Paratanytarsus										
Tanytarsus	14	1	4		5				2	
									2	
Cylorrhaphous										
Brachycera										
·										
Orthocladinae										
Corynonneura									1	
Orthocladius										
Tanypodinae	8		1							
Ablabesmyia	1		1			1		2	1	
Clinotanypus		2				1				
Larsia										
Procladius	1				1	1				
Psectrotanypus					1		2			
Tanypus	3		2		2	2	1		5	1
.,,										
Culicidae										
Pupae										
Anopheles										
Culex										-
Culiseta										
Sciomyzidae										
Syrphidae										
Tabanidae										
Chrysops										
Diachlorus										

	C-1 Trap Dec 2009	C-1 Net Nov 2009	C-2 Trap Dec 2009	C-2 Net Nov 2009	C-4 Trap Dec 2009	C-4 Net Nov 2009	C-5 Trap Dec	C-5 Net Nov	C-7 Trap Dec	C-7 Net Nov
TAXA						2009	2009	2009	2009	2009
BIVALVIA										
Sphaeriidae		1		4	1	5				
GASTROPODA		1			·	3	4	4		13
Ancylidae										
Limnaeidae										1
Physidae	26	7	14	7	9	12				
Planorbidae						12		2	9	8
HIRUDINEA	1						5			
OLIGOCHAETA		1		6	3	6	5	1	1	
NEMATODA						0	24	7		6
HYDRACARINA							24			
DECAPODA						3			2	
Cambaridae									_	1
Procambarus					1					
MEGALOPTERA										
Corydalidae										
Chauliodes								2		
Sialidae										
Sialis										8
EPHEMEROPTERA										
Baetidae										
Baetis						4				
Callibaetis										<u> </u>
Procloeon		4	1	8					1	
Caenidae										
Caenis										
ODONATA										
Anisoptera										
Aeshnidae										
Aeshna										

	Trap Dec 2009	C-1 Net Nov 2009	C-2 Trap Dec 2009	C-2 Net Nov 2009	C-4 Trap Dec 2009	C-4 Net Nov 2009	C-5 Trap Dec 2009	C-5 Net Nov	C-7 Trap Dec	C-7 Net Nov
Anax						6	2009	2009	2009	2009
Boyeria						0				
Corduliidae										
Epitheca										
Neurocordulia						-				
Somatochlora										
Somatochiora										
Libellulidae										
Erythemis								4		
Erythrodiplax								4		
Libellula										
Pachydiplax						2				_
Perithemis										
Plathemis		7		19		31	2	7		15
Sympetrum										1.0
Zygoptera										
Coenagrionidae						3				
Amphiagrion										
Chromagrion										
Enallagma/Coenagrion				2			1			
Ischnura			1	7	14	51		6		3
Nehalenniae							2		1	
Telebasis		1								
Lestidae										
Lestes		1				2				
		,								
TRICHOPTERA										
MEHOPTERA										
Hydroptilidae										
Hydroptila										
Leptoceridae										
Oecetis										

	C-1 Trap Dec 2009	C-1 Net Nov 2009	C-2 Trap Dec 2009	C-2 Net Nov 2009	C-4 Trap Dec 2009	C-4 Net Nov 2009	C-5 Trap Dec	C-5 Net Nov	C-7 Trap Dec	C-7 Net
Phryganeidae						2009	2009	2009	2009	Nov 2009
Ptilostomis										
HEMIPTERA										2
Belostomatidae										
Belostoma				3		1		1		1
Notonectidae										·
Buenoa			1		2					
Notonecta	1	32	3	23	11	18		1	16	14
Corixidae			1							
Hesperocorixa	4	8	5	6	9	1	6	2	66	13
Ramphocorixa									00	13
Trichorixa		4	3					1		3
Gelastocoridae										
Gelastocoris										
Gerridae										
Gerris										
Trepobates										
Hydrometridae										
Hydrometra										
Mesoveliidae										
Mesovelia						5				
Microveliidae										
Microvelia						2				
Nepidae										
Ranatra						2				
Pleidae								11		
Neoplea								11		

	Trap Dec 2009	Net Nov 2009	C-2 Trap Dec 2009	C-2 Net Nov 2009	C-4 Trap Dec 2009	C-4 Net Nov 2009	C-5 Trap Dec	C-5 Net Nov	C-7 Trap Dec	C-7 Net Nov
COLEOPTERA						2009	2009	2009	2009	2009
Dytiscidae										
Larvae				7						
Acilius										
Agabetes										
Agabus sp 1 (Red)	1		3		28	1		1		
Agabus sp 2 (Striped)	11	7	19	5	31	1	6			
Agabus larvae	1	5	3			·	0		19	
Bidessonotus										
Coptotomus	1	5	1	1		2				
Cybister									5	
Desmopachria										
Dytiscus									1	
Heterostemuta										
Hydroporus										
Hydrovatus									33	
Hygrotus				1		12				
Laccornis						1.2				
Laccophilus		20		10	2	6	1	8		
Neoporus	9	12	13	10	44	6	5	1	28	1
Pachydrus		12	1.7	10						
Thermonectus		1		1	1	2				
rnermonectus		'								
Gyrinidae										-
Dineutus										
Haliplidae										
Haliplus									1	
Peltodytes					5	8	3	3	2	
renogytes	1	9								_
Hydrophilidae										
Berosus		8								
Derallus										
Dibolocelus										
Hydrobius										
Hydrochus										
Paracymus						1		10	5	
Tropisternus	2	30	5	14	4	17		10		

	C-1 Trap Dec 2009	C-1 Net Nov 2009	C-2 Trap Dec 2009	C-2 Net Nov 2009	C-4 Trap Dec 2009	C-4 Net Nov 2009	C-5 Trap Dec	C-5 Net Nov	C-7 Trap Dec	C-7 Net Nov
Noteridae						2009	2009	2009	2009	2009
Hydrocanthus										
Suphisellus										
Scirtidae										
Cyphon	-									
Scirtes				1						
										1
Staphylinidae										-
DIPTERA										
Larva										
Рира						2				
-										
Ceratopogonidae										
Alluaudomyia							1			
Bezzia		2								
Culicoides								1		
Mallochohelea					1					
Probezia										
Serromyia										
Sphaeromias										
Chaoboridae										1
Chaoborus					2		1	6		
Chironimidae										
Chironominae										
Chironomini				1						-
Chironomini Genus I										
Chironomus									1	1
Dicrotendipes										10
Endochironomus		1	1						4	19
Glyptotendipes		1		2						
Goeldichironomus		2	1		1	3			4	14
Kiefferulus		-	,	1			2	28	4	,
Parachironomus									1	2
Polypedilum				2	30		8		,	
Zavreliella	-									

	C-1 Trap	C-1 Net	C-2 Trap	C-2	C-4	C-4	C-5	0		
	Dec 2009	Nov 2009	Dec 2009	Net Nov 2009	Trap Dec 2009	Net Nov 2009	Trap Dec	C-5 Net Nov	C-7 Trap Dec	C-7 Net Nov
Tanytarsini						2007	2009	2009	2009	2009
Paratanytarsus		-								
Tanylarsus										1
Cylorrhaphous										
Brachycera										
Orthocladinae										
Corynonneura										-
Orthocladius				5						
Tanypodinae										
Ahlahesmyia					1		2			
Clinotanypus						1				
Larsia							1			
Procladius										
Psectrotanypus										
Tanypus										1
Culicidae										
Pupae				1						
Anopheles		1		25						1
Culex								2		1
Culiseta								1		
Sciomyzidae										2
Syrphidae										1
Tahanidae						1				-
Chrysops										1
Diachlorus										

	C-1 Trap April 2010	C-1 Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April 2010	C-5 Trap April 2010	C-5 Net April	C-7 Trap April	C-7 Net April
TAXA						-10	2010	2010	2010	2010
BIVALVIA										
Sphaeriidae		3		5	2	4				
GASTROPODA						-	5	3	1	24
Ancylidae										
Limnaeidae										
Physidae	9	2	42	3	3	3	10			
Planorbidae						- 3	18	5	54	11
HIRUDINEA			2				11			
OLIGOCHAETA		4		7	25	30	310	3	1	_
NEMATODA						.50	9	10		-
HYDRACARINA	19		68	2			30	7		-
							30	1	8	-
DECAPODA				2					2	,
Cambaridae					8					
Procambarus					2				1	
MEGALOPTERA										
Corvdalidae										
Chauliodes										
Sialidae										_
Stalis									1	-
EPHEMEROPTERA										
Bactidae										
Baetis										
Callibactis										-
Proclocon		1		11						
		,								
Caenidae										
Caenis										
ODONATA										
ODONATA										
Anisoptera		2								-
Aeshnidae										
Aeshna										

	C-1 Trap April 2010	C-1 Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April 2010	C-5 Trap April	C-5 Net April	C-7 Trap April	C-7 Net
Anax				20		2010	2010	2010	2010	April 2010
Boyeria										
Boyers						1				
Corduliidae										
Epitheca										
Neurocordulia										1
Somatochlora		_						-		1
5000								-		
Libellulidae										_
Erythemis								,		
Erythrodiplax						1		1		
Libellula										
Pachydiplax								1	1	
Perithemis			1					1	1	
Plathemis	7	11	1			8			3	10
Sympetrum									-	
Zygoptera										
										-
Coenagrionidae						-	_	-		
Amphiagrion							-	-		
Chromagrion										-
Enallagma/Coenagrion		1						-		-
Ischnura				1		11		33		
Nehalenniae				2				-		
Telebasis										
Lestidae								+		
Lestes										
Unidentified										
TRICHOPTERA										
Hydroptilidae						_				
Hydroptila										

	C-1 Trap April 2010	C-1 Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April 2010	C-5 Trap April	C-5 Net April	C-7 Trap	C-7 Net
Leptoceridae						2010	2010	2010	April 2010	April 2010
Oecetis										
Phryganeidae										1
Ptilostomis								,		
HEMIPTERA								1		
Belostomatidae										
Belostoma								1		
Notonectidae			1				4		3	_
Виепоа		7	9	11	4	5			1	18
Notonecta		3	2	3				1	·	6
Corixidae	4	1	4		2		83		32	8
Hesperocorixa	2	2	6	1	2	1	2	2	7	10
Ramphocorixa					2				,	23
Trichorixa						1				
Gelastocoridae										
Gelastocoris										
Gerridae										
Gerris										1
Trepobates										
Hydrometridae										
Hydrometra										1
Mesoveliidae										
Mesovelia										
Microveliidae										
Microvelia										
Nepidae										
Ranatra								1		

	Trap April 2010	Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April	C-5 Trap April	C-5 Net April	C-7 Trap	C-7 Net
Pleidae					2010	2010	2010	2010	April 2010	April 2010
Neoplea	1									2010
Inter_							36	19		
COLEOPTERA										
Dytiscidae										
Larvae										
Acilius								3	1	
Agabetes							4	1		
Agabus sp 1 (Red)			1			1				
Agabus sp 2 (Striped)	1		7			,	1			
Agabus larvae	1	4		7		5	2	22	3	
Bidessonotus							2		.5	
Coptotomus		4		2		1	-	2		
Cyhister								-		
Desmopachria										
Dytiscus							1			
Heterostemuta										
Hydroporus							1			
Hydrovatus						1	1			
Hygrotus										
Laccornis									1	
Laccophilus	2	9	6	4	2	3	5	- 3	6	
Neoporus		8	1	2	3	10	6	1.5	7	1
Pachydrus										
Thermonectus							1	4		
Gynnidae										
Dineutus		2	1	2						
Haliplidae										
Haliplus								4		1
Peltodytes	1	3	4			1		-		
Hydrophilidae										
Berosus	1	3								
Derallus										
Dibolocelus			1							

	C-1 Trap April 2010	C-1 Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April	C-5 Trap April	C-5 Net April	C-7 Trap April	C-7 Net
Hydrobius					2010	2010	2010	2010	2010	April 2010
Hydrochus										
Paracymus						1				
Tropisternus	1	1	3	2	4					
					7		7	15		
Noteridae										
Hydrocanthus										
Suphisellus					1		1		2	
Scirtidae										
Cyphon				2						
Scirtes							The State of the S			
Staphylinidae										
DIPTERA								1		
Larva										
Рира	1		1				1		1	
•										
Ceratopogonidae										
Alluaudomyia										
Bezzia						2			2	
Culicoides						1				
Mallochohelea										
Probezzia										
Serromyia	1						22			
Sphaeromias										
Chaoboridae										
Chaoborus	2		21		3	3	79		4	
	-									
Chironimidae										
Chironominae										
Chironomini								-		
Chironomini Genus 1							12		7	2
Chironomus					1		24	-	-	
Dicrotendipes							1			
Endochironomus			2				3			

	C-1 Trap April 2010	C-1 Net April 2010	C-2 Trap April 2010	C-2 Net April 2010	C-4 Trap April 2010	C-4 Net April	C-5 Trap April	C-5 Net April	C-7 Trap	C-7 Net
Glyptotendipes			1	-010	2010	2010	2010	2010	April 2010	April 2010
Goeldichironomus									6	2010
Kiefferulus			3						-	
Parachironomus					2		16	5		1
Paracini one Paracini Polypedilum		2	1							
			1			3	13	2	1	5
Zavreliella										
Tanytarsini										
Paratanytarsus										
Tanytarsus										
								1		
Cylorrhaphous										
Brachycera										
Orthocladinae										
Corynonneura										
Orthocladius			1							
Tanypodinae										
Ablabesmyia							1			
Clinotanypus		1								
Larsia			1							
Procladius		3	6						1	
Psectrotanypus		2	2		5	1	4			
Tanypus		1			3		3		1	
										_
Culicidae										_
Pupae										
Anopheles										
Culex										
Culiseta								-		
								-		
Sciomyzidae										
Sympl: 1										_
Syrphidae										-
Tabanidae										1
Chrysops	-									_
Diachlorus		1								

	NC-5 Trap June 2009	NC-5 Net June 2009	NC-6 Trap June 2009	NC-6 Net June 2009	NC-7 Trap June 2009	NC-7 Net June 2009	NC-8 Trap June	NC-8 Net June	NC-9 Trap June	NC-9 Net
TAXA						2009	2009	2009	2009	June 2009
BIVALVIA								-		
Sphaeriidae	12	10	3				,			
GASTROPODA							1			
Ancylidae			8		1			-		
Limnaeidae			17	1				-		
Physidae	9	3			9				2	3
Planorbidae					4				1	-
HIRUDINEA	40	8		13	7	2				-
OLIGOCHAETA	39	5	71	5	73	5	5		5	-
NEMATODA		2	1		1		1		31	-
HYDRACARINA	1	1					8	+	1	
							0		7	
Decapoda										
Cambaridae										
Procambarus										
7 TOCUMAN TO										
MEGALOPTERA										
Corydalidae										
Chauliodes				1						1
Sialidae										
Sialis	15								1	1
EPHEMEROPTERA										-
Baetidae										+
Procloeon							1	-	-	+
										-
ODONATA								_		+
Anisoptera									1	+
										_
Aeshnidae								-		
Aeshna								+		
								+		
Corduliidae						-	-			1
Epicordulia								+		
Gomphidae										

	NC-5 Trap June 2009	NC-5 Net June 2009	NC-6 Trap June 2009	NC-6 Net June 2009	NC-7 Trap June 2009	NC-7 Net June	NC-8 Trap June	NC-8 Net June	NC-9 Trap	NC-9 Net
Arigomphus	2				2009	2009	2009	2009	June 2009	June 2009
Libellulidae		1		1						
Erythemis							1	-		
Erythrodiplax					1	1	_	1		
Libellula						1	-			
Pachydiplax				1						
Perithemis										-
Plathemis	1					2				-
Sympetrum				1		-				1
										+
Zygoptera										
Coenagrionidae	1						,			
Amphiagrion							1			+
Argia										+
Chromagrion										+
Coenagrion										+
Enallagma										_
Ischnura	1	6		1						
Nehalenniae	1	0		<u> </u>						+
Telebasis							1			
TETEDASIS							,			
TRICHOPTERA										
							-			-
Leptoceridae						-	-			
Oecetis	29									
Phryganeidae										
Ptilostomis										+
HEMIPTERA										
Beloston		_								-
Belostomatidae		7			1	3				1
Belostoma	1				1					-
Notonectidae							-			2
Виспоа				7		9				

	NC-5	NC-5	NC-6	NC-6	NC-7	No				
	Trap June 2009	Net June 2009	Trap June 2009	Net June 2009	Trap June 2009	NC-7 Net June 2009	NC-8 Trap June	NC-8 Net June	NC-9 Trap June	NC-9 Net
Notonecta	3	2	3			2009	2009	2009	2009	June 2009
Corixidae	2				3					5
Hesperocorixa	2	5			3	1				
Trichorixa						1				3
Gerridae										
Gerris	-									
Hebridae										
Merragata								1		
Hydrometridae										
Hydrometra										
Mesoveliidae										
Mesovelia								1	1	1
Microveliidae										
Microvelia			1							1
Naucoridae	+									
Pelocoris							5	1		
Nepidae										
Ranatra		2				3			1	
Pleidae										
Neoplea		8	1	5		3	21	3	49	4
Unidentified	1									
COLEOPTERA										
							-			
Dytiscidae	6						-		2	
Acilius	2		3		3		-			
Agabetes	2		2				_			
Agabus sp 1 (Red)	1									
Agabus Larvae										

	NC-5 Trap June	NC-5 Net June	NC-6 Trap June	NC-6 Net June	NC-7 Trap	NC-7 Net	NC-8 Trap	NC-8	NC-9	NC-9
	2009	2009	2009	2009	June 2009	June	June	Net June	Trap June	Net
Bidessonotus				2	2009	2009	2009	2009	2009	June 2009
Celina			3	1					2	
Copelatus			4						1	
Coptotomus	2					_				
Cybister	1		2							
Desmopachria			1						3	2
Dytiscus			1	1						
Graphoderus										
Hydaticus									2	1
Hydroporus				1						+
Hydrovatus									-	+
llybius							<u> </u>		13	+
Laccophilus	3	1		1	1					-
Neoporus	9		2		1		1	6	7	3
Rhantus			1				1	0	7	+-
Thermonectus			1							+-
Uvarus										
0.44.11										+
Gyrinidae										+
Dineutus	3					1			2	
Haliplidae										
Peltodytes		6		6						2
Hydrophilidae										
Berosus		1								
Cymbiodyta								1		
Derallus		2								_
Dibolocelus	1	-		2	2	1				
Enochrus	1			1						+
Hydrochus		1				2				5
Hydrobiomorpha		1								
Hydrophilus										1
Laccobius										1
Paracymus									5	1
Tropisternus		0		1	2	1	1		5	+
Protettius		9		Ė						+-
Noteridae										

	Trap June 2009	NC-5 Net June 2009	NC-6 Trap June 2009	NC-6 Net June 2009	NC-7 Trap June 2009	NC-7 Net June 2009	NC-8 Trap June 2009	NC-8 Net June	NC-9 Trap June	NC-9 Net June
Hydrocanthus	_			3		1	2009	2009	2009	2009
Suphisellus	_					<u> </u>		1		
								-		
Scirtidae	_									
Cyphon			1							
Scirtes									1	1
Staphylinidae		3								
DIPTERA										
Larvae	1				1					
Pupac										
r of ac										
Ceratopogonidae					1					
Bettia	1	3	2		1					-
	1		-				1		1	-
Ceratopogon Mallochohelea	<u>'</u>				,					+
	14				1	1				-
Sulobezzia	1.4							-		-
Serromyta									-	-
Cultionles		2								-
				-						
Chaoboridae	-			-					1	-
Charlerus	16	6		1	4	1	61	1	114	12
				-						+
Chironimidae				-						+
Chironominae										-
Chironomini	13		2		1				,	
Chironomini Genus 1	1		1		4,1				2	4
( himmowing	6	3	0	1	1	6			-	
Dicrotendipes	1		4						2	
Endochtronomus									1	
(asytotendipes	5				17	4			1-	12
Kiegerulus	10	10	1	1	56	34	1		1	
( heart and	43		26	1	131	-	1		1	
Parachironomus										1
Polypedilum	1	,	31		Î		9		10	:
	-4	1			48				,	-
Astrehella	4		34		45					

	NC-5 Trap June 2009	NC-5 Net June 2009	NC-6 Trap June 2009	NC-6 Net June 2009	NC-7 Trap June 2009	NC-7 Net June 2009	NC-8 Trap June 2009	NC-8 Net June	NC-9 Trap June	NC-9 Net June
Tanytarsini						1007	2009	2009	2009	2009
Paratanytarsus	1									
Orthocladinae						-				
Corynoneura							16			-
Tanypodinae									1	
	2					-				
Ablabesmyia	1						29	6		1
Cinotanypus Gluttipelopia	_	1				-		-		
Giunipetopia Labrundinia	1	· -							2	
	,		15			-	2			
Larsia	10					-			1	
Procladius	10		1	-					2	1
Psectrotanypus	,			-	1					-
Tanypus	.4				1		1	-	12	2
Culicidae					1					
Ardes										
Anopheles	1		2							
Culer			1						6	9
Mansonia										
Uranotaenia		1	1	1	1					
										-
Sciomyzidae								-		
Stratiomyidae										
Struttomys										
Labanidae										
Haematopota										
<i>Y</i>										
Tipulidae										
Enoptera chlorophylla										
- and any min										

	NC-5 Trap Dec 2009	NC-5 Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov	NC-8 Trap Dec	NC-8 Net Nov	NC-9 Trap	NC-9 Net
TAXA				2007	2009	2009	2009	2009	Dec 2009	Nov 2009
BIVALVIA										2007
Sphaeriidae			1							
GASTROPODA						1				
Ancylidae										
Limnaeidae	1									
Physidae										1
Planorbidae	1							1		
HIRUDINEA						2			1	
OLIGOCHAETA	12		51	4	11	3			2	
NEMATODA			4	·	- 11	3	21	5		9
HYDRACARINA			,						2	
Decapoda										
Cambaridae										
Procambarus										
Procambarus										
MEGALOPTERA										
Corydalidae										
Chauliodes	-									
Sialidae										
Sialis		5				3				2
EPHEMEROPTERA										
Baetidae										
Procloeon									1	_
ODONATA										
Anisoptera										
Aeshnidae										
Aeshna										
Corduliidae										
Epicordulia Epicordulia										
Gomphidae										

	Trap Dec 2009	NC-5 Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov 2009	NC-8 Trap Dec	NC-8 Net Nov	NC-9 Trap Dec	NC-9 Net
Arigomphus						2009	2009	2009	2009	Nov 2009
Libellulidae										
Erythemis										
Erythrodiplax							1	2		
Libellula		7		12						1
Pachydiplax	1	4	2			19				
Perithemis						1		3		7
Plathemis						10				
Sympetrum						10				
Zygoptera										
Coenagrionidae										
Amphiagrion										
Argia								4		
Chromagrion								·		
Coenagrion		2								1
Enallagma				8			1		1	
Ischnura		1							4	9
Nehalenniae			1	6			5	1		
Telebasis										
TRICHOPTERA										
Leptoceridae										
Oecetis		1	2							
Phryganeidae										
Ptilostomis	1									
HEMIPTERA										
Belostomatidae										
Belostoma										
Notonectidae										
Buenoa								1		

	NC-5 Trap Dec 2009	NC-5 Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov	NC-8 Trap Dec	NC-8 Net Nov	NC-9 Trap Dec	NC-9 Net
Corixidae						2009	2009	2009	2009	Nov 2009
Hesperocorixa					11					
Trichorixa						5			2	
										1
Gerridae										
Gerris										
Hebridae										
Merragata										
Hydrometridae										
Hydrometra		1								
Mesoveliidae										
Mesovelia										
Microveliidae										
Microvelia										
Naucoridae										
Pelocoris								13		
Nepidae										
Ranatra										
Pleidae										
Veoplea						1		1		11
Unidentified										
COLEOPTERA										
Dytiscidae										
Acilius	3	2			3	1				
Agabetes									2	1
Agabus sp 1 (Red)	2	1		1		1				
Agabus Larvae										
Bidessonotus										
Celina										
Copelatus										

	Trap Dec 2009	Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov 2009	NC-8 Trap Dec	NC-8 Net Nov	NC-9 Trap Dec	NC-9 Net
Coptotomus		1			1		2009	2009	2009	Nov 2009
Cybister					·	1				
Desmopachria										
Dytiscus										1
Graphoderus										
Hydaticus						1				
Hydroporus						1	1			
Hydrovatus										
Ilybius										
Laccophilus	1	1		1						
Neoporus				10			2		10	1
Rhantus					1		2		10	
Thermonectus	1					1				
Uvarus						·				
Gyrinidae										
Dineutus						2				
Direction										
Haliplidae										
Peltodytes	1					1	1		1	1
Hydrophilidae										
Berosus										
Cymbiodyta										
Derallus										
Dibolocelus										
Enochrus										
Hydrochus										
Hydrobiomorpha		1								
Hydrophilus										
Laccobius										
Paracymus										
Tropisternus	1								2	
Protettius	1									
Noteridae										
Hydrocanthus										
Suphisellus										

	NC-5 Trap Dec 2009	NC-5 Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov	NC-8 Trap Dec	NC-8 Net Nov	NC-9 Trap Dec	NC-9 Net
Cyphon					200)	2009	2009	2009	2009	Nov 2009
Scirtes										
Staphylinidae										1
DIPTERA								1		
DIFTER										
Larvae										
Pupae										
Ceratopogonidae										
	2		2							
Bezzia	2		2							
Ceratopogon										
Mallochohelea										
Stilobezzia										
Serromyia										
Culicoides	1									-
Chaoboridae										
Chaoborus	1		5		1	6	2	1	1	3
Chironimidae										
Chironominae										
Chironomini										
Chironomini Genus 1										
Chironomus	1		7				1			
Dicrotendipes							1			
Endochironomus	1						1		1	
Glyptotendipes										<del> </del> .
Kiefferulus	2	5		28	1	17		1		1
Omisus	1	3	12	2	1	1				1
Parachironomus									12	
Polypedilum	1		2				32	1	12	
Zavreliella	2		4							
Tanytarsini										-
Paratanytarsus										
stanytursus										
Orthocladinae										
Corynoneura	2									

	NC-5 Trap Dec 2009	NC-5 Net Nov 2009	NC-6 Trap Dec 2009	NC-6 Net Nov 2009	NC-7 Trap Dec 2009	NC-7 Net Nov 2009	NC-8 Trap Dec 2009	NC-8 Net Nov	NC-9 Trap Dec	NC-9 Net Nov
Tanypodinae							2009	2009	2009	2009
Ablabesmyia	9		7							
Cinotanypus		1		2			4	1	2	1
Gluttipelopia			8							
Labrundinia	1		1							
Larsia	1									
Procladius			3							
Psectrotanypus										
Tanypus	1		2				1			1
Culicidae										
Aedes								1		
Anopheles						2				
Culex										
Mansonia										
Uranotaenia										
Sciomyzidae	1									
Stratiomyidae							1			
Stratiomys										
Tabanidae										
Настаtороtа		1								
Tipulidae										
Erioptera chlorophylla		2								

	Trap April 2010	Net April 2010	NC-6 Trap April 2010	NC-6 Net April 2010	NC-7 Trap April 2010	NC-7 Net April 2010	NC-8 Trap April	NC-8 Net April	NC-9 Trap April	NC-9 Net April
TAXA						2010	2010	2010	2010	2010
BIVALVIA										
Sphaeriidae		1	4	1	1					
GASTROPODA					,	1				
Ancylidae										
Limnaeidae		1								
Physidae					2					
Planorbidae	1	_								
HIRUDINEA	3	1	15	1	12	2				
OLIGOCHAETA	7		6	1	40				1	
NEMATODA				1	10		44	13	8	1
HYDRACARINA							,			
							1		42	
Decapoda										
Cambaridae										
Procambarus										
MEGALOPTERA										
Corydalidae										
Chauliodes							1			
Sialidae										
Sialis						2				
EPHEMEROPTERA										
Baetidae										
Procloeon										
ODONATA										
Anisoptera										
Aeshnidae										
Aeshna		1								
Corduliidae										
Epicordulia										

	Trap April 2010	NC-5 Net April 2010	NC-6 Trap April 2010	NC-6 Net April 2010	NC-7 Trap April 2010	NC-7 Net April 2010	NC-8 Trap April	NC-8 Net April	NC-9 Trap April	NC-9 Net
Gomphidae	_					2010	2010	2010	2010	April 2010
Arigomphus										
Libellulidae										
Erythemis										
Erythrodiplax										
Libellula	1	1	4	1	1					
Pachydiplax			4	8	1	_				5
Perithemis				0	1	7		3	3	16
Plathemis					2	12				
Sympetrum					2	13				1
0,111										
Zygoptera										
Coenagrionidae										
Amphiagrion										
Argia										
Chromagrion										
Coenagrion				3						
Enallagma			2							
Ischnura				2						2
Nehalenniae										
Telebasis										
										-
TRICHOPTERA										
Leptoceridae										
Oecetis			15							
Phryganeidae										-
Ptilostomis				1		1				2
HEMIPTERA										
Belostomatidae										
Belostoma										
Notonectidae										
Buenoa										

	Trap April 2010	Net April 2010	NC-6 Trap April 2010	NC-6 Net April 2010	NC-7 Trap April 2010	NC-7 Net April 2010	NC-8 Trap April	NC-8 Net April	NC-9 Trap April	NC-9 Net April
Notonecta				1		2	2010	2010	2010	2010
Corixidae	1		7					1		1
Hesperocorixa		1	,	1	63		8		2	
Trichorixa				1				3	2	3
THE						2				
Gerridae										
Gerris										
								1		
Hebridae										
Merragata										
Hydrometridae										
Hydrometra										
Mesoveliidae										
Mesovelia										
Microveliidae										
Microvelia										
	-									
Naucoridae								2		
Pelocoris								3		
N. C.										
Nepidae				2						1
Ranatra				2						
Pleidae										
Neoplea	1	18		4			4	11	68	30
Unidentified	1	10								
COLEOPTERA										
IERA										
Dytiscidae									3	2
Acilius										
Agabetes										1
Agabus sp 1 (Red)								20		
Agabus Larvae								1	1	1
Bidessonotus	1									

	NC-5 Trap April 2010	NC-5 Net April 2010	NC-6 Trap April 2010	NC-6 Net April 2010	NC-7 Trap April 2010	NC-7 Net April	NC-8 Trap April	NC-8 Net April	NC-9 Trap April	NC-9 Net
Celina	2				-510	2010	2010	2010	2010	April 2010
Copelatus										
Coptotomus										
Cybister								1		
Desmopachria								1		
Dytiscus										
Graphoderus										
Hydaticus										
Hydroporus					1					
Hydrovatus	1						1		2	
Ilybius	2									
Laccophilus							2			
Neoporus			2	3			2	1		1
Rhantus									5	2
Thermonectus						1				_
Uvarus						1				
O TATALO										1
Gyrinidae										
Dineutus										
Haliplidae										
Peltodytes				2			1	2		5
Hydrophilidae										
Berosus										
Cymbiodyta										
Derallus										
Dibolocelus										
Enochrus										<u> </u>
Hydrochus										1
Hydrobiomorpha										
Hydrophilus		1								
Laccobius		1								1
Paracymus									3	3
Tropisternus	1						2		3	-
protettius	1									
Noteridae							1			
Hydrocanthus							1			
Suphisellus		1								

	NC-5 Trap April 2010	NC-5 Net April 2010	NC-6 Trap April 2010	NC-6 Net April 2010	NC-7 Trap April 2010	NC-7 Net April	NC-8 Trap April	NC-8 Net April	NC-9 Trap April	NC-9 Net
Scirtidae						2010	2010	2010	2010	April 2010
Cyphon		5								
Scirtes										1
Staphylinidae										
DIPTERA										
Larvae										
Pupae			3		3		1			2
Ceratopogonidae										
Bezzia	1						1	1		
Ceratopogon							1	1		
Mallochohelea										
Stilobezzia										
Serromyia					4					
Culicoides										
Chaoboridae										
Chaoborus	9		10	2	55		23	19	63	1
Chironimidae										
Chironominae										
Chironomini										
Chironomini Genus I										
Chironomus	4		1	1	4	1	15	1	3	1
Dicrotendipes										
Endochironomus	1					1	1			
Glyptotendipes			1	1	4	10	1			5
Kiefferulus		1	6	13	15	28	7			
Omisus	1	1	11	34	2	2				
Parachironomus							1		1	2
Polypedilum	5			2	1		1			
Zavreliella	40		10		7					
Tanytarsini										
Paratanytarsus										

	NC-5 Trap	NC-5 Net	NC-6 Trap	NC-6 Net	NC-7 Trap	NC-7	NC-8	NC-8		
	April 2010	April 2010	April 2010	April 2010	April 2010	Net April 2010	Trap April	Net April	NC-9 Trap April	NC-9 Net April
Orthocladinae						2010	2010	2010	2010	2010
Corynoneura										
Tanypodinae										
Ablabesmyia		1	1							
Cinotanypus			1							26
Gluttipelopia			1							
Labrundinia										
Larsia										
Procladius					1					
Psectrotanypus					2		2			
Tanypus									1	
Culicidae										
Aedes										1
Anopheles										
Culex										
Mansonia	1									
Uranotaenia										
Sciomyzidae										
Stratiomyidae										
Stratiomys										1
Tabanidae										
Haematopota										
Tipulidae										
Erioptera chlorophylla										

Laura Ashlie Farmer was born in Clarksville, Tennessee on 24 June 1978 to Cathleen Darnell and Jerry Rawlings of Clarksville, TN and Woodlawn, TN respectively. She has one older sister Natalie Ann Adams. Ashlie was raised throughout most of her school years on her family's farm in North Clarksville. She and her mother moved to Dover, TN for a short period of time where she graduated from Stewart County High School. Following high school, Ashlie enrolled at Austin Peay State University (APSU) where she majored in Biology and minored in Agriculture. During her junior and senior years at APSU she interned as a veterinary technician for the Animal Medical Center. Her degree was conferred in 2004.

After college Ashlie moved to Nashville, TN where she worked for four years as a Project Manager for Resolution, Inc, a private environmental consulting firm. During this period Ashlie married her husband Robert Farmer and had their son Trebor Cash Farmer. After their son was born, Ashlie and Robert moved to Ashland City, TN where they currently reside.

In 2009 Ashlie returned to APSU and enrolled in the Master of Science degree program under the tutelage of Dr. Steven W. Hamilton. Ashlie began her thesis work as a Graduate Research Assistant through the Center of Excellence for Field Biology and has presented her thesis work at the 2009-10 Tennessee Entomological Society meetings and the 2009-10 Tennessee Academy of Science meetings, where, in 2009, she was awarded 2<sup>nd</sup> place for her poster presentation in the Zoology section. She has also presented at the 2010 Tennessee Water Resources Symposium, 2010 North American Benthological

Society Summer meeting, the 2010 APSU Graduate Research and Creative Activity Extravaganza and the 2010 APSU Research Forum, where she was awarded best poster presentation. Ashlie was also recently awarded the Sara and Floyd Ford Scholarship for the 2010-11 academic years. In addition to her thesis work, Ashlie also performed a concurrent research project on Meerkat behavior at the Nashville Zoo. She was invited to present her findings to the staff and her resulting paper is currently archived at the Nashville Zoo's library. After graduation Ashlie hopes to continue research with water quality and work in the Ecology field with macroinvertebrates and other aquatic organisms.