Water Chemistry and Aquatic Insect Assemblages of Ephemeral Ponds in the Munson Sand Hills Region of the Apalachicola National Forest, Florida

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Abstract - Ephemeral ponds in the Munson Sand Hills region (MSH) of Apalachicola National Forest (ANF) are an essential resource in the life cycles of a variety of amphibian species, a number of which are threatened or endangered. Various types of human activities have disturbed some of these ponds threatening their survival. Although extensive research has been done on the biology of amphibians in the ponds, little is known of the invertebrates and to what extent the water quality may be affected by human impacts. We monitored 4 ponds, representing a spectrum of sizes, natural settings, and anthropogenic disturbance, in terms of water chemistry and aquatic insect assemblages seasonally for 2 years. Pond waters were characterized by acidic pH, low ionic strength, low buffering capacity, low nutrient concentrations, and phosphorus-limiting conditions. The water quality of studied ponds was similar to those reported for natural wetlands in west-central Florida. The chemistry, as compared to a nearby sinkhole, indicated that these ponds were mainly recharged with rain and had no connectivity to groundwater. Aquatic beetles (Coleoptera), dragonflies and damselflies (Odonata), and aquatic bugs (Heteroptera) were the most diverse groups of aquatic insects recorded. Species collected included many common, predatory species adapted to exploit resources in fishless, temporary ponds. Water chemistry and aquatic insect composition showed minor spatial–temporal variations among ponds. The results of this study indicate that human disturbances have not had a significant effect on pond water quality, posing no threat to amphibian and other wildlife species, and the sampled ponds had abundant and diverse aquatic insect fauna. The aquatic insect assemblages documented in this study provide evidence that pond type and the top-down effects of aquatic insects as predators are important determinants of community structure, which is a common theme observed in temporary ponds found in other regions within temperate biomes.

Introduction

Ephemeral ponds are relatively small and shallow, isolated water bodies that undergo natural seasonal wet and dry cycles (Collinson et al. 1995). These features are also known in the literature as ephemeral wetlands, seasonal ponds, temporary ponds, vernal pools, isolated wetlands, depression marshes, and depression wetlands (Tiner 2003). Ephemeral ponds are a common landscape feature in the southeastern US (Tansey and Cost 1990). These water bodies support a large diversity of plants, aquatic insects, reptiles, and amphibians despite their small size and seasonal hydroperiod (Semlitsch and Bodie 1998). Many amphibians, in particular, use ephemeral ponds exclusively as breeding sites due to the absence of fish

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predators (Dodd 1992, Sutter and Kral 1994). Recently, ephemeral ponds have received increasing attention from ecologists, biologists, and environmentalists due to their critical role in maintaining regional biological diversity and providing ecosystem services to humans, aquatic species, and wildlife (William 2006). Despite their ecological significance, these water bodies are not currently protected by the United States Federal Clean Water Act due to their isolated and non-navigable nature (McCauley et al. 2013, Stokstad 2006). This leaves these water bodies potentially more vulnerable to contamination and degradation. Water quality of ephemeral ponds is one of a number of factors that has raised substantial concern because it is not only essential for proper biological growth and reproduction, but also directly interacts with other components across multiple spatial and temporal scales (Yu et al. 2015). Previous studies have reported that poor water quality has contributed to the decline in many amphibians, reptiles, and other wildlife species (De Solla et al. 2002, Laposata and Dunsom 2000, McKibbin et al. 2008, Semlitsch 2002).

This study focuses on ephemeral ponds in the Munson Sand Hills (MSH) region located south of the City of Tallahassee, in the Florida Panhandle. Most of the MSH region falls within the boundary of the Apalachicola National Forest (ANF; Fig. 1), which is managed by the United States Department of Agriculture Forest Service (USDA-FS). The ephemeral ponds in this region provide habitat for a number of species of greatest conservation need (SGCN) including: *Ambystoma cingulatum* Cope (Flatwoods Salamander), *Notophthalmus perstriatus* (Bishop) (Striped Newt), *Pseudacris ornate* (Holbrook) (Ornate Chorus Frog), *Rana capito* LeConte (Gopher Frog), and *Ambystoma tigrinum* (Green) (Tiger Salamander) (Meegan 2007). These amphibian species depend on ephemeral pond habitats for part of their life cycles due to the absence therein of predators, such as fish, that might eat their eggs and larvae. These species are thus more vulnerable to changes in these habitats than other generalist species. At least 2 species, Gopher Frog and the Striped Newt, are considered to be declining (Franz and Smith 1999, Moler 1992). Various human influences such as logging, road construction, changes in the frequency and duration of prescribed fires, and off-road vehicular use in and around the ponds have adversely impacted some of the pond habitats, threatening the survival of sensitive amphibian species.

Ephemeral ponds in the MSH have been the subject of detailed research on pond-breeding amphibians by Bruce Means and his research team at the Coastal Plains Institute (CPI), Crawfordville, FL (Means 1999, 2001, 2007a, 2007b; Means and Means 2005; Means and Prentiss 1996; Means et al. 2004). Their research has focused on documenting the decline of ephemeral-pond–obligate vertebrates, studying their life cycles, and developing conservation and management recommendations and strategies. Much of the data collected by the CPI exist in unpublished reports and documents prepared for state and federal agencies. During a workshop hosted by the CPI in cooperation with United States Geological Survey (USGS) at the Florida Integrated Science Center in Gainesville, FL, on 5 March 2007, a group of amphibian biologists identified water quality as one of the threats and research gaps pertaining to ephemeral ponds in Florida that host amphibian communities (Meegan 2007).

While the amphibians of the MSH have been the focus of much research, aquatic macroinvertebrate biodiversity has not received the same attention. To date, there have been no scientific papers published that characterize the aquatic insect fauna of these unique habitats. The most closely related studies of macroinvertebrate assemblages were conducted in lime sink wetlands in southwest Georgia (Battle and Golladay 2001, Golladay et al. 1997). In general, despite often being biodiversity hotspots, ephemeral ponds have not been studied much in terms of their aquatic insect assemblages. The current state of knowledge of invertebrates in temporary ponds of temperate biomes was reviewed by Jeffries et al. (2016) as part of a larger work on the invertebrates of freshwater wetlands throughout the world (Batzer and Boix 2016).

This study is part of a project that was developed in collaboration with and support from the USDA-FS to study and characterize the status of ephemeral pond habitats in the MSH region of ANF. For this study, we selected 4 ephemeral ponds in the Apalachicola National Forest for evaluating water chemistry and aquatic insect fauna. The results of this study will be useful to understand the present conditions of these habitats and will provide baseline data for future studies related to environmental assessment and impact of forest management activities.

Study Area

The study area is located in the Munson Sand Hills (MSH) region of the Woodville Karst Plain in the Apalachicola National Forest (Fig. 1). The MSH is a physiographic province characterized by numerous shallow depressions that represent karst features at depth mantled by overlying sands, silts, and clays. The ephemeral ponds in the MSH (Fig. 1) are formed within these well-drained depressions when seasonal rains saturate the surface sands and exceed the infiltration rate of the soils. During periods of low rainfall, the ponds gradually lose water, leaving behind dry depressions ringed by distinctive bands of vegetation resulting from fluctuating water levels.

Figure 1 shows the distribution of ponds in and around the ANF (black dots) studied by Means and Prentiss (1996). We selected 4 ephemeral ponds (stars) for studying water chemistry and aquatic insect assemblages for a 2-year period from November 2008 to November 2010. We designated the pond sites as Pond1, Pond5, Pond6, and Pond266, following the naming convention of previous work (Fig. 2; Means 2008). We chose these ponds for study because they are representative of the spectrum of sizes, natural settings, and degrees of human impacts of ponds located within the ANF (see individual pond description below).

The selected ponds vary in size from 0.37 to 2.1 ha. In this study, we consider pond areas to represent the area of maximum inundation as determined by measuring the area enclosed by the sandy ring surrounding each pond that marks a transition from wetland to upland vegetation. They have variable hydroperiods that depend on seasonal precipitation. Water depths vary from zero during prolonged drought to 1.5–2.4 m (5–8 ft) during periods of high precipitation. In addition, to determine if the study ponds have any connectivity with groundwater, we sampled a water body (Blue sink), which has connectivity with the groundwater, and compared its water chemistry with those of the ephemeral ponds.

Individual pond-site descriptions

Pond1, 0.37 ha. This pond is located directly next to a major highway (US 319) and has experienced disturbances on the southeastern portion of its drainage area from road grading and construction (Fig. 2). It is a known breeding pond of the Gopher Frog and Striped Newt and thus has been the focus of extensive long-term study into the life cycles of pond-obligate amphibians by Means (2001).

Pond5, 0.82 ha. Pond5 has a longer hydroperiod than other ponds selected for this study. A forest road transects the northern portion of the drainage area of this pond (Fig. 2). During the sampling period, this road was incised into the landscape which may have an impact on the normal pond hydrologic functions. The pond was blocked off to vehicle access and was only minimally affected by off highway vehicle (OHV) traffic in the region immediately surrounding the pond.

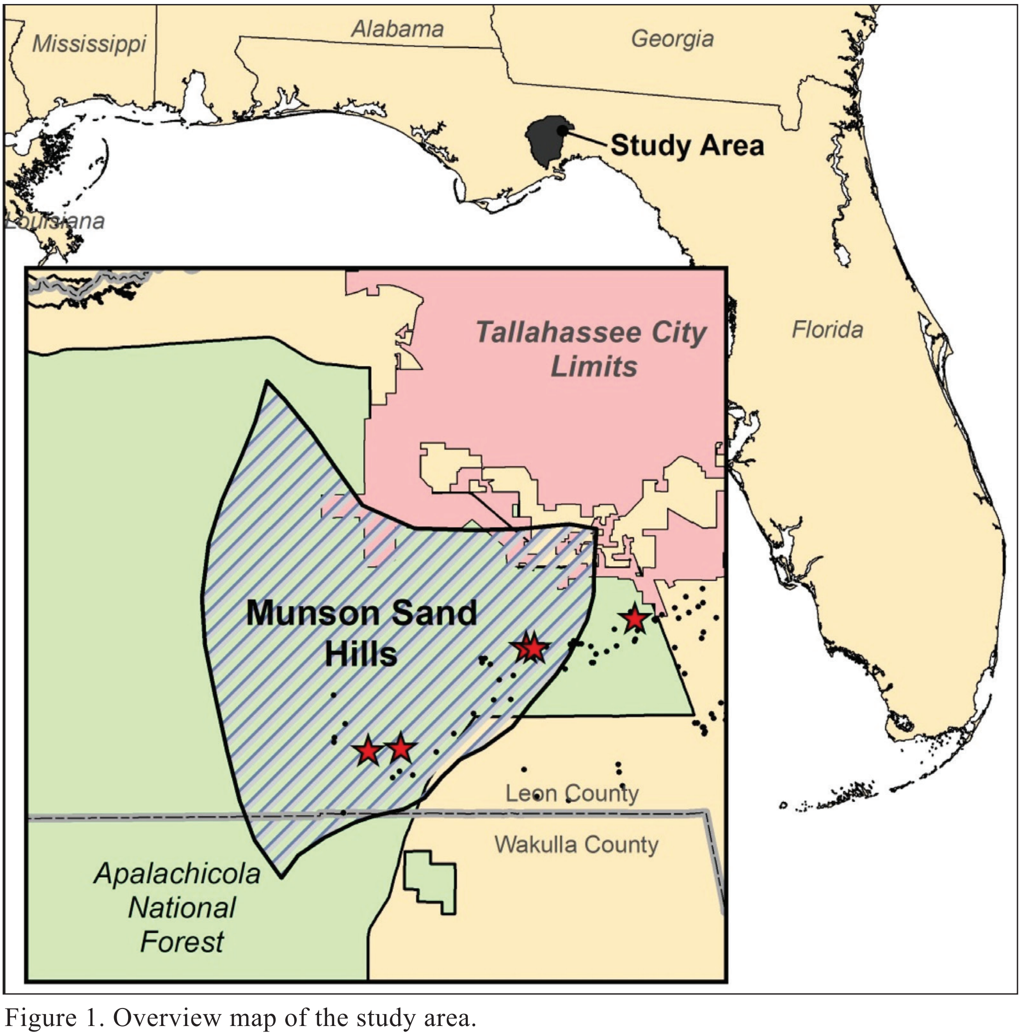

Figure 1. Overview map of the study area.

Pond6, 1.0 ha. Pond 6 is located west of a power-line right-of-way that crosses the ANF (Fig. 2). The right-of-way was used by OHV riders to access the pond site for illegal "mud-bogging". This activity led to significant destruction of vegetation surrounding the pond and severe disturbance of soils in and around the pond. Means (2007b) documented the degradation of this pond between 1993 and 2004. By the time of this study, the Forest Service had installed a fence around the pond, and vegetation was in recovery.

Pond266, 2.1 ha. Pond 266 is a relatively large pond located at the eastern edge of the MSH region (Fig. 2). An east–west trending forest road is located ~125m south of the pond, and has no physical influence on the water body or its vegetation. Although Pond266 is relatively secluded, track marks on aerial photos indicate that it experiences at least occasional impact from vehicle traffic; however, there has been minimal physical impact to the pond, and vegetative communities are largely intact.

Blue sink, 0.33 ha. Blue sink is a sinkhole located ~200 m to the east of Pond1 (Fig. 2). Blue sink has direct connectivity to the groundwater table.

Methods

Water chemistry

Water sampling and field parameters. We collected 1-L water samples (grab) quarterly in each season at 3 widely spaced locations in each of the ephemeral ponds using acid-washed high density polyethylene amber bottles during November 2008–November 2010. Immediately after collection, we composited and placed the samples in a cooler packed with ice. We measured temperature, pH, and specific conductivity in situ at each sampling site using a portable multimeter (Oaklon, Vernon Hills, IL) and dissolved oxygen using a dissolved oxygen meter (YSI, Yellow

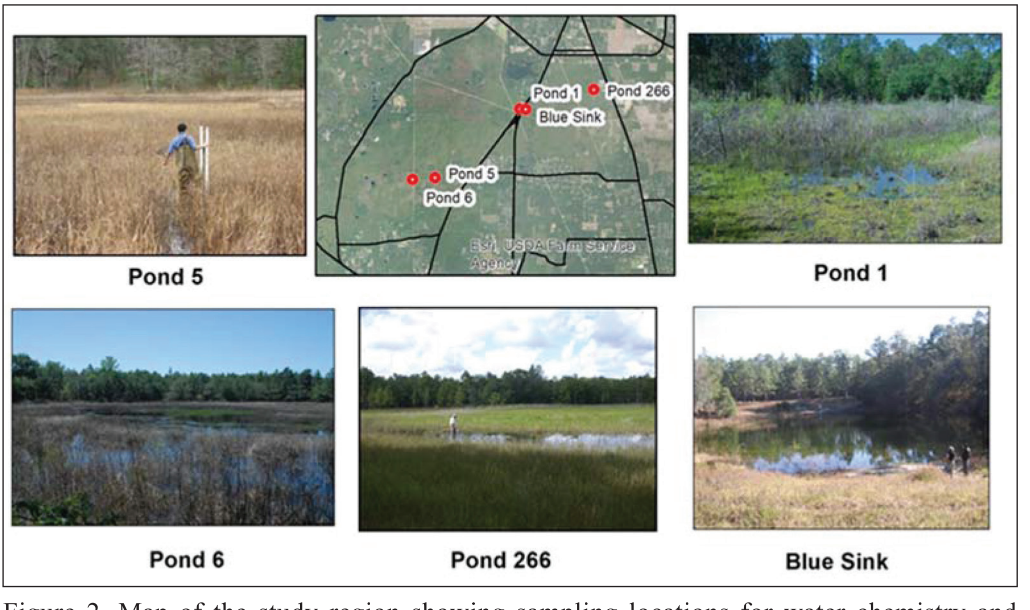

Figure 2. Map of the study region showing sampling locations for water chemistry and aquatic insect fauna, and photographs of each of the 5 waterbodies studied.

Springs, OH). Upon return to laboratory, 100 mL of the sample was preserved with 0.2% H_2SO_4 and stored at 4 °C for total kjeldahl nitrogen (TKN) and total phosphorus (TP) analyses. We filtered the other portion of the water samples using 0.45-µm cellulose acetate membrane filters. We stored a portion of the filtered sample at 4°C for analysis of nitrate+nitrite, ammonia, and orthophosphate, which we completed within 48 hours of sample collection. We preserved separate sub-samples of filtered samples with 0.2% HNO_3 for trace metal analysis and with 0.2% H_2SO_4 for dissolved organic carbon (DOC), and stored at 4 °C.

Laboratory analyses. We analyzed the collected water samples for hardness, alkalinity, dissolved organic carbon, nitrate+nitrite, orthophosphate, ammonia, TKN, TP, and trace metals. We calculated hardness from calcium and magnesium concentrations determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES; 7300DV, Perkin-Elmer, Waltham, MA). We determined alkalinity by potentiometric titration and analyzed nitrate+nitrite, orthophosphate, and ammonia using a continuous-flow auto analyzer (AA3; Bran+Luebbe, Norderstedt, Germany) following the EPA methods 353.1, 365.1, and 350.1, respectively. The method detection limits were 5 µg L^{-1} for nitrate+nitrite-N and orthophosphate-P, and 20 µg L^{-1} for ammonia-N. For the determination of TKN and TP, we digested the samples in the presence of H_2SO_4, K_2SO_4, and $HgSO_4$ using a Bran +Luebbe BD 50 block digestor and analyzed the digested samples using the AA3 continuous-flow auto analyzer following EPA methods 351.2 and 365.4, respectively. The method detection limits for TKN-N and TP-P were 0.18 mg N $L^{-1}$and 0.010 mg P L^{-1}, respectively. We analyzed DOC as non-purgeable organic carbon using the high-temperature combustion method on a TOC-V analyzer (Shiamdzu, Kyoto, Japan). The method detection limit for DOC was 0.04 mg L^{-1}. We analyzed trace metals using an atomic absorption spectrometer coupled with a graphite furnace (AAnalyst 300, HGA 850; Perkin-Elmer) and the ICP-OES. For all water chemistry laboratory analyses, blanks, spikes, and certified quality control samples were analyzed in each run to ensure data quality.

Statistical analyses. We statistically analyzed water chemistry data for all sampling sites to determine significant difference in spatial and temporal (seasonal) variations in water quality parameters, i.e., pH, DO, specific conductivity, hardness, alkalinity, nitrate, orthophosphate, TKN, TP, and DOC. We used the general linear models (GLM) procedure to perform a two-way analysis of variance (ANOVA) of the data. We performed Tukey's honestly significantly different (HSD) tests to determine significant difference in mean water quality parameters across sampling sites and sampling dates at the 0.05-level of significance. All statistical analyses were performed using SAS v. 9.4.

Aquatic insect fauna sampling and analysis

We sampled aquatic insects from each of the 4 pond sites 8 times on a seasonal basis, from November 2008 through September 2010, providing us with 2 full years of data that we used to characterize the aquatic insect fauna of the 4 ponds, compare the aquatic insect fauna among ponds, and track changes in taxonomic composition over time.

Insect sampling method. We used aquatic D-frame dipnets (600-micron mesh) to sweep pond substrates. We field-picked material from each sweep by placing the dipnet contents in white plastic pans for closer examination and removing insect specimens from the pans and placing them in sample bottles containing 80% ethanol. Each sampling event was carried out by 3 people who spent 1 hour each (a total of 3 person-hours) sweeping and field-picking specimens. An effort was made to collect from as many microhabitats as possible so that as many species as possible were collected. Because the samples were field-picked using the naked eye, smaller macroinvertebrates (e.g., early instars, smaller dipterans) were not collected.

Sample sorting and taxonomic identification. We rough sorted specimens of aquatic insects contained in each sample in the laboratory using a stereomicroscope (Olympus SZX16; Tokyo, Japan). We then more closely examined the sorted material under the microscope and identified specimens to lowest practical taxonomic level using current taxonomic keys, with the exception of the Diptera, which were only identified to family/genus level. The number of individuals of each taxon was recorded on a datasheet for each sample and entered into an electronic spreadsheet. Voucher specimens representing the various taxa will be deposited in the aquatic insect collection at Florida A&M University.

Data analyses. We analyzed the taxonomic composition and abundance data to investigate both underlying spatial variation between ponds and temporal seasonal patterns. The sampling of each of the 4 ponds seasonally over a 2-year period resulted in 8 samples from each pond site and 8 samples from each season. We used these data to compare mean abundance (# of individuals) and mean taxa richness (# of taxa) for each pond and each season. We performed a Levene's test to examine the homogeneity of variances and graphed residuals to evaluate normality of data. The data were subsequently square-root transformed to meet parametric assumptions. We used one-way ANOVA analysis to search for significant influences of site or season on richness and on abundance. Upon detection of significant results, we performed HSD tests to examine pairwise interactions between season or site on richness/abundance. Comparative composition and abundance analyses as described above were performed using XLSTAT 2019.1.1. Additionally, we used taxonomic composition and abundance data from the 8 samples collected at each pond to compare the overall diversity and similarity of aquatic insect assemblages among ponds. Specifically, we used PAST v. 3.25 (Hammer et al. 2001) to calculate Shannon–Wiener (H') diversity and Sorensen similarity indices.

Results

Water chemistry

The water quality parameters measured in situ and in the laboratory for all 5 sampling sites are presented in Figure 3 and Table 1. During the sampling period from November 2008 through November 2010, the temperature measured at sampling time varied from 11.7 °C in Pond266 in January 2009 to 32.5 °C in Pond266 in early September of 2010 (Table 1). The water temperature in Blue sink varied from 14.2 °C in January 2009 to 34.4 °C in September 2010.

Dissolved oxygen. All 4 ponds showed large variations in dissolved oxygen (DO) level from 1.8 to 9.5 mg L^{-1}, corresponding to 22% to 95% saturation, respectively, during the sampling period. Both minimum and maximum DO values were

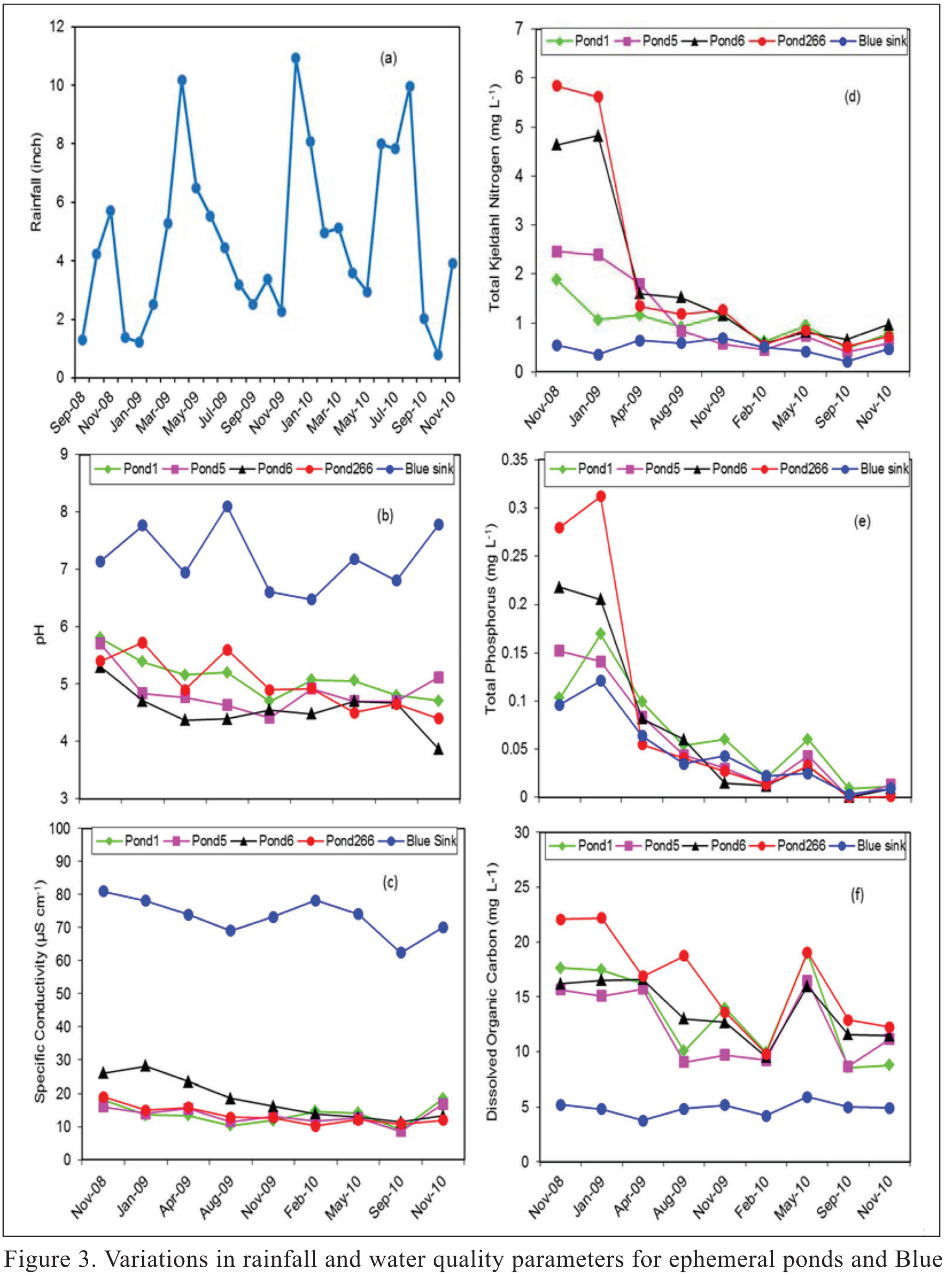

Figure 3. Variations in rainfall and water quality parameters for ephemeral ponds and Blue sink. (a) Rainfall, (b) pH, (c) specific conductivity, (d) total kjeldahl nitrogen, (e) total phosphorus, and (f) dissolved organic carbon.

Table 1. Median and min–max (in parentheses) values for water quality parameters for sampling sites. Water quality parameters for natural wetlands of west-central Florida with no connectivity to groundwater are shown for comparison purpose (Lee et al. 2009). * indicates significantly different from studied ephemeral ponds (Tukey's studentized range test: $P < 0.001$ in all cases).

Water quality parameters	Pond1	Pond5	Pond6	Pond266	Blue sink	Natural wetlands of west central Florida
Temperature (°C)	17.6 (12.9–29.3)	18.0 (13.4–31.4)	19.2 (12.2–29.9)	21.1 (11.7–32.5)	20.6 (14.2–34.4)	–
DO (mg L^{-1})	4.3 (1.8–9.5)	3.9 (2.7–8.2)	3.6 (2.0–6.3)	5.2 (2.9–8.4)	9.4 (7.5–11.8)*	3.8 (1.5–8.5)
pH	5.07 (4.70–5.80)	4.77 (4.41–5.70)	4.55 (3.86–5.30)	4.90 (4.40–5.72)	7.14 (6.47–8.10)*	4.50 (3.90–5.70)
Specific conductivity (µS cm^{-1})	13.6 (9.2–18.5)	13.2 (8.7–16.9)	16.2 (11.4–28.1)	12.7 (10.2–19.0)	73.9 (62.4–81.0)*	41.15 (32.0–85.0)
Hardness (mg L^{-1} as $CaCO_3$)	3.7 (2.1–5.2)	2.6 (1.6–3.0)	1.8 (1.4–2.5)	2.4 (1.3–5.7)	25 (21.9–31.1)*	–
Alkalinity (mg L^{-1} as $CaCO_3$)	2.1 (1.3–3.0)	2.1 (1.0–2.8)	1.1 (0.5–1.4)	1.7 (1.1–5.8)	28.2 (24–37.4)*	1.75 (0–5.3)
Nitrate+nitrite-N (µg L^{-1})	40 (<5–70)	50 (<5–110)	50 (<5–90)	60 (20–80)	40 (<5–80)	0 (0–60)
Ammonia-N (µg L^{-1})	51 (<20–180)	60 (21–160)	140 (31–240)	80 (32–260)	20 (<20–110)	–
Orthophosphate (µg L^{-1})	6 (<5–30)	<5 (<5–10)	<5 (<5–10)	<5 (<5–10)	<5 (<5–10)	0 (0–75)
TKN (mg L^{-1})	0.93 (0.49–1.16)	0.73 (0.42–2.46)	0.97 (0.55–4.82)	1.18 (0.51–5.84)	0.51 (0.22–0.69)	1.5 (0.5–3.5)
TP (mg L^{-1})	0.06 (0.01–0.17)	0.04 (<0.01–0.15)	0.06 (<0.01–0.22)	0.07 (<0.01–0.31)	0.03 (<0.01–0.12)	0.01 (<0.008–0.023)
DOC (mg L^{-1})	13.94 (8.64–18.99)	11.13 (8.55–16.47)	12.98 (9.51–16.56)	16.85 (9.79–22.17)	4.99 (3.77–5.90)*	37.5 (14.0–50.3)

measured in Pond1 indicating greater variation there compared to other ponds in the study (Table 1). The median DO values for Pond1, Pond5, Pond6, and Pond266 were 4.3, 3.9, 3.6, and 5.2 mg L^{-1}, respectively. Pond6 had maximum DO value of 6.3 mg L^{-1}, whereas the other ponds had maximum DO greater than 8 mg L^{-1}, corresponding to close to 100% saturation (Table 1). In general, Pond6 had relatively lower DO values compared to other ponds in the study. Compared to the ephemeral ponds, Blue sink had higher DO values throughout the sampling period, varying from 7.5 to 11.8 mg L^{-1}, corresponding to 100% to 117% saturation, respectively.

pH. Water pH in the ephemeral ponds varied from 3.86 in Pond6 to 5.80 in Pond1 with a median pH of 4.79 (Table 1). Considering all ponds, Pond6 had the lowest minimum (3.86) and maximum (5.30) pH values, and pond1 had the highest minimum (4.70) and maximum (5.80) pH values. The ponds showed modest variation in pH across the sampling period but exhibited no seasonal trend (Fig. 3). The pH of Blue sink water was circumneutral to basic (pH 6.47–8.10 with a median pH of 7.14). The pH below 7.0 was observed only in the wet season due to the input of rainwater, which usually has pH near 5.6.

Specific conductivity, hardness, and alkalinity. The specific conductivities of the ephemeral pond waters were low and varied from 8.7 µS cm^{-1} in Pond5 to 28.1µS cm^{-1} in Pond6 with a median value of 13.4 µS cm^{-1}, indicating low total dissolved solids (Table 1). Median specific conductivities of Pond1, Pond5, and Pond266 were close, varying only from 12.7 to 13.6 µS cm^{-1}, whereas Pond 6 had a value of 16.2 µS cm^{-1}. The ponds showed only small variation in conductivity values over the sampling period (Fig. 3). The ponds had very low hardness and alkalinity,with values of 1.3–5.7 mg L^{-1} as $CaCO_3$ and 0.5–5.8 mg L^{-1} as $CaCO_3$, respectively (Table 1). Blue sink had substantially higher specific conductivity (62.4–81.0 µS cm^{-1}), hardness (21.9–31.1 mg L^{-1} as $CaCO_3$), and alkalinity (24.0–37.4 mg L^{-1} as $CaCO_3$) than ephemeral pond waters.

Nutrients. The concentrations of nitrate+nitrite and orthophosphate were very low at all the sampling sites, with values of <5 to 110 µg N L^{-1} and <5 to 30 µg P L^{-1} (Table 1). The median nitrate+nitrite concentrations varied from 40 to 60 µg N L^{-1}, whereas the median orthophosphate concentrations were either below or very close to the detection limit of 5 µg P L^{-1} for all the ponds. The ammonium concentrations varied from 20 µg N L^{-1} at Pond1 to 260 µg N L^{-1} at Pond266 (Table 1). The highest median ammonium concentration of 140 µg N L^{-1} was observed at Pond6. Blue sink also had low median concentrations of nitrate+nitrite (40 µg N L^{-1}), ammonium (20 µg N L^{-1}), and orthophosphate (<5 µg P L^{-1}).

The concentrations of TKN and TP in the ponds varied from 0.42–5.84 mg N L^{-1} with a median value of 0.95 mg N L^{-1} and <0.01–0.31 mg P L^{-1} with a median value of 0.042 mg P L^{-1}, respectively (Table 1). Pond5 had the lowest median TKN (0.73 mg N L^{-1}) and TP (0.04 mg P L^{-1}) concentrations, and Pond266 had the highest median TKN (1.18 mg N L^{-1}) and TP (0.07 mg P L^{-1}) concentrations of all the ponds. The highest concentrations of TKN and TP were measured in samples collected in November 2008 and January 2009 for each of the 4 ponds, which most likely reflect evaporative concentrations resulting from the drought conditions of summer 2008.

In subsequent sampling in April 2009, we recorded a sharp decline in TKN and TP concentrations due to dilution by rainwater (Fig. 3). Thereafter, the concentrations showed small variation, only fluctuating from 0.42 to 1.26 mg N L^{-1} for TKN, and <0.01 to 0.06 mg P L^{-1} for TP over rest of the sampling period. The heavy rainfall in February and September 2010 resulted in a noticeable drop in TKN and TP concentrations (Fig. 3). Compared to the ponds, Blue sink had significantly lower TKN and TP concentrations: 0.22 to 0.69 mg N L^{-1} with median value of 0.51 mg N $L^{-1}$and <0.01 to 0.12 mg P L^{-1} with median value of 0.03 mg P L^{-1}, respectively.

Dissolved organic carbon. In ephemeral ponds, the concentrations of dissolved organic carbon varied from 8.55 mg C L^{-1} in Pond5 to 22.17 mg C L^{-1} in Pond266 with a median concentration of 13.77 mg C L^{-1} (Table 1). Noticeable decreases in DOC concentrations were observed in April 2009, February 2010, and September 2010 due to heavy rainfall (Fig. 3). The ponds showed large variation in DOC concentrations throughout the sampling period but exhibited no seasonal trend. Blue sink had significantly lower DOC concentrations (3.77–5.90 mg L^{-1}) and exhibited much less variation during the sampling period compared to the ephemeral ponds.

The concentrations of heavy metals were either very low or below the detection limits (data not shown).

Spatial and seasonal variations in water quality parameters. Differences in water quality parameters (pH, DO, specific conductivity, hardness, alkalinity, nitrate, orthophosphate, TKN, TP, and DOC) among the 4 ephemeral ponds in this study were not significant. Ephemeral ponds were significantly different from Blue sink with regard to pH, DO, specific conductivity, hardness, alkalinity, and DOC (Tukey's studentized range test: $P < 0.001$ in all cases). Seasonal variations in water quality parameters were not significant for all 5 sampling sites.

Aquatic insect fauna

We compiled our field survey data from the 32 samples of aquatic insects into a master taxa list (Appendix 1). We plotted taxa richness and relative abundance of the major aquatic insect groups for the 4 ponds to show the contribution of the different aquatic insect orders to overall aquatic insect community composition (Figs. 4, 5). Change over time in abundance (# of individuals) and taxa richness (# of taxa) recorded from each sampling event are shown in Figures 6 and 7. Abundance and taxa richness by pond site and by season and H' values for each of the 4 ponds are given in Table 2. Sorensen similarity values are provided in Table 3.

A total of 129 distinct taxa of aquatic insects were identified in the samples (Appendix 1). The most speciose groups of aquatic insects were the Coleoptera (60 species), Heteroptera (32 species), and Odonata (21 species). Other insect groups, represented by Ephemeroptera, Trichoptera, Megaloptera, Neuroptera, Lepidoptera, and Diptera, accounted for a relatively small proportion of the total number of species identified. Coleoptera, Odonata, and Heteroptera were the dominant aquatic insect groups in all 4 ponds, both in terms of taxa richness (Fig. 4) and relative abundance (Fig. 5). Table 4 lists the species that accounted for ≥1% of the total collections; these 17 species collectively accounted for ~71% of the total catch. Information regarding these species is presented in the discussion section.

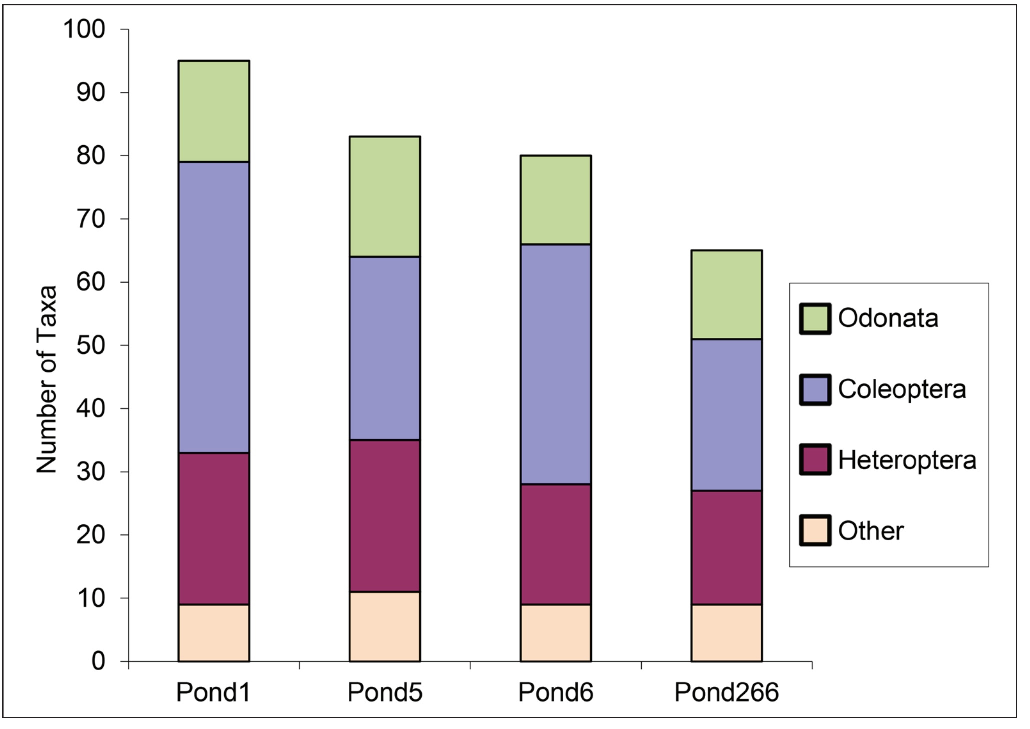

Figure 4. Taxa richness of major groups of aquatic insects collected in survey.

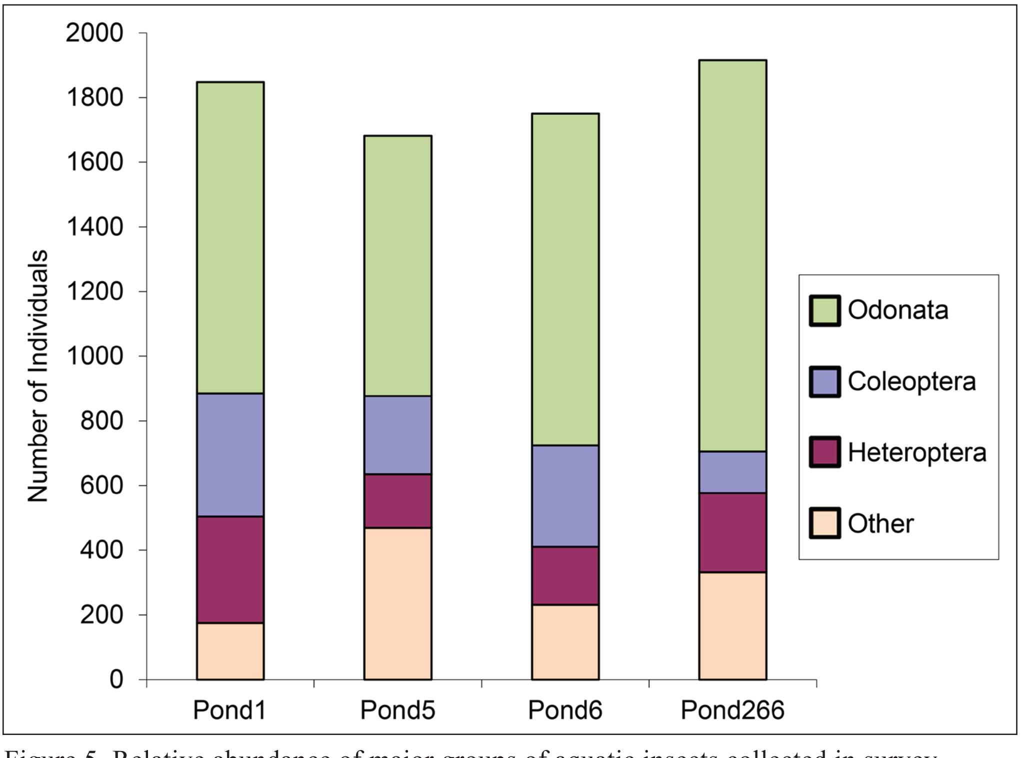

Figure 5. Relative abundance of major groups of aquatic insects collected in survey.

The similar taxonomic composition among the 4 ponds (Tables 3, 4) suggests that these shared species are common inhabitants of ephemeral ponds throughout the MSH. The 2 largest ponds (Pond6, Pond266) had the most similar (0.816 Sorensen Similarity) assemblages of aquatic insects (Table 3). These 2 ponds are essentially large marshes, so it was expected that they support very similar aquatic insect assemblages. Pond1 and Pond5 had the second highest similarity value

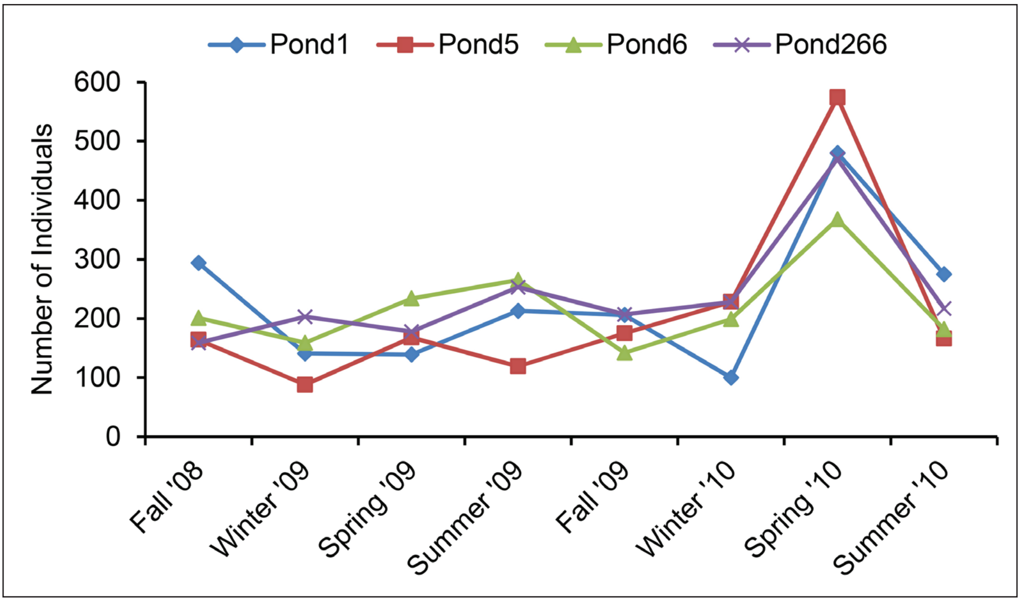

Figure 6. Abundance of aquatic insects collected in samples at the 4 pond sites.

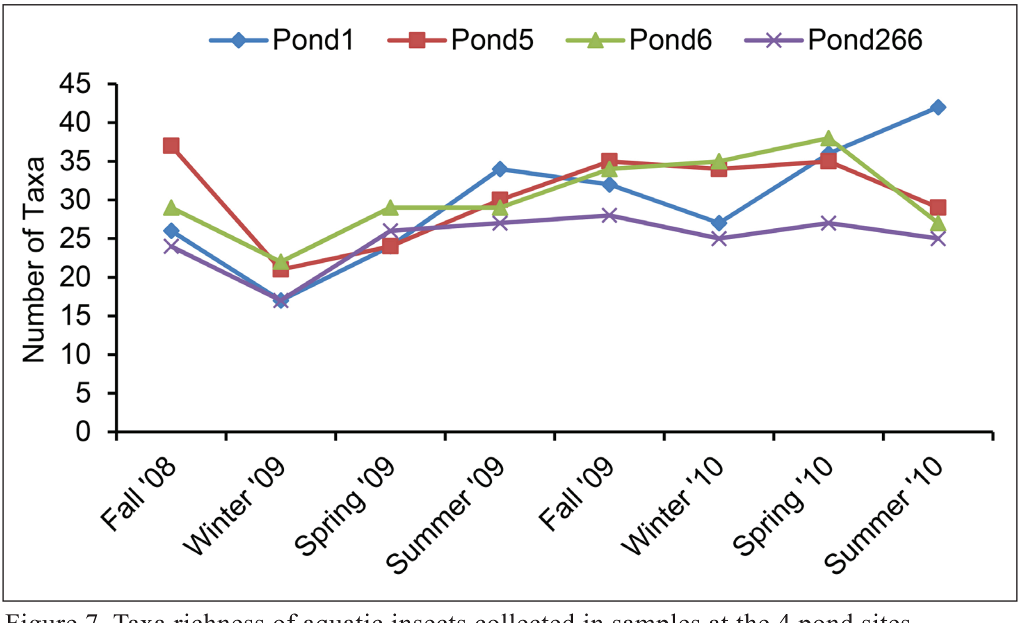

Figure 7. Taxa richness of aquatic insects collected in samples at the 4 pond sites.

(0.702 Sorensen Similarity), which likely reflects a shared fauna that is adapted to the more pond-like basin characteristics of these 2 ponds.

Site comparison. Total abundance varied from 1682 individuals collected from Pond5 to 1915 individuals collected from Pond266 (Table 2, Fig. 5). Statistically, there was no significant difference between the pond sites in terms of the mean number of individuals collected. Aquatic insect taxa richness varied from 65 taxa at Pond266 to 95 taxa at Pond1 (Fig. 4, Table 2). Pond266 had the lowest taxa richness for 6 of 8 sampling events, varying from 17 to 28 taxa. In comparison, Pond1, Pond5, and Pond6 had higher numbers of total taxa and greater variation in numbers of taxa collected. However, the mean number of taxa collected in the 8 samples from each of the 4 sites did not differ on a statistical basis. Shannon–Weiner diversity scores (Table 2) were higher in Pond1 (3.286) and Pond5 (3.116), as compared to the 2 larger, marsh-like ponds, Pond 6 (2.778) and Pond266 (2.747).

Seasonal comparison. Total abundance was highest (2611 individuals) in the samples collected in spring (Table 2) due mainly to the large uptick in abundances across all 4 ponds recorded during the Spring 2010 sampling event (Fig. 6). The overall larger spring abundance was in contrast to winter samples that had the

Table 2. Abundance (# individuals/sample ±SD) and taxa richness (# taxa/sample, and range) of aquatic insects by pond site and season. Also shown are Shannon–Wiener diversity index (H') values for each pond. * indicates mean abundance was statistically lower (*P* = 0.015) in winter as compared to spring.

		Abundance		Taxa richness		Diversity
	n	Total # individuals	# individuals per sample (±SD)	Total # taxa	taxa per # sample (min–max)	H'
Site						
Pond1	8	1848	231 (± 121)	95	29.8 (17–42)	3.286
Pond5	8	1682	210 (± 153)	83	30.6 (21–37)	3.116
Pond6	8	1750	219 (± 72)	80	30.4 (22–38)	2.778
Pond266	8	1915	239 (± 98)	65	24.9 (17–28)	2.747
Season						
Fall	8	1548	194 (± 47)	91	30.6 (24–37)	
Winter	8	1346	168 (± 55)*	71	24.8 (17–35)	
Spring	8	2611	326 (± 168)*	77	29.9 (24–38)	
Summer	8	1690	211 (± 54)	73	30.4 (25–42)	

Table 3. Pair-wise comparison of Sorensen similarity index values based on data from the 8 samples collected from each pond.

	Sorensen similarity index			
	Pond1	Pond5	Pond6	Pond266
Pond1	1.000	0.702	0.580	0.627
Pond5	0.702	1.000	0.628	0.657
Pond6	0.580	0.628	1.000	0.816
Pond266	0.627	0.657	0.816	1.000

lowest total abundance (1346 individuals) and statistically lower (P= 0.015) mean number of individuals collected in the 8 samples as compared to the spring period. Aquatic insect taxa richness was highest in the fall samples (91 taxa), versus lower taxa richness for winter (71 taxa), spring (77 taxa) and summer (73 taxa). Interestingly, the higher overall taxa richness (91 taxa) in the fall samples was not reflected in the mean, minimum, and maximum taxa richness values, which were very similar to corresponding values from the other seasons (Table 2).

Discussion

Water chemistry

The water quality parameters that varied the most among ephemeral ponds during the sampling period were DO, pH, TKN, TP, and DOC. Pond1 had higher pH (median pH 5.07) and showed larger variation in DO level (1.84–9.5 mg L^{-1}) compared to other ponds in the study. This could be related to higher fluctuation in water level in Pond1, which is relatively smaller in size and shallower than the other ponds. Pond6 had the lowest median pH (4.55) and DO level (3.6 mg L^{-1}), and the highest specific conductivity (16.2 µS cm^{-1}) among all 4 ponds. Pond6 was most impacted by the use of off-highway vehicles, which might have negatively impacted soil and vegetation; decaying vegetation could result in low DO level, low pH, and higher specific conductivity. Dissolved oxygen concentrations were typically lower in summer than in winter for all the ponds. DO values less than 3

Table 4. Percent contribution of the most abundant species (≥1% of total catch) for Pond1, Pond, Pond5, Pond6, Pond266, and for all ponds combined. Data from 8 seasonal samples per pond were combined for the analysis. Common names and authorities for species are given in Appendix 1.

Species	Pond1	Pond5	Pond6	Pond266	All Ponds
Tramea carolina/lacerata	16.0	11.9	18.6	22.0	17.3
Ischnura posita	12.2	9.2	14.6	8.4	11.0
Lestes australis	4.9	9.8	8.9	17.0	10.2
Caenis diminuta	0.9	11.8	2.7	5.6	5.4
Anax longipes	3.3	2.8	1.7	5.4	3.3
Buenoa confusa	2.7	3.1	2.2	5.2	3.3
Pachydiplax longipennis	6.4	1.2	4.1	0.8	3.1
Nehalennia integricollis	0.2	5.7	4.8	0.7	2.7
Anax junius	5.1	0.8	1.3	3.0	2.6
Thermonectus basillaris	1.5	2.3	5.8	0.0	2.3
Triaenodes dendyi	1.1	5.4	1.0	0.1	1.8
Notonecta indica	2.9	1.0	1.7	1.1	1.7
Hydrocanthus oblongus	1.8	2.0	1.4	1.1	1.6
Erythrodiplax minuscula	0.6	2.7	1.4	1.4	1.5
Ischnura hastata	0.9	0.8	0.1	2.9	1.2
Erythemis simplicicollis	1.4	0.5	2.2	0.5	1.1
Coptotomus interrogatus	1.1	1.5	1.1	0.4	1.0
Total	60.0	72.5	73.4	75.6	71.2

mg L^{-1}, a commonly used support threshold for aquatic life (USEPA 2016) were observed in all the ponds during late spring through early fall for sampling dates of August 2009, May 2010, and September 2010, which could be attributed to higher microbial activity and subsequent higher oxygen demand for the decaying vegetation in these ponds. Blue sink had oxygen-saturated conditions during most of the sampling events, which could be attributed to the groundwater inflow at the sink's surface resulting in mixing of air with water as well as the scarcity of aquatic vegetation therein.

The waters in ephemeral ponds were acidic, and relatively low pH (median pH of 4.79) seems to be predominantly controlled by humic substances formed as a result of decomposition of plant material. Humic substances can decrease the pH of low alkalinity waters such as the ephemeral ponds in this study by 0.5 to 2.5 pH units and thus act as a natural source of acidity (Lydersen 1998). Additionally, humic substances, due to the presence of acid functional groups, play a significant pH-buffering role in aquatic systems between pH 4 and 5 (Kullberg et al. 1993, Roila et al. 1994). The ponds showed no seasonal trend with regard to pH over the 2-year sampling period (Fig. 3). Previous studies reported similar results for ephemeral wetlands that exhibited no seasonal trend with regard to pH despite high variability (Boeckman and Bidwell 2007, Pickens and Jagoe 1996, Scholnick 1994). Low pH, specific conductivity, hardness, and alkalinity of ephemeral pond waters indicate that rain water was the main source of water in these ponds. Conversely, basic pH, higher specific conductivity, hardness, and alkalinity of Blue sink water reflect dominance of groundwater from the upper Floridian Aquifer, which is mainly composed of calcite and dolomite.

The ammonium concentrations were little higher than the nitrate+nitrite concentrations in the ponds. The nitrate form of nitrogen is more efficiently and readily removed from surface water by aquatic plants than the ammonium form (Miller 1990). Pond6 had the highest median ammonium concentration of 140 µg N L^{-1} among all 4 ponds. This result could be associated with the relatively low oxygen level observed at Pond6, which likely limited the nitrification process and conversion of ammonium to nitrite and nitrate. The low nutrient concentrations in ponds are most likely a consequence of high biological activity and N and P requirements. This finding might also suggest that external nutrient loading to these ponds by runoff or human activities is minimal. Organic nitrogen and organic phosphorus were the predominant forms of nitrogen and phosphorus in all the 4 ponds. TKN and TP concentrations for all the ponds exhibited very similar trends during the study period (Fig. 3) and were strongly correlated ($r^2 = 0.75$ to 0.99), suggesting the same source of origin such as decomposed plant detritus. The nitrogen to phosphorus ratio (N/P) is used to characterize aquatic ecosystem to assess nutrient limitations and trophic status. According to previous studies (Downing and McCauley 1992, Redfield 1958), N is limiting when TN:TP ratio by mole is <10, and P is limiting when TN:TP by mole is >16. TN:TP ratios between 10 and 16 reflect nutrient-balanced conditions. In this study, the concentrations of nitrate+nitrite were very low at all sites, hence TKN concentrations were used for total nitrogen (TN) for calculating the TN:TP ratio. For ephemeral ponds, the calculated median nitrogen to phosphorus ratio varied from 37 at Pond1 to 64 at Pond266. Based upon the above criteria, the ponds may be characterized by phosphorus-limiting conditions and plant communities in these ponds may be phosphorus limited. Similar results have been reported for a large proportion of North American wetlands, where plant communities were found to be phosphorus limited (Bedford et al. 1999). The lower TN:TP ratio at Pond1 compared to the other ponds could be associated with its shorter hydroperiod because desiccation promotes mineralization of organic material resulting in nitrogen loss through nitrification and denitrification processes but no significant loss of phosphorus (Busnardo et al. 1992, De Groot and Golterman 1994, Fernandez-Alaez and Fernandez-Alaez 2010). Previous studies also reported that TN:TP ratios were higher in ponds with longer hydroperiod than ponds with short hydroperiod (Sahuquillo et al. 2012).

Similar to TKN and TP results, Pond5 had the lowest median DOC (11.13 mg L^{-1}) and Pond266 had the highest median DOC (16.85 mg L^{-1}) concentrations. Variations in DOC concentrations among ponds could be attributed to the type and abundance of aquatic and terrestrial vegetation in and around the ponds. The higher DOC concentrations were observed in November 2008 and January 2009 for all the pond sites, which is consistent with the higher TKN and TP concentrations at the same time due to drought conditions in summer 2008. The lower concentrations and less variation in DOC in Blue sink could be related to the presence of little to no aquatic vegetation and the dominance of groundwater, which has lower DOC concentrations than surface waters (Lee et al. 2009).

Florida has not established water quality standards for ephemeral ponds/wetlands against which the water quality of the studied ephemeral ponds could be assessed. We compared water quality parameters measured for ephemeral ponds in this study with those of natural wetlands in west-central Florida (Table 1). The pH, DO, alkalinity, and nutrients concentrations in ephemeral ponds were similar to those reported for natural wetlands of west-central Florida (Lee et al. 2009). However, the specific conductivity and DOC concentrations in the studied ponds were lower compared to the west-central Florida wetlands, which probably is due to the accumulation of natural organic matter from shorter residence times in the studied ponds compared to wetlands.

The significant differences in water chemistry between the ephemeral ponds and the Blue sink with regard to pH, DO, specific conductivity, hardness, alkalinity, and DOC reflect that these water bodies are recharged from different water sources. The ephemeral ponds were mainly recharged with rainwater and have no connectivity to groundwater, whereas Blue sink is dominated by groundwater due to its connectivity with the Floridian aquifer.

Water chemistry in the ephemeral ponds was found to be characterized by acidic pH, low ionic strength, low buffering capacity, low nutrient concentrations, and phosphorus-limiting conditions. These results suggest that the pond water chemistry was largely controlled by natural settings and processes such as type of soils and vegetation, hydroperiod, evapotranspiration, rain, and biological activities and was not affected by forest management and human activities.

Habitat indicator taxa

The most abundant aquatic insects collected in the study (Table 4) are mostly predatory species that are common and widely distributed in the southeastern United States, often in association with lentic habitats and aquatic vegetation. These species are likely to be ecologically important as top predators in these fishless ponds. Discussed below are their occurrences within the 4 ponds.

Odonata (dragonflies and damselflies). The 21 species of Odonata recorded in the study accounted for ~56% of the total catch of individuals, including 10 of the 17 most abundant (≥1% of total catch) species (Table 4). The overall most abundant taxon collected comprised 2 closely related migratory species of *Tramea* (*T. carolina* [Carolina Saddlebags], *T. lacerata* [Black Saddlebags]) (Dunkle 2000). These 2 species are difficult to distinguish in the nymphal stage, and so we lumped them together. Of the 10 most abundant Odonata species, 6 are dragonflies [*T. carolina/lacerata*, *Anax longipes* (Comet Darner), *Pachydiplax longipennis* (Blue Dasher), *A. junius* (Common Green Darner), *Erythrodiplax minuscula* (Little Blue Dragonlet), *Erythemis simplicicollis* (Eastern Pondhawk)] and 4 are damselfies (*Ischnura posita* [Fragile Forktail], *Lestes australis* [Southern Spreadwing], *Nehalennia integricollis* [Southern Sprite], *I. hastata* [Citrine Forktail]). Seven of the odonate species were recorded during the first collection event (November 2008), which took place shortly after the end of a prolonged drought period when ponds began to refill. Later colonizers, 3 species (*A. longipes*, *N. integricollis*, *I. hastata*), were first collected on the second collection event (February 2009). Of note, *P. longipennis* and *A. junius* occurred in the highest abundance in the first collection event and were far less abundant in subsequent collections. This finding suggests these 2 species are effective rapid colonizers (pioneers) with high reproductive rates in ephemeral ponds after they fill following dry conditions.

Aquatic Coleoptera (water beetles). The aquatic Coleoptera were highly diverse in our pond samples, comprising 60 distinct taxa and ~14.8% of the total individuals collected in the study. Dytiscidae (predacious diving beetles) was by far the most diverse family, with a total of 30 distinct taxa collected. Three species of water beetles (*Thermonectus basillaris*, *Hydrocanthus oblongus*, and *Coptotomus interrogatus*) were abundant (≥1% of total catch) over the course of the study (Table 4). All 3 species were recorded from all 4 ponds, with the exception of *T. basillaris*, which was absent from collections from Pond266. *Thermonectus basillaris* is the most common species of *Thermonectus* in Florida, occurring in many types of lentic habitats (ponds, lakes, swamps, ditches, and even temporary puddles; Epler 2010). *Coptotomus interrogatus*, another dytiscid beetle, is also a common inhabitant of lentic habitats, especially on vascular macrophytes. The second most abundant aquatic beetle collected in the study was *H. oblongus* (Noteridae). It is considered to be the most abundant and common *Hydrocanthus* species in Florida, occurring in most lentic habitats having floating mats of algae and other vegetation (Epler 2010).

*Heteroptera (aquatic bugs).*The Heteroptera, the second most diverse group collected from the 4 ponds, comprised 32 distinct taxa and ~12.8% of the total individuals collected in the study. Two species of backswimmers (Notonectidae) were abundant (≥1% of total catch), namely *Buenoa confusa* and *Notonecta indica* (Table 4). Both species were recorded in modest numbers from all 4 ponds throughout most seasons. Backswimmers were observed often swimming (diving) in the water column. *Buenoa* have the ability to maintain a neutral buoyancy and can rest underwater, whereas *Notonecta* species float to the surface when not swimming (Epler 2006).

Ephemeroptera (mayflies). Only 3 species of mayflies were recorded in the study (Appendix 1). All 3 species are known from a very wide range of habitats and environmental conditions. They are able to survive and thrive in low dissolved oxygen and high temperature environments where most other mayfly species are not found (Berner and Pescador 1988). The especially tolerant species *Caenis diminuta* was the 4th most abundant species collected in the study (Table 4) accounting for ~5.4% of the total individuals collected. This species was collected from all 4 ponds but was absent from the first collection event (November 2008) and reached its highest total abundance in the May 2010 samples. Berner and Pescador (1988) described the nymphs of *C. diminuta* as sprawlers that are most abundant on the mud and silt of pond beds where they crawl about plant stems.

Trichoptera (caddisflies). As with the mayflies, only 3 taxa of Trichoptera were recorded in the study (Appendix 1). Of these, only *Triaenodes dendyi* was collected in abundance, accounting for ~1.9% of the total catch (Table 4). *Triaenodes dendyi* (a shredding/chewing herbivore) has been recorded from Alabama, Florida, Georgia, and South Carolina (Manuel 2010). This species appears to be a specialist of ephemeral ponds and is known to occur in high numbers on aquatic macrophytes such as emergent aquatic sedges within Carolina bays, prairie ponds, and other natural temporary ponds (Morse et al. 2017, Pescador et al. 2004). We captured the highest numbers of this species from Pond5, which is the most permanent of the 4 ponds sampled, suggesting that *T. dendyi* is not a rapid colonizer (pioneer) of newly flooded temporary ponds as are many species of Odonata, Coleoptera, and Heteroptera.

Temporal-spatial variation of aquatic insect assemblages

The temporary pond habitats within the MSH can be considered as habitat islands in a biogeographic sense. The typical species–area relationship of most habitats is one in which the number of species increases as the area of the habitat increases. However, the 4 ponds surveyed in this study showed an inverse species–area relationship, with Pond1, the smallest pond (0.37 ha), having the most taxa (95 taxa) and Pond266, the largest pond (2.1 ha), having the fewest taxa (65 taxa). The other 2 ponds were intermediate in terms of their area and number of taxa. The unusual species–area relationship uncovered in this study is likely related to differences in the pond basin characteristics. Pond6 and Pond266 are flatter marsh-like systems, in contrast to Pond1 and Pond5, which have better-defined basins. This distinction between the 2 pond types is reflected in both the Sorensen similarity values (Table 3) and Shannon–Wiener diversity scores (Table 2).

That taxa richness was highest in Pond1 was in part due to the contribution of the rich aquatic beetle fauna (46 taxa), whereas the other ponds had 24–38 beetle taxa. It is possible that the steeper sidewalls to the basin create a sharper water/shore ecotone that in turn supports a higher diversity of beetles. Pond266, in contrast, had a less well-defined shoreline transition and appeared to support few aquatic beetle taxa. Additionally, Pond266 supported large populations of *Tramea carolina*/*lacerata* and *Lestes australis* (Appendix 1), which may have limited taxa richness due to interspecific competition and/or predation.

The sharp uptick in abundances across all 4 ponds in the spring 2010 (Fig. 6) was explained in part by the substantial numbers of the 4 most abundant damselfly species (*Ischnura posita*, *Lestes australis*, *Nehalennia integricollis*, and *Ischnura hastata*). All of these species are classified as climbers and were found in abundance on submerged macrophytes such as *Utricularia* (bladderworts) which had become well established throughout open-water habitats. Because of the life-history phenology of these species, wherein adults emerge in late spring/summer, the abundance numbers of the nymphs were much reduced by late summer when the final sampling took place. Also collected in much higher numbers in the Spring 2010 were the mayfly species *Caenis diminuta* and the caddisfly species *Triaenodes dendyi*. Both of these species were very abundant in Spring 2010 in Pond 5, which historically is the most permanent of the 4 ponds. Population sizes of these 2 species appeared to experience year-to-year growth in Pond5.

Another seemingly incongruent result of this study was the high taxa richness in the fall season (91 taxa), in contrast to the other seasons (71–73 taxa). The reasons for this are not entirely clear, but are at least parlty due to 23 taxa that were recorded only in fall samples. Of note, out of those 23 taxa, 14 taxa were collected only in the Fall 2008 after the ponds exited a dry-phase and began to refill as a result of Tropical Storm Fay in August 2008. Nine of the 14 taxa were beetles, and all of these Fall-only taxa were collected in small numbers (5 or less individuals). These taxa may have been early successional pioneers that did not persist due to interspecific competition and/or predation, which caused them to perish or emigrate out of the ponds.

Based on the results of our water chemistry and aquatic insect surveys, we conclude that the ephemeral ponds studied are healthy and productive, and water quality in these ponds do not present any threat to amphibians and other wild life. The results of this study will serve as baseline data for future assessment and monitoring of the ephemeral ponds in the ANF. These results have implications for ecologists, conservationists, and USDA-FS officials in developing conservation and management strategies for these habitats to continue providing ecosystem services.

Ephemeral ponds are vital to the survival of many amphibians. To date, only a few studies appear in the peer-reviewed literature regarding systematic characterization of water chemistry and aquatic insect assemblages in ephemeral ponds of the southeastern US. The ponds host endemic and threatened species that have adapted to rely on a complex interplay of physical, chemical, biological, climatic, and landscape variables that occur in these wetland habitats. This study serves to increase our understanding of these habitats by adding to the body of knowledge regarding water chemistry and aquatic insect communities for north-central Florida. Expanding the knowledge base now is especially important to meet the challenges of changing climate trends in the region.

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Appendix 1. Aquatic insect taxa collected from 4 ponds that were sampled quarterly for 2 years (November 2008 to September 2010) (*n* = 8/pond).

Aquatic insect taxa	Pond1	Pond5	Pond6	Pond266
Odonata (dragonflies and damselflies)				
Anisoptera (dragonflies)				
Aeshnidae				
Anax junius (Drury)	95	14	23	58
Anax longipes Hagen	61	47	30	103
Gomphidae				
Arigomphus pallidus (Rambur)	2	1		
Libellulidae				
Celithemis ornata (Rambur)/*verna* Pritchard		2		1
Erythemis simplicicollis (Say)	25	8	38	9
Erythrodiplax berenice (Drury)		1		
Erythrodiplax minuscula (Rambur)	11	46	24	27
Ladona deplanata (Rambur)	1	18	7	
Libellula incesta Hagen	1	13	6	7
Pachydiplax longipennis (Burmeister)	118	20	71	16
Tramea carolina (L.)/*lacerata* Hagen	296	200	326	422
Zygoptera (damselfies)				
Coenagrionidae				
Unidentified *Enallagma*		1		
Ischnura hastata (Say)	16	13	1	55
Ischnura kellicotti Williamson		2		
Ischnura posita (Hagen)	226	154	255	160
Ischnura prognata (Hagen)	5	4	1	
Ischnura ramburii (Selys)	11	1	2	11
Nehalennia integricollis Calvert	4	96	84	13
Lestidae				
Lestes australis Walker	90	164	155	325
Lestes rectangularis Say	1			
Lestes vigilax Hagen				3
Aquatic Coleoptera (water beetles)				
Curculionidae				
Unidentified *Bagous*		1		
Lissorhoptrus lacustris Kuschel	4	4	20	12
Dytiscidae				
Unidentified *Agabus*	1			
Anodocheilus exiguus (Aubé)	1			
Unidentified *Bidessonotus*	2		1	
Celina angustata Aubé		2	1	
Copelatus caelatipennis princeps Young	3	2	1	3
Copelatus chevrolati Aubé			1	
Coptotomus interrogatus (Fabricius)	21	26	19	7
Coptotomus longulus lenticus Hilsenhoff	19	11	9	5
Coptotomus loticus Hilsenhoff	6	11	5	
Coptotomus venustus (Say)	6	7	5	
Unidentified *Coptotomus*	5	2		2
Cybister fimbriolatus (Say)	12	5	5	11
Desmopachria seminola Young	2			
Unidentified *Desmopachria*	1		1	
Graphoderus liberus (Say)	1		6	

Aquatic insect taxa	Pond1	Pond5	Pond6	Pond266
Hydaticus bimarginatus (Say)		3	8	3
Unidentified *Hydroporus*		1		
Hydrovatus pustulatus (Melsheimer)	6	2		3
Unidentified *Hydrovatus*	1		1	1
Ilybius oblitus Sharp			1	
Unidentified *Ilybius*	2	1	1	
Laccophilus fasciatus rufus (Melsheimer)			1	
Laccophilus proximus Say	26	7	20	3
Unidentified *Laccophilus*	18	1		1
Liodessus noviaffinis Miller	1			
Matus bicarinatus (Say)			1	
Matus ovatus blatchleyi Leech			2	
Unidentified *Matus*			1	
Neobidessus pullus (LeConte)	1			1
Neoporus cimicoides (Sharp)	15	27	8	13
Neoporus lobatus (Sharp)	1		2	
Neoporus lynceus (Sharp)	6			
Unidentified *Neoporus*	4	4	3	18
Platambus astrictovittatus (Larson & Wolfe)			1	
Rhantus calidus (Fabricius)	2	1	6	
Thermonectus basillaris (Harris)	27	39	101	
Thermonectus nigrofasciatus ornaticollis (Aubé)		2		
Unidentified *Thermonectus*	38	4	2	2
Gyrinidae				
Dineutus carolinus LeConte	12		5	2
Unidentified *Dineutus*	3	1		
Gyrinus elevatus LeConte		1		1
Gyrinus woodruffi Fall			1	
Unidentified *Gyrinus*	1			
Haliplidae				
Haliplus punctatus Aubé	1			
Haliplus triopsis Say	7			
Peltodytes dietrichi Young	1			
Peltodytes oppositus Roberts	1	2		1
Peltodytes sexmaculatus Roberts	2			
Hydrochidae				
Hydrochus rugosus Mulsant	2			1
Hydrochus simplex LeConte	2			
Unidentified *Hydrochus*			2	
Hydrophilidae				
Berosus aculeatus LeConte	1	3		
Berosus corrini Wooldridge	3	1		
Berosus sayi Hansen	1			
Unidentified *Berosus*			1	
Derallus altus (LeConte)				1
Enochrus ochraceus (Melsheimer)	9		4	1
Helochares maculicollis Mulsant			1	
Unidentified *Helochares*				1
Hydrophilus triangularis Say			2	
Paracymus confusus Wooldridge	1			
Paracymus nanus (Fall)	1			
Tropisternus blatchleyi Orchymont	12	5	6	

Aquatic insect taxa	Pond1	Pond5	Pond6	Pond266
Tropisternus collaris (Fabricius)		1	1	3
Tropisternus lateralis nimbatus (Say)	19	8	15	3
Tropisternus natator Orchymont			3	
Unidentified *Tropisternus*	7	4	3	
Noteridae				
Hydrocanthus atripennis Say	1	1		1
Hydrocanthus oblongus Sharp	33	34	24	21
Unidentified *Hydrocanthus*	17	5	5	5
Suphis inflatus (LeConte)	1	4	8	
Suphisellus gibbulus (Aubé)	1			
Suphisellus puncticollis (Crotch)				2
Aquatic Heteroptera (water bugs)				
Belostomatidae				
Belostoma flumineum Say				1
Belostoma lutarium (Stål)		1	1	1
Belostoma testaceum (Leidy)		2		
Unidentified *Belostoma*	5	2	7	
Lethocerus uhleri (Montandon)	1	1	1	
Corixidae				
Hesperocorixa interrupta (Say)	5	4		
Hesperocorixa martini (Hungerford)		1	3	
Hesperocorixa nitida (Fieber)	1	4		
Sigara hubbelli (Hungerford)			1	
Sigara sigmoidea (Abbott)	17	1	8	3
Sigara zimmermanni (Fieber)	13	2		
Unidentified *Sigara*	10	1		
Trichocorixa verticalis (Fieber)				1
Gerridae				
Limnoporus canaliculatus (Say)	1	1		
Neogerris hesione (Kirkaldy)	1	1		
Trepobates subnitidus Esaki	1			
Hebridae				
Merragata brunnea Drake				3
Hydrometridae				
Hydrometra australis Say	5	5	6	16
Unidentified *Hydrometra*				1
Mesoveliidae				
Mesovelia mulsanti White	7		2	4
Unidentified *Mesovelia*	1			1
Naucoridae				
Pelocoris balius La Rivers	1	5	33	1
Pelocoris carolinensis Torre-Bueno		12	3	
Pelocoris femoratus (Palisot de Beauvois)	1	1	1	1
Unidentified *Pelocoris*	2	3		
Nepidae				
Ranatra australis Hungerford	2	1	2	
Ranatra drakei Hungerford	1	1	4	
Unidentified *Ranatra*		5	3	2
Notonectidae				
Buenoa artafrons Truxal	27	8	10	25
Buenoa confusa Truxal	50	52	39	99
Buenoa scimitra Bare	40	3		6

Aquatic insect taxa	Pond1	Pond5	Pond6	Pond266
Unidentified *Buenoa*	23		7	
Notonecta indica L.	53	17	29	22
Notonecta irrorata Uhler	8			
Unidentified *Notonecta*	27	5	3	15
Pleidae				
Unidentified *Neoplea*	10	12	5	35
Veliidae				
Microvelia hinei Drake	2		1	1
Microvelia pulchella Westwood	1			
Platyvelia brachialis (Stål)	3			1
Unidentified *Rhagovelia*		1		1
Ephemeroptera (mayflies)				
Baetidae				
Callibaetis floridanus Banks	5			
Callibaetis pretiosus Banks	38			22
Caenidae				
Caenis diminuta Walker	16	199	48	122
Trichoptera (caddisflies)				
Hydroptilidae				
Unidentified *Oxyethira*			1	
Leptoceridae				
Oecetis sp. E Floyd		2	2	
Triaenodes dendyi Manuel	21	90	17	2
Megaloptera (fishflies)				
Corydalidae				
Chauliodes rastricornis Rambur		1	4	1
Aquatic Neuroptera (spongillaflies)				
Sisyridae				
Sisyra vicaria (Walker)				1
Aquatic Lepidoptera (aquatic moths)				
Crambidae				
Unidentified *Elophila*	1	3		3
Parapoynx allionealis Walker		1		
Parapoynx seminealis (Walker)		1		
Aquatic Diptera (aquatic flies)				
Chaoboridae				
Unidentified *Chaoborus*	9	9	7	16
Chironomidae	78	157	142	152
Culicidae	5	5	9	13
Sciomyzidae	2	1		
Tabanidae			1	