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Characterizing Wetland Hydrology, Chemistry, And Soils For An Endemic Crayfish, The Piedmont Blue Burrower

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ABSTRACT

Anthropogenic alteration of Earth's ecosystems has pushed global biodiversity into a state of crisis. Freshwater species have been particularly vulnerable, with crayfish ranking as the second most imperiled taxonomic group in North America. Primary burrowers represent only 15% of crayfish species in North America; however, they constitute 32% of imperiled crayfish. Despite their conservation importance, little is known about the ecology of burrowing crayfish. To advance our knowledge about the habitat characteristics of burrowing crayfish, groundwater hydrology, water chemistry, and soil properties were assessed to determine their importance for the state-listed endangered, primary burrower *Cambarus harti*. Groundwater hydrology and chemistry were monitored from shallow wells installed among *C. harti* burrows and in similar areas without burrows (<50 m away) at 4 sites across Meriwether County (GA). Groundwater depth and temperature were automatically recorded every 30 min, whereas dissolved oxygen and pH were measured manually every 1-4 weeks from 6/6/2013-8/1/2014. Water chemistry (K^+, $Mn^{2+/3+}$, Cl^-, $Fe^{2+/3+}$, SiO_2) was analyzed from samples every 1-4 weeks (1/16/2014-8/1/2014) from wells and crayfish burrows. To assess crayfish soil preferences, 3 soil cores were collected from random locations within 10 m of each well. Groundwater levels for areas with *C. harti* burrows were ~ 3 times shallower than areas lacking burrows. Groundwater was acidic (pH=5.60, mean) and experienced hypoxia (<3 mg/L dissolved oxygen) for at least 48% of the study. Wells and burrows had similar water chemistry except for Cl^-, which was on average 16-18 times more concentrated in burrow water. Soils were sandy (85.5%) with minor amounts of silt/clay (11.3%) and only differed slightly among sites. Thus, this study found that *C. harti* inhabits wetlands with shallow groundwater (> 1 m) that are acidic and often hypoxic. While this burrower can tolerate variation in groundwater level, anthropogenic activities that depress groundwater could extirpate populations of *C. harti*.

For my daughter Raegan and wife Molly: without your inspiration, I would not have accomplished my goals of completing a Master's Thesis.

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INTRODUCTION

Anthropogenic alteration and deterioration of Earth's environments have pushed global biodiversity into a state of crisis (Wilson, 1988; Vitousek, 1994), where extinction rates have accelerated to a pace greater than other extinction events recorded in the fossil record (Singh, 2002). Freshwater ecosystems have experienced tremendous environmental degradation/destruction by human activities (Ricciardi & Rasmussen, 1999; Dudgeon *et al*., 2006). These changes have caused a high percentage of freshwater taxa to be imperiled and many species to go extinct (Sala *et al*., 2000).

North American freshwater fauna are highly imperiled, with a majority of these taxa being invertebrates (Master, 1990; Master *et al*., 2000; Wilcove & Master, 2005; Strayer, 2006). Crayfish are ranked as the second most imperiled taxonomic group in North America (Master, 1990). Primary burrowing crayfish (Hobbs, 1981) inhabit underground burrows to maintain connectivity to groundwater (Grow & Merchant, 1980). While these hypogean crustaceans comprise only 15% of crayfish species in North America, they constitute 32% of imperiled crayfish (Taylor *et al.*, 1996; Welch & Eversole, 2006; Taylor *et al*., 2007). Endemism, poor dispersal capabilities, and lack of scientific knowledge (due to the elusiveness of primary burrowers) contribute to the high degree of imperilment among primary burrowers (Gibert *et al*., 1994; Welch & Eversole, 2006; Lefébure, 2007).

Survival and success of primary burrowing crayfish depend on their ability to maintain connectivity with groundwater (Grow & Merchant, 1980; Stoeckel *et al*., 2011; Helms *et al*., 2013a; Helms *et al*., 2013b). One factor affecting burrowers' ability to maintain connectivity is changes in groundwater depth. Crayfish dig deeper burrows to access water during times of receding groundwater (Grow & Merchant, 1980; Johnston & Figiel 1997; Welch *et al.*, 2008; Helms *et al.*, 2013b). While groundwater is likely to be an important determinant of burrowing crayfish success, little information exists in the scientific literature quantifying groundwater variation in crayfish habitats over extended periods of time. Because wetlands are delineated based on groundwater levels and the presence of crayfish burrows, it is critical to understand the association between these two important wetland indicators (Wakeley *et al.*, 2010).

Crayfish burrowing could be affected by soil properties (Stoeckel *et al.*, 2011). For example, one could hypothesize that soils must have some cohesiveness to maintain burrow structural integrity, but too much cohesiveness could compromise crayfish digging capacity. Crayfish can burrow in a variety of soil types, for example, *Cambarus diogenes* can burrow in fine-grained, clay-rich (Grow, 1982; Helms *et al.*, 2013a) and sand-rich soils (Helms *et al.*, 2013a) as long as there is some clay content. Stoeckel *et al.* (2011) reported *Cambarus striatus* rarely reached groundwater levels (15 cm below surface) when burrowing in sand. Additional data on crayfish soil preferences are needed because studies have focused on a limited number of species at only a few locations. These data are essential to determine if generalizations can be made about soil properties at sites with burrowing crayfish.

Physical and chemical characteristics of groundwater may also influence the survival and distribution of hypogean crayfish, as has been documented from studies of epigean species (Crawshaw, 1974; Capelli & Magnuson, 1983; France, 1985, 1993; Smith *et al.*, 1996; Cairns & Yan, 2009). The metabolism, physiology, and behavior of crayfish are predominantly influenced by water temperature (Crawshaw, 1974; Whiteley *et al.*, 1997), pH (France, 1985; Wood & Rogano, 1986; Patterson & deFur, 1988), and oxygen concentration (Wiens & Armitage, 1961; Gäde, 1984; Reiber & McMahon, 1998). Shallow groundwater environments, such as those inhabited by primary burrowers, differ from surface waters in that they exhibit minimal daily and annual fluctuations in temperature (Parsons, 1970; Silliman & Booth, 1993) and are commonly depleted of oxygen (Malard & Hervant, 1999). The chemistry of shallow groundwaters is predominantly a function of the rock composition for the area (Back, 1960; Frape *et al*., 1984; Nordstrom *et al*., 1989; Guo & Wang, 2005). Little is known about how spatio-temporal variation in groundwater physicochemical parameters (e.g., temperature, pH, dissolved oxygen) influence primary burrowers. These data are critical to develop effective conservation strategies for protecting imperiled hypogean crayfish.

In order to address unanswered questions regarding the ecology of primary burrowing crayfish, I assessed the hydrology, chemistry, and soils at sites inhabited by *Cambarus harti*, a state-listed endangered primary burrower found in Meriwether and Troup Counties in Georgia, USA (Figiel, 2009; Keller *et al*., 2011; Helms *et al*., 2013b). Like most primary burrowers, general knowledge of this species and its associated habitat are lacking. Conservation of *C. harti* relies on the ability to define its critical habitat. The goal of this research was to characterize groundwater and soil parameters for the habitat of *C. harti*. To characterize *C. harti* habitat, hydrology, chemistry and soils were compared between *C. harti* habitats and similar areas lacking evidence of *C. harti*. I hypothesize 1) *C. harti* will be associated with areas having groundwater close to the surface because burrow excavation to shallow groundwater requires less energy than to deeper groundwater; 2) chemistry for *C. harti* habitats will be similar to that of areas without *C. harti* because their close proximity results in similar underlying rock composition; and 3) Soils among sites will be similar to those reported by Helms *et al*. (2013b) for the type locality.

METHODS

Study sites

To assess the habitat characteristics of *C. harti*, field investigations were conducted at 4 locations within Meriwether County, GA (Fig. 1). Meriwether County, a rural area that represents the southernmost county of the Atlanta Metropolitan area, resides in both the Chattahoochee and Flint River drainage basins. All study sites were located within the Piedmont ecoregion (Omernik, 1987) and were underlain by crystalline metamorphic rocks. Study sites were selected based on accessibility and the presence of *C. harti* populations.

The eastern-most site is owned by Stan Cartwright (Cartwright Property; Fig. 2) and is located within the Flint River drainage basin. The land is covered by second growth deciduous forest and sandy loam soil (USDA NRCS, 2010). According to USGS maps, the underlying bedrock is mica schist (Lawton *et al.*, 1976). *Cambarus harti* populations are generally found inhabiting areas located along seepage zones at the base of slopes. Burrows are also concentrated on the western side of a second-order tributary of Cold Springs Brook, with a few burrows by an unknown species on the eastern side of the floodplain.

Site 2 is the northern-most site and is owned by Jack Chandler (Chandler Property; Fig. 2). The land cover is composed of deciduous forest and stony loamy coarse sand soil (USDA NRCS, 2010). The underlying bedrock is undifferentiated granitic bedrock (Lawton *et al.*, 1976) *Cambarus harti* populations are located along seepage zones at the base of a steep slope. Sampling locations are within a few meters of a first-order tributary of Walnut Creek (Flint River Watershed).

The southern-most site, the Warm Springs Fish Hatchery, is located adjacent to the U.S. Fish and Wildlife Service Warm Springs Regional Fisheries Center (Fig. 2). This site is within the Flint River drainage basin and is the type locality for *C. harti.* The dominant cover type is mixed deciduous forest. Soils are classified as loamy sand (USDA NRCS, 2010) and are underlain by quartzite bedrock (Lawton *et al*., 1976). Burrows of *C. harti* are concentrated in seepage zones positioned at the base of slopes located in close proximity to a first-order tributary of Cold Springs Brook.

The western-most site, White Sulphur Springs (Fig. 2), is a mixed deciduous forest underlain by amphibolite bedrock (Lawton *et al*., 1976) and dominated by silty loam soils (USDA NRCS, 2010), and is the only study site residing in the Chattahoochee River drainage basin. White Sulphur Springs has been a site of human occupation for an extensive period of time (Steven Stewart, pers. comm.). Home to four natural springs, the site has undergone recent small-scale (~3000 m^2) deforestation (November of 2013) for the construction of a new bottling water facility (April of 2013). *Cambarus harti* burrows are located in forested riparian zones >50 m downstream from the spring boils.

Groundwater level and temperature

To test the hypothesis that groundwater levels for areas inhabited by *C. harti* were closer to the ground surface than that of surrounding areas, 2 groundwater monitoring wells were installed at each of the 4 study sites (n=8). At each site, an experimental groundwater monitoring well was installed in areas of active *C. harti* burrows (burrow well), and a control groundwater monitoring well (non-burrow well) was installed in a nearby area (10 to 45 meters) without crayfish burrows.

Monitoring wells were constructed of PVC pipes (10.2 cm diameter, ~ 150 cm long) that were perforated on two sides, with 1 cm diameter holes spaced 12 cm apart. These perforations were used to improve flow through wells to ensure that well water was similar to groundwater. Wells were covered with a nylon silt sleeve to exclude suspended particulates. Wells were installed to depths ≥ 30 cm below the water table to ensure water was always available during sampling (Fig 3).

To monitor fluctuations in groundwater level and temperature, SOLINST® Junior (Solinst Canada LTD) submersible pressure transducer leveloggers were installed at the bottom of each well (n=8) (Fig. 3). Leveloggers were programmed to record water pressure (kPa) and temperature (°C) every 30 minutes and recorded data from 6/6/2013-8/1/2014 (n=20,207 records/site). Preliminary well monitoring indicated that recording intervals of 30 minute were appropriate for capturing groundwater level fluctuations.

Because pressure transducers in the wells measure both water and atmospheric pressure, all measurements required a barometric pressure correction to calculate groundwater level (Fig. 3). Barometric pressure was monitored every 30 min using a SOLINST® Barologger deployed in the non-burrow (control) well located at the Warm Springs Fish Hatchery (Fig. 3). To ensure the barologger did not come in contact with groundwater, it was stationed near ground level.

Groundwater data recordings for monitoring well datasets had varying start and end dates. To ensure all datasets had the same time ranges, recordings prior to 6/7/2014 were omitted from analyses. During the collection of groundwater samples, leveloggers were temporarily removed from wells. During this time, leveloggers recorded surface pressure and temperatures.

These values were replaced by interpolated values calculated by averaging the previous and following recordings.

To meet the assumption of independence among data points for statistical analysis, daily averages separated by 2 weeks were extracted from each well dataset. This 2-week interval was determined from autocorrelation analysis of time-series well data. The initial daily average was randomly chosen to be 6/29/2014. Comparisons between treatments and across study sites were performed using a fully factorial Two-Way Analysis of Variance (ANOVA). Pairwise comparisons were performed using a Tukey HSD. Statistical analyses were performed using IBM SPSS Statistics (ver. 21, IBM Corp., 2012).

In order to analyze the dynamic nature of time-series of groundwater level, 2 approaches were used: exceedance probabilities and time-series spectral analysis. Exceedance probabilities, the proportion of time groundwater exceeds a given depth, were constructed using methods modified from Gore (1996). Spectral analysis identifies frequencies of oscillations in time-series data (e.g., seasonality). Spectral analysis was performed after the application of a fast Fourier transformation that decomposes data into a series of sine and cosine functions (Keller *et al*., 2001). Data were detrended and demeaned before performing the analysis using R version 3.1.1 (R Core Team, 2014). To reduce noise within the periodogram, artificial smoothing was performed using a Daniell smoother (weighting coefficient=20).

The selection process of groundwater temperature data was the same protocol used for groundwater level (i.e., 2-week daily averages). Temperature data did not have normally distributed errors and were not homoscedastic. Mathematical functions were unsuccessful in transforming temperature to meet the assumptions of a Two-Way ANOVA. Therefore, temperature was compared between treatments and across study sites using a non-parametric statistical approach, the Shierer-Ray-Hare extension of the Kruskal-Wallis Test (Dytham, 2011). All pairwise comparisons were tested using a Tukey HSD on ranked values from each dataset (Sokal & Rohlf, 1995).

Groundwater chemistry

Groundwater pH, dissolved oxygen, and temperature (used to correct dissolved oxygen and pH) were measured for areas with and without *C. harti.* Measurements were performed using a water quality multi-parameter sonde (Yellowsprings, Inc 6920-V2) at least 10 cm below the water surface of each well. Measurements were recorded every 1-4 weeks from 6/1/2013-8/1/2014 (n=23). Sonde calibrations were performed less than 24 hours prior to each sampling event.

Statistical analysis of dissolved oxygen and pH included only data from sampling dates (n=20) when measurements were collected from all 8 wells. Comparisons of pH between treatments and across study sites were statistically analyzed using a fully factorial Two-Way ANOVA (n=160). Pairwise comparisons were tested using a Tukey HSD. Statistical analysis was performed using IBM SPSS Statistics (ver. 21, IBM Corp., 2012).

Dissolved oxygen did not meet the assumptions of the Two-Way ANOVA model and could not be successfully transformed. Thus, data were analyzed using a non-parametric statistical method, the Scheirer-Ray-Hare extension of the Kruskal-Wallis Test (Dytham, 2011). All pairwise comparisons were tested using Tukey HSD on ranked values (Sokal & Rohlf, 1995). Exceedance probabilities were used to quantify the proportion of time groundwater exceeds a given dissolved oxygen concentration (modified from Gore, 1996). To assess if a relationship existed between groundwater temperatures and dissolved oxygen, a two-tailed Spearman's rank-order correlation was used. A non-parametric model was used because dissolved oxygen did not meet the assumptions of parametric statistics. Statistics were performed using IBM SPSS Statistics (ver. 21, IBM, Corp., 2012).

To characterize and compare groundwater chemistry for *C. harti* habitats and areas without *C. harti*, groundwater samples were collected from non-burrow wells, burrow wells and crayfish burrows (n=3) at each study site (n=4). Groundwater samples were collected every 1-4 weeks from 1/16/2014 to 8/1/2014 (n==10). Groundwater samples were collected after YSI measurements were taken. Groundwater samples were extracted from crayfish burrows located in close proximity to burrow wells (<10 m). Samples were collected from crayfish burrows by digging a hole around a burrow deep enough to fill with water from a side burrow chamber. A different crayfish burrow was excavated for water samples each sampling trip. When groundwater was sufficiently close to the ground surface a siphon pump was used to collect burrow water. Water samples were placed in 500 mL bottles and stored in a refrigerator ($T^o \leq 6$ ^oC) until analysis.

Groundwater samples were analyzed for chloride, potassium, iron, manganese, and silica. The chemicals chosen for analysis were selected from a set of inorganic chemicals that provide insight into the natural solute characteristics of the groundwater (Berndt *et al.*, 2005). Groundwater samples were filtered using grade B glass microfiber filter media (pore size= 1µm). Groundwater chemistry was analyzed with colorimetric methods using spectrophotometers (HACH DR/2700). Iron, manganese, and potassium were analyzed within 1-2 weeks of collection; chloride and silica were refrigerated at <6 ^oC and analyzed within 28 days. In order to analyze groundwater samples for iron and manganese, samples were digested within 1-2 weeks after collection following a modified version of the EPA's mild digestion with hot plate for metals analysis (Hach, 1999). Digestions were performed by acidifying 40 mL of groundwater sample to a pH≤2 by adding 0.05 mL (2 drops) of concentrated nitric acid and 2mL of 6N hydrochloric acid. Samples were heated on a hot pan overnight (12 hours) at 120 °C until 10-15mL of sample remained. Samples were neutralized with 5N sodium hydroxide and were refilled to their original volume (40 mL) using deionized water (Millipor Ultrapure).

Potassium, iron, and silica concentrations were compared between wells and crayfish burrows (n=3) and across study sites (n=4) using a fully factorial Two-Way MANOVA. Potassium and silica did not meet the assumptions of the MANOVA model. Therefore, potassium and silica were transformed using a log transformation (ln + 1). Pairwise comparisons were tested using a Tukey HSD. Statistical analyses were performed using IBM SPSS Statistics (ver. 21, IBM Corp., 2012).

Transformations were unsuccessful in conforming chloride and manganese data to meet the assumptions of parametric statistics. Thus, these data were analyzed using a non-parametric statistical method equivalent to a Two-Way ANOVA, the Shierer-Ray-Hare extension of the Kruskal-Wallis Test (Dytham, 2011). All pairwise comparisons were tested using a Tukey HSD on ranked values (Sokal & Rohlf, 1995). Statistical analyses were performed using IBM SPSS Statistics (ver. 21, IBM Corp., 2012) and Microsoft Excel 2007.

Soil collections and analysis

In order to test the hypothesis that *C. harti* burrows in soils having a similar grain-size distribution as the type locality, 3 soil cores were collected near each well (n=6 per site). Cores were taken from randomly selected points at each study site using a 99 cm long, 1.75 cm diameter soil probe. Soil samples were transported and stored in plastic bags at 22 °C until analysis.

Soil samples were oven dried (10 hr at 150 °C) before being weighed for dry mass using a Ohaus balance (0.1 mg). Samples were sorted using an Humboldt automated sieve shaker (3 min) using sieve diameters > 2 mm (gravel), 1- 2mm (sand), 0.5-1 mm (sand), 0.25-0.5 mm (sand), 0.125-0.25 mm (sand), 0.06-0.125 mm (sand), and hardpan deposits < 0.0 6 mm (silt/clay). Once sieving was complete, the mass of each sieve size class was measured (0.1 mg).

A fully factorial Two-Way MANOVA was used to compare percent sand and silt/clay content between areas with and without *C. harti* burrows and across study sites. Percent sand and silt/clay were calculated separately for each sample as the mass of sand or silt/clay divided by the total sample mass. A Tukey HSD was used to test all pairwise comparisons. Statistics were performed using IBM SPSS Statistics (ver. 21, IBM Corp., 2012).

RESULTS

Study Sites

During the course of this study, the Pine Mountain area, which includes Meriwether County, experienced warmer than normal temperatures (Georgia Automated Environmental Monitoring Network). The mean temperature for the area from 6/6/2013-8/1/2014 was 17.57 °C, 0.73 °C warmer than the mean annual temperature for the area (Georgia Automated Environmental Monitoring Network). The Pine Mountain area received an average rainfall of 0.37 cm/day from 6/6/2013-8/1/2014 (Georgia Automated Environmental Monitoring Network), which is equal to the mean rainfall for the area (Georgia Automated Environmental Monitoring Network).

Groundwater level and temperature

Groundwater temperature and depth were monitored from 6/6/2013-8/1/2014 to assess the ecological importance of fluctuations in groundwater depth and temperature for areas with and without *C. harti*. Variance in groundwater depths between burrow and non-burrow wells were significantly different (Levene's test, F=199.16, P<0.001), where overall burrow well groundwater depths displayed a higher degree of fluctuations (Coefficient of variation=0.74) compared to non-burrow wells (Coefficient of variation=0.33; Fig. 4). Spectral analysis indicated that groundwater depths oscillated at low frequencies for areas with and without *C. harti*, where the period of the oscillations occurred on time-scales ≥6 weeks (Table 2).

To test the hypothesis that *C. harti* inhabits areas with groundwater depths closer to the ground surface than areas without *C. harti*, groundwater depths were compared between burrow and non-burrow wells across the 4 study sites (Table 1). *Cambarus harti* inhabited areas where mean groundwater depths were nearly 3 times shallower than areas lacking *C. harti* burrows (Two-Way ANOVA, $F_{1, 168}$=256.58, P<0.001; Fig. 5). Groundwater depths differed significantly across study sites (Two-Way ANOVA, $F_{3, 168}$=10.539, P<0.001); mean groundwater depth for the Cartwright Property was 14-20 cm deeper than the other study sites (Tukey HSD, P≤0.001; Fig. 6). Patterns in mean water depth differed among well types and study sites as indicated by the statistically significant interaction term (Two-Way ANOVA, $F_{3, 168}$=18.724, P<0.001).

Differences in groundwater depths among well types were also evident in exceedance probability plots of the time series data (Fig 6). Groundwater depths for non-burrow wells were shallower than 49.1 cm for 10%, 77.2 cm for 50%, and 99.0 cm for 90% of the study period. In contrast, wells in areas with burrows had water levels shallower than 9.7 cm for 10%, 29.5 cm for 50%, and 44.8 cm for 90% of the time (Fig. 6).

Groundwater temperatures were positively correlated with air temperature (Spearman's rank-order, N=421, ρ=0.776, P<0.001; Fig. 7). The highest temperatures were recorded during summer months while the lowest temperatures were observed during the winter (Fig. 8). While air temperatures during the course of study ranged 36.4 ^{o}C (-9.0 to 27.4 ^{o}C), groundwater temperatures varied only 12.5 ^{o}C (8.4 to 20.9 ^{o}C). Comparisons of mean groundwater temperature showed no significant differences between wells in areas with and without *C. harti* burrows (Sheirer-Hare-Ray, df=1, H=0.55, P>0.5) or among study sites (Sheirer-Hare-Ray, df=3, H=0.55, P<0.5).

Groundwater chemistry

One goal of this research was to determine if water chemistry within *C. harti* habitats is similar to that of areas without *C. harti*. Groundwater in wells maintained acidic conditions throughout the study (pH 5.0-6.3). Mean groundwater in areas with *C. harti* were 50% (as measured by differences in hydrogen ion concentrations) more acidic than groundwater in areas lacking *C. harti* (Two-Way ANOVA, $F_{1,144}$=18.017, P<0.001; Fig. 9). Study sites also differed significantly in groundwater pH (Two-Way ANOVA, $F_{3,144}$=13.077, P<0.001; Fig. 9). Groundwater at White Sulphur Springs was 71% more basic than the Cartwright Property and 92% more basic than the Warm Springs Fish Hatchery (Tukey HSD, P<0.001 for both). Groundwater at the Chandler Property was 56% more basic than at Warm Springs Fish Hatchery (Tukey HSD, P=0.003). Comparisons of pH between treatments and across study sites indicated a significant treatment by site interaction (Two-Way ANOVA, $F_{3,144}$=9.233, P<0.001).

Dissolved oxygen concentrations displayed a high degree of variability over space and time (Coefficient of Variation= 0.81). Groundwater in wells exhibited hypoxic and oxic conditions (Fig. 10). Wells stationed among crayfish burrows exhibited hypoxic conditions (<3 mg/L dissolved oxygen) 39% of the study time (Fig. 11). The lowest (0.24 mg/L) and highest (8.31 mg/L) well water dissolved oxygen concentrations were measured at the Cartwright Property (June and January 2014 respectively).

Despite the fact that dissolved oxygen concentrations differed on average by 11% between treatments (Fig. 10), no statistically significant differences were detected (Sheirer-Hare-Ray, df=1, H= 0.996, P>0.1). In contrast, mean dissolved oxygen concentrations in differed significantly across study sites (Sheirer-Hare-Ray, H=16.02, df=3, P<0.005; Fig. 10). Mean dissolved oxygen concentration at the Cartwright Property was 32% higher than the Chandler Property (Tukey HSD, P=0.010), 73% higher than the Warm Springs Fish Hatchery (Tukey HSD, P≤0.001), and 77% higher than White Sulphur Springs (Tukey HSD, P≤0.001). White Sulphur Springs also had 48% lower groundwater well dissolved oxygen concentrations than the Chandler Property (Tukey HSD, P=0.010). A significant site by treatment interaction term for groundwater dissolved oxygen was also detected in the analysis (Sheirer-Hare-Ray, df=3, H=9.25, P<0.05). Groundwater dissolved oxygen concentrations shifted seasonally. The highest dissolved oxygen concentrations were recorded during the cooler months and decreased during the summer (Fig. 12). This seasonal shift may have been caused by changes in temperature, as groundwater temperature and dissolved oxygen concentrations were negatively correlated (Spearman's rank-order correlation, n=152, ρ= -0.67, P<0.001; Fig. 13).Groundwater temperature and percent dissolved oxygen saturation were also negatively correlated (Spearman's rank-order correlation, n=152, ρ= -0.63, P<0.001).

To assess the natural solute characteristics of groundwater in *C. harti* habitats, water samples were analyzed from wells with and without burrows and from burrows directly at each of the study sites (1/16/14-8/1/2014). In general, groundwater chemistry (as indicated by potassium, iron, and silica) did not statistically differ among treatments (Two-Way MANOVA, Wilks' Lambda, $F_{8, 210}$=0.543, P=0.823; Fig. 14) but did statistically differ across study sites (Two-Way MANOVA, Wilks' Lambda, $F_{12, 278.095}$=3.242, P=0.012). The differences among sites were primarily attributed to potassium (Two-Way ANOVA, $F_{3, 108}$=3.80, P=0.012; Fig. 15), where groundwater at White Sulphur Springs had an average concentration 2 times greater than the Cartwright Property (Tukey HSD, P=0.009; Fig. 15). Groundwater chemistry also showed a significant treatment by site interaction (Two-Way MANOVA, Wilks' Lambda, $F_{24, 367.511}$=1.696, P=0.023).

Statistical analysis of chemical analytes that failed to meet the assumptions of the parametric models (i.e, chloride and manganese) indicated that chloride concentrations differed significantly among treatments (Sheirer-Hare-Ray, df=2, H=27.32, P>0.001; Fig. 14). Water in crayfish burrows contained 16 times higher concentration than non-burrow wells (Tukey HSD, P<0.001) and 18 times higher concentration than burrow wells (Tukey HSD, P<0.001). Chloride concentrations in groundwater also differed statistically across study sites (Sheirer-Hare-Ray, df=3, H=34.95, P<0.001; Fig. 15). Groundwater at White Sulphur Springs contained, on average, 4 times higher chloride concentrations than groundwater at the Chandler Property (Tukey HSD, P=0.028), 7 times higher concentrations than the Cartwright Property (Tukey HSD, P=0.009), and 10 times higher concentrations than the Warm Springs Fish Hatchery (Tukey HSD, P=0.006). No statistically significant interaction term was detected between treatments and study sites in their chloride concentrations (Sheirer-Hare-Ray, df=6, H=5.36, P>0.1). In contrast to chloride, groundwater manganese concentrations did not significantly differ among treatments (Sheirer-Hare-Ray, df=2, H=3.02, P>0.1). However, manganese concentrations did statistically differ across study sites (Shierer-Hare-Ray, df=3, H=49.56, P<0.001; Fig. 15). Groundwater at White Sulphur Springs contained 2 times the concentration of manganese as the Cartwright Property (Tukey HSD, P<0.001) and nearly 2 times the concentration as the Chandler Property (Tukey HSD, P<0.001). Water from Warm Springs Fish Hatchery contained half the concentration of manganese as White Sulphur Springs (Tukey, HSD, P=0.027) and nearly 1.5 times the amount of manganese as the Cartwright (Tukey HSD, P<0.001) and Chandler Properties (Tukey HSD, P=0.006). Manganese did not show a significant interaction term between treatments and study sites (Sheirer-Hare-Ray, df=6, H=672.05, P<0.001).

Soil Texture

Soils collected from all sites were dominated by sand-sized particles (79-92%) and contained minor amounts of silt-clay (10-20%). Sand and silt/clay content did not statistically differ between areas with and without *C. harti* (Two-Way MANOVA, Wilks' Lambda, $F_{2, 15}$= 0.801, P=0.467, Fig. 16). Despite the absence of treatment differences, sand and silt/clay content differed statistically across study sites (Two-Way MANOVA, Wilks' Lambda, $F_{6, 30}$= 5.767, P<0.001). Soils from the Warm Springs Fish Hatchery contained ~ 50 % lower silt/clay as the other study sites (Tukey HSD, P<0.05; Fig. 16). Soil sand and silt/clay content showed no significant treatment by site interactions (Two-Way MANOVA, Wilks' Lambda, $F_{6, 30}$=0.646, P=0.693).

DISCUSSION

Groundwater level and temperature

The survival of primary burrowing crayfish may well depend on their ability to maintain access to water sources below ground (Grow & Merchant, 1980; Stoeckel *et* al., 2011; Helms *et al.*, 2013a; Helms *et al.*, 2013b). One of the primary goals of this research was to compare groundwater levels between *C. harti* habitats and areas without crayfish. Results showed that groundwater levels for *C. harti* habitats were on average 3 times shallower than surrounding areas. Well water levels near burrows never exceeded 90 cm below the ground surface at any of the habitats studied (Fig. 6). In contrast, water in wells without burrows regularly exceeded 90 cm below ground surface (Fig. 5). Thus, this study found support for the hypothesis that *C. harti* dig burrows in habitats with relatively shallow groundwater (i.e., less than 1 m).

This study also documented that groundwater levels in *C. harti* habitats varied considerably over time. Low-frequency oscillations in groundwater levels were shown to be the dominant form of groundwater fluctuations. These oscillations occurred at time scales of 6 weeks or greater, suggesting that groundwater levels shift monthly or seasonally. Relatively rapid changes in groundwater were also recorded regularly in the data (Fig. 4). However the most rapid shifts (hours to days) in water level occurred when groundwater was rising. Declines in groundwater appeared to occur more slowly, taking days to weeks to return to previous levels.

These groundwater level fluctuations are likely to affect the ecology and conservation of these crayfish. Primary burrowers have been shown to increase excavation activities during times of declining groundwater levels or during drought conditions (Johnston & Figiel 1997; Stoeckel *et* al., 2011; Helms *et al.*, 2013a; Helms *et al.*, 2013b). Crayfish need to expend energy to excavate burrows; thus, animals burrowing in areas with deeper groundwater or levels that fluctuate widely could experience smaller broods, slower growth, and reduced survival.

Groundwater level fluctuations appeared to be driven by local processes at each study site. For 3 sites, groundwater levels covaried in wells with *C. harti* burrows and areas without (Fig. 4). This correlation among wells suggests that processes controlling groundwater levels were local because water levels in burrow and non-burrow wells vacillated synchronously. The Warm Springs Fish Hatchery was the only site where wells that did not show groundwater level covariance. At this site, the well installed near burrows regularly exceeded the ground surface. This truncated the data (i.e., eliminated the highest water levels) making it impossible to determine whether both wells were truly synchronized.

In addition to supporting burrowing crayfish populations, groundwater level fluctuations have implications for other important ecological processes, e.g. the maintenance of wetlands. One legal definition of a wetland requires groundwater levels to be greater than or equal to 30 cm in depth for at least 50% of the study period (Wakeley *et al.*, 2010). Because groundwater data are rarely available and almost never distributed spatially, other hydrologic indicators, e.g., hydrophytic vegetation, hydric soils, hydrogen sulfide odors, and the presence of crayfish burrows, are often used to delineate wetlands (Wakeley *et al.*, 2010). The exceedance probabilities of groundwater levels from all study sites indicated that only two areas meet the hydrologic definition of a wetland (Fig. 6): burrow wells located at the Chandler Property and Warm Springs Fish Hatchery. *Cambarus harti* habitats located at the Cartwright Property and White Sulphur Springs have mean groundwater depths around -40 cm and -37 cm, slightly deeper than the 30 cm criteria for jurisdictional wetlands. These study sites have strong hydrogen sulfide odors suggesting that they experience regular soil saturation and could be designated as wetlands. Thus, the presence of *C. harti* does appear to be a reliable indicator of the presence of wetland habitats.

Temperature influences physiologic and behavioral characteristics of crayfish (Crawshaw *et al*., 1983; Whiteley *et al*., 1997). Crayfish can tolerate a wide range of temperatures (Aiken, 1968; Mundahl, 1989; Mundahl & Benton, 1990; Nakata *et al*., 2002; Payette & McGaw, 2003). In the present study, groundwater temperatures varied little over short time-scales, while the dominant temperature fluctuations occurred seasonally (Fig. 8). Groundwater for areas inhabited by *C. harti* and surrounding areas exhibited similar temperature ranges (6.8-22 °C) and fluctuation patterns (Fig. 8). Behavioral preference trials generally report that crayfishes select temperatures between 18-26°C, which appears to be the optimal temperature range for growth and survival (Crawshaw, 1974; Peck, 1985; Mundhal & Benton, 1990; Payette & McGraw, 2003). Temperatures above and below this range resulted in decreasing growth rate and alteration of crayfish behaviors (Mundhal & Benton, 1990; Payette & McGaw, 2003). Decreasing growth rates of crayfish at suboptimal temperatures are not uncommon since crayfish generally molt less frequently during cooler, winter months (Mundhal and Benton, 1990). At extremely high temperatures (>30 °C), abnormal post-molting mortality is common (Mundhal & Benton, 1990; Nakata *et al*., 2002).

Groundwater chemistry

Acidic waters can affect crayfish behavior, metabolism, and physiology (Appelberg, 1985; France, 1985; Wood & Rogano, 1986; Patterson & deFur, 1988; Allison *et al*., 1992; France, 1993) and ultimately, their growth and survival (Malley, 1980; Berrill *et al*., 1985; France & Collins 1993). The most apparent effect that acidic waters can have on crayfish is a decrease in post-molt calcification, resulting in increased stress and mortality (Malley, 1980; Morgan & McMahon, 1982; France, 1993; Zanotto & Wheatly, 1993). *Cambarus harti* inhabit acidic groundwater (pH=4.8-6.4, Fig. 9), suggesting that this species can tolerate low pH conditions.

Crayfish tolerance for acidic conditions may be species dependent. Newcomber (1975) found *Parastacoides tasmanicus* (a burrowing crayfish) inhabited waters with a mean pH of 4.5 and a minimum of 3.8. *Cambarus diogenes*, another primary burrower, inhabited mildly acidic groundwater with a mean pH of 6.25 (Grow & Merchant, 1980). In soft waters, the surface dwelling *Orconectes rusticus* and *O. propinquus* experienced pH toxicity at values ranging from 5.4-6.1. In contrast, *Cambarus robustus*, another surface dwelling crayfish, survived at pH values near 4 (Berrill *et al.*, 1985). Morgan & McMahon (1982) tested the median lethal pH of *Procambarus clarkii* and *O. rusticus* and found their tolerances were roughly equivalent (2.8 and 2.5 respectively). Clearly, crayfishes differ in their tolerance to acidic conditions. Results from this study indicate that while *C. harti* tolerates long-term exposure to mildly acidic waters, it does not experience extremely low pH in its natural habitats.

Results from this study also showed that groundwater near burrowing crayfish experienced oxygen depleted conditions (minimum 0.24 mg/L) consistent with Grow and Merchant (1980) and Noro and Buckup (2010). Grow and Merchant (1980) found dissolved oxygen concentrations in *C. diogenes* burrows ranged from 0.40-3.20 mg/L. Noro and Buckup (2010) observed *Parastacus defossus* inhabiting burrow waters with dissolved oxygen concentrations ranging from 0.70-1.93 mg/L. This study also found that groundwater environments inhabited by burrowing crayfish can also become oxygenated (maximum 8.31 mg/L). The variation in dissolved oxygen was seasonal, with oxic conditions occurring during cooler months and suboxic-hypoxic levels during summer (Fig. 12). There was a negative correlation (ρ= -0.67) between groundwater temperature and dissolved oxygen concentrations and a similar negative relationship (ρ= -0.63) between groundwater temperature and percent dissolved oxygen saturation. Thus, the observed variation in dissolved oxygen concentration may be attributed to elevated microbial respiration of organic materials at higher temperatures (Starr & Gillham, 1993; Malard & Hervant, 1999; Datry *et al.*, 2004) rather than temperature-driven changes in oxygen saturation.

Oxygen depletion can have physiological and behavioral effects on crayfish (Wiens & Armitage, 1961; McMahon *et al.*, 1974; Wilkes & McMahon, 1982a; Wilkes & McMahon, 1982b; Gäde, 1984; Reiber & McMahon, 1998; McMahon, 2001). To persist in oxygen depleted waters, *C. harti* and other primary burrowing crayfish may have evolved physiological and behavioral adaptations to tolerate suboxic-hypoxic conditions (Dickson & Franz, 1980). Crayfish mitigate oxygen depletion by reducing oxygen uptake and lowering their metabolism or by holding these 2 attributes constant until a minimum dissolved oxygen threshold is reached (Larimer & Gold 1961; Wiens & Armitage, 1961; Reiber, 1994, 1995). *Cambarus harti* and other primary burrowers can cope with low oxygen concentrations by positioning their gills at the air-groundwater interface (Grow & Merchant, 1980; Stoeckel *et al.*, 2011; Helms *et al.*, 2013a; Helms *et al.*, 2013b) using water retained in the branchial chamber to diffuse atmospheric oxygen across the gills (Taylor *et al.*, 1973; Taylor & Wheatly, 1980).

Burrowing crayfish may have also evolved morphological adaptations for tolerating hypoxia. A possible morphological adaptation observed in primary burrowing species is an obliterated areola (Hobbs, 1981), which may be a result of increased gill surface area and branchial chamber volume (Swain *et al*., 1988). Larger gill surface area and expanded branchial chamber volume allow for increased oxygen diffusion across the gills.

Except chloride, groundwater chemistry exhibited similar concentrations between *C. harti habitat* and surrounding areas (Fig. 14). The dominant factor controlling chemical constituents in groundwater is chemical weathering of underlying bedrock geology (Back, 1960; Back, 1966; White *et al*., 1980; Frape *et al*., 1984; Thomas *et al*., 1989; Güler & Thyne, 2004; Glynn & Plummer, 2005). These similarities in water chemistry found among wells are possibly an effect of relatively homogenous soil and bedrock compositions across small spatial scales (<100 m).

Chloride, potassium, and manganese concentrations in groundwater differed across study sites. White Sulphur Springs contained 4-10 times higher chloride concentrations in comparison to other study sites and 2 times the amount of potassium relative to the Cartwright Property. Groundwater at White Sulphur Springs contained 0.5-2 times higher manganese concentrations in comparison to other study sites, while the Warm Springs Fish Hatchery contained nearly 1.5 times the concentration as the Cartwright and Chandler Properties.

Crayfish burrows contained on average 16-18 times the chloride concentration as burrow and non-burrow wells. The small amount of chloride measured in non-burrow wells is likely the consequence of chemical weathering of bedrock (Kuroda & Sandell, 1953; Mullany *et al*., 2009) and/or atmospheric deposition (Feth, 1981; Neal & Kirchner, 2000). The higher concentrations found in crayfish burrows could indicate that excess chloride results from biological processes. Kristiensen and Hensen (1992) found that the crayfish *Astacus astacus* excreted significantly higher amounts of nutrients when fed shrimp pellets compared to those fed potatoes. Gonzalo and Camargo (2012) found bioaccumulation of fluoride in the crayfish *Pacifasticus leniusculus* lead to the release of excess fluoride. These studies suggest it is conceivable that food sources consumed by *C. harti* could contain chloride, resulting in a net efflux of chloride-rich waste into burrow water and elevating chloride levels. Further experiments would be required to determine if crayfish burrowing activity explains the elevated chloride concentration observed in burrow water.

Soil texture

Survival and success of primary burrowing crayfish depends on their ability to reach groundwater, which is in part affected by soil properties such as sediment texture (Dorn & Volin, 2009; Stoeckel *et al.*, 2011). The current study found *C. harti* habitats consist of sandy soils (79-92%) containing minor components of silt and clay (10-20%). In contrast, Grow (1982) found *C. diogenes* burrowed most efficiently in fine-grained soils (100% silt and clay), while Loughman *et al*. (2012) found *Fallicambarus fodiens* were generally associated with soils containing less than 27% clay.

The current study found similar sand and silt/clay content between areas with and without *C. harti.* These results suggest soil texture is not the exclusive environmental factor controlling the distribution of this primary burrowing crayfish. While Helms *et al*. (2013b) reported *C. harti* excavated larger chambers in sandy loam soils from the type locality, crayfish also burrowed in clayey loam test soils. *Cambarus striatus* burrowed in both sand- and clay-dominated soils but was less effective in burrowing in sand-dominated substrates (Stoeckel *et al*., 2011). These studies support the hypothesis that primary burrowing crayfish do not appear to select burrow locations based solely on soil texture.

CONCLUSION

Effective conservation of imperiled species depends on the protection of their habitat. Outside of the type locality, little is known about the habitat characteristics of *C. harti.* To address this deficiency, this study characterized groundwater levels, water chemistry, and soil texture at sites occupied by known populations of *C. harti*. *Cambarus harti* inhabited forested wetlands with shallow groundwater (i.e., depths less than 1 m below the ground surface). While this species can tolerate variation in groundwater level, anthropogenic alteration of groundwater hydrology (e.g., water extraction, land cover alterations, climate shifts) could extirpate populations of *C. harti*.

Suitable habitat for *C. harti* appears to include areas where groundwaters are acidic (pH=4.77-6.36) and can be hypoxic (<3 mg/L dissolved oxygen) during summer months. The results of this study showed no evidence *C. harti* require groundwater with specific chemical signatures (based on potassium, iron, manganese, and silica). Although soil texture does not appear to be the primary factor affecting burrow locations for *C. harti*, soils must be cohesive and malleable. Results from this study provide much needed insights into groundwater environments endured by primary burrowing crayfish, helps characterize suitable *C. harti* habitat, and provides fundamental information necessary for the conservation of this endemic species. Conditions measured may have required primary burrowing crayfish to evolve special adaptations (e.g., increased gill area and branchial chamber volume) for the tolerance of environmental characteristics (hypoxia and acidic conditions) that would be uninhabitable by their epigean counterparts.

FIGURES

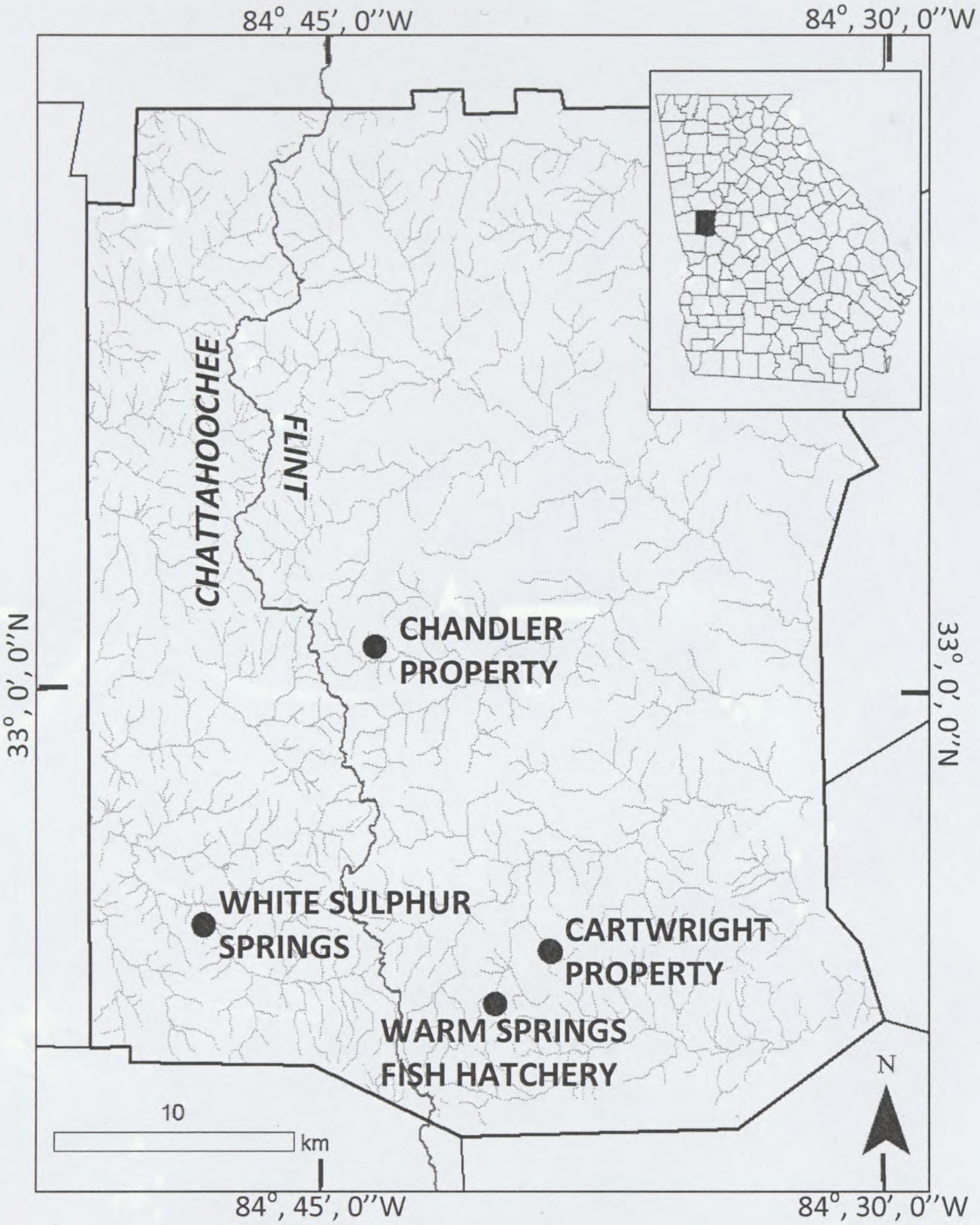

Figure 1. Map of Meriwether County river networks with study locations (dots) (inset map) and Georgia counties map indicating the location of Meriwether County (top right). Dark line trending N-S through Meriwether County represents the watershed boundary between the Chattahoochee (left) and Flint (right) River drainage basins.

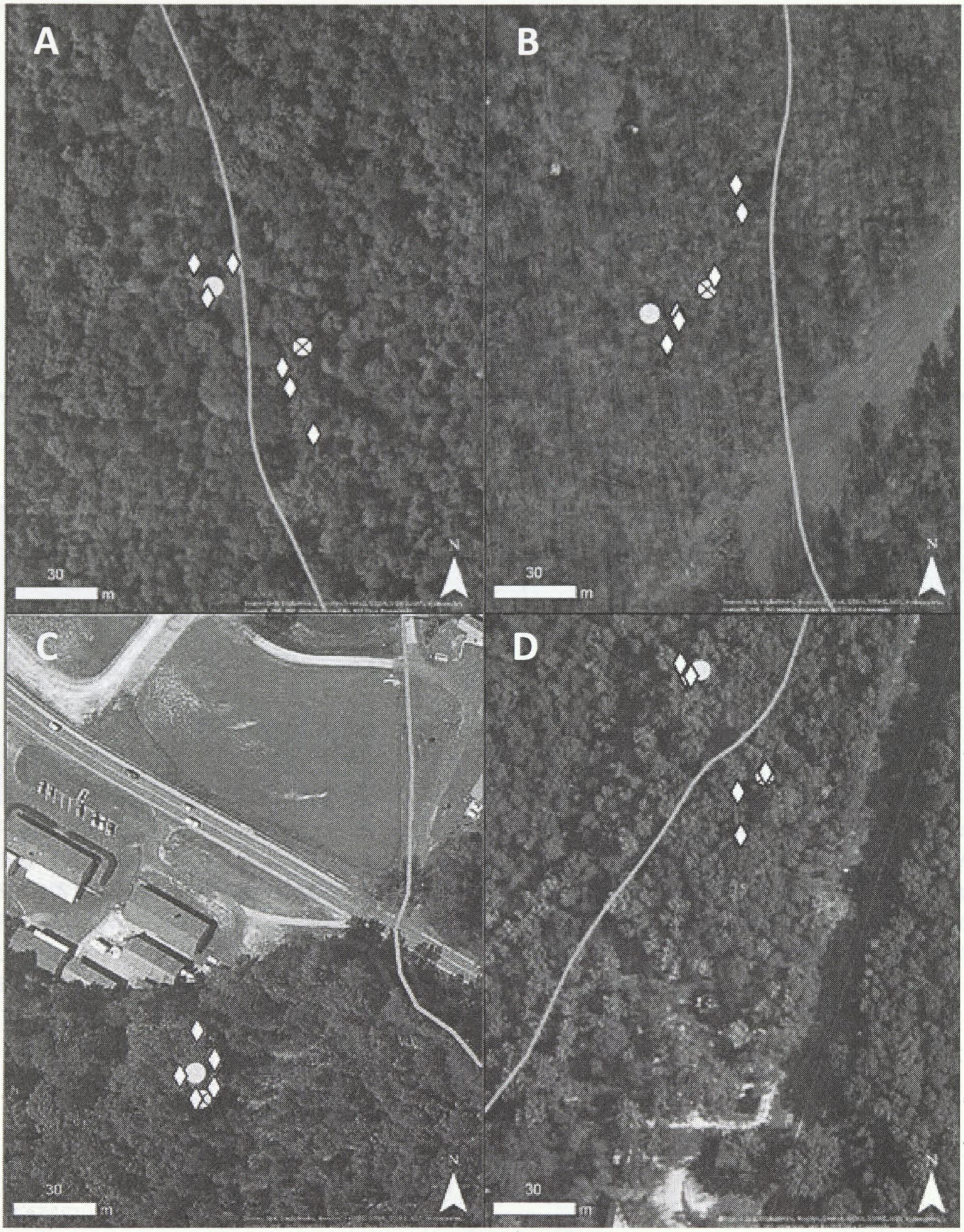

Figure 2. Image of the 4 study sites in Meriwether County, GA: (A) Cartwright Property; (B) Chandler Property; (C) Warm Springs Fish Hatchery; (D) White Sulphur Springs. Circles with crosses inside: non-burrow wells; circles without crosses: burrow wells; diamonds: soil extraction sites; and continuous lines represent streams.

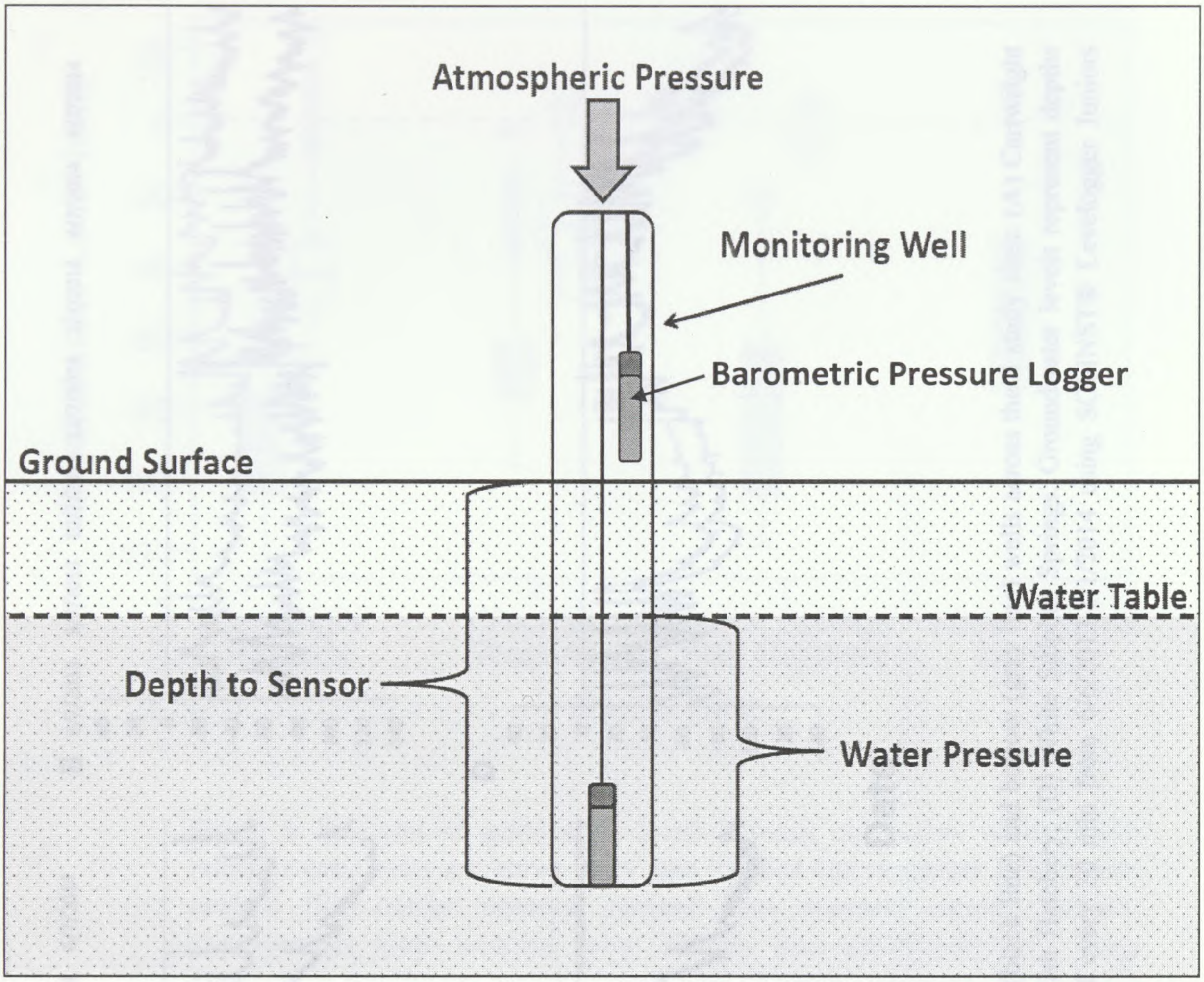

Figure 3. Cross-section of groundwater monitoring well set up. Wells were installed to depths greater than water table depths. SOLNST® Levelogger Juniors were stationed at the bottom of wells. A SOLNST® Barologger was installed in the upper portion of the non-burrow well at the Warm Springs Fish Hatchery. Leveloggers recorded water pressure + overlying air pressure and water temperature. Leveloggers were programmed to record groundwater pressure and temperature every 30 min from 6/6/2013-8/1/2014.

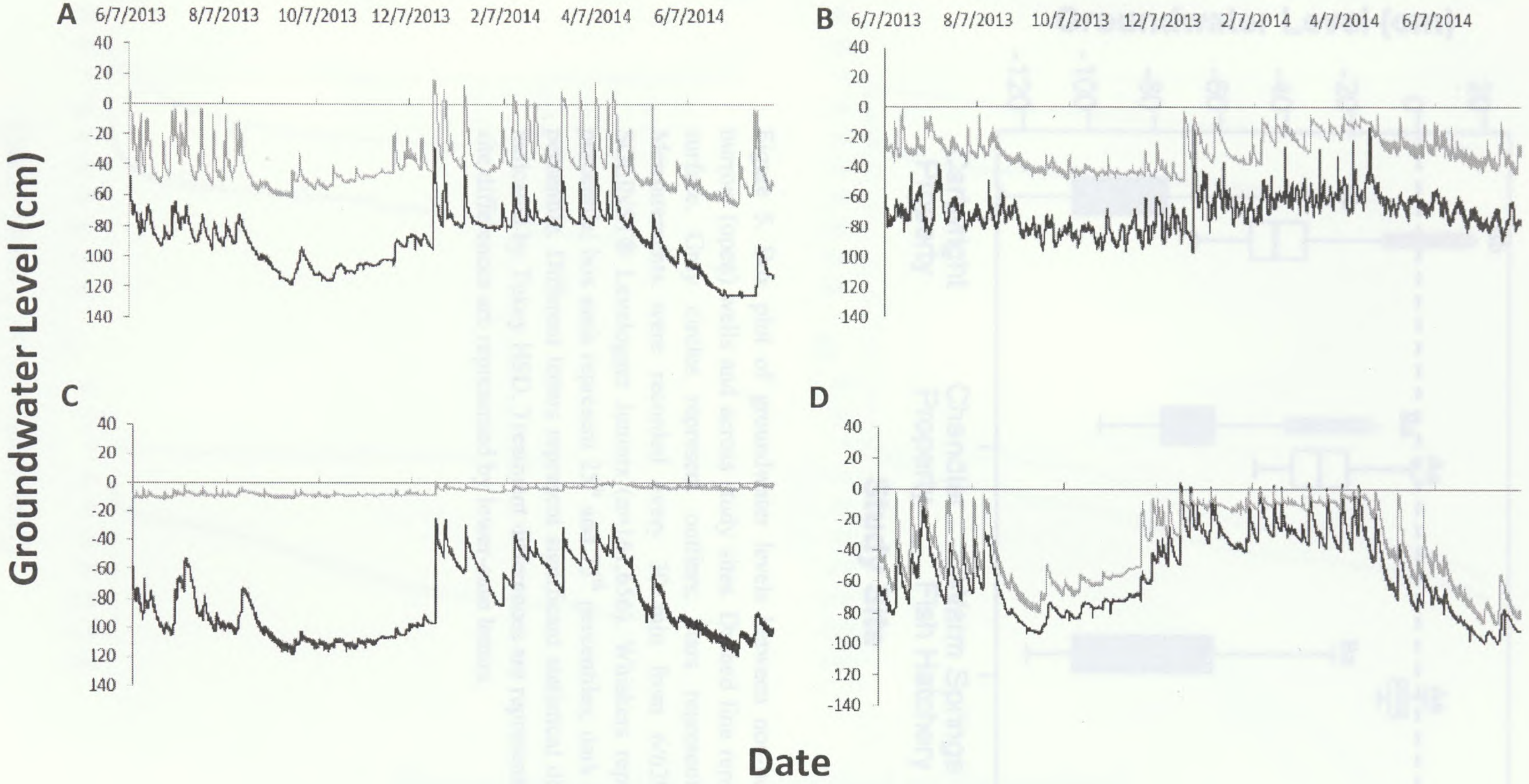

Figure 4. Time-series of groundwater level for non-burrow (black line) and burrow (gray line) wells across the 4 study sites: (A) Cartwright Property (A); (B) Chandler Property; (C) Warm Springs Fish Hatchery; (D) White Sulphur Springs. Groundwater levels represent depths below/above ground surface. Measurements were recorded every 30 min from 6/6/2013-8/1/2014 using SOLINST® Levelogger Juniors (n=161,656).

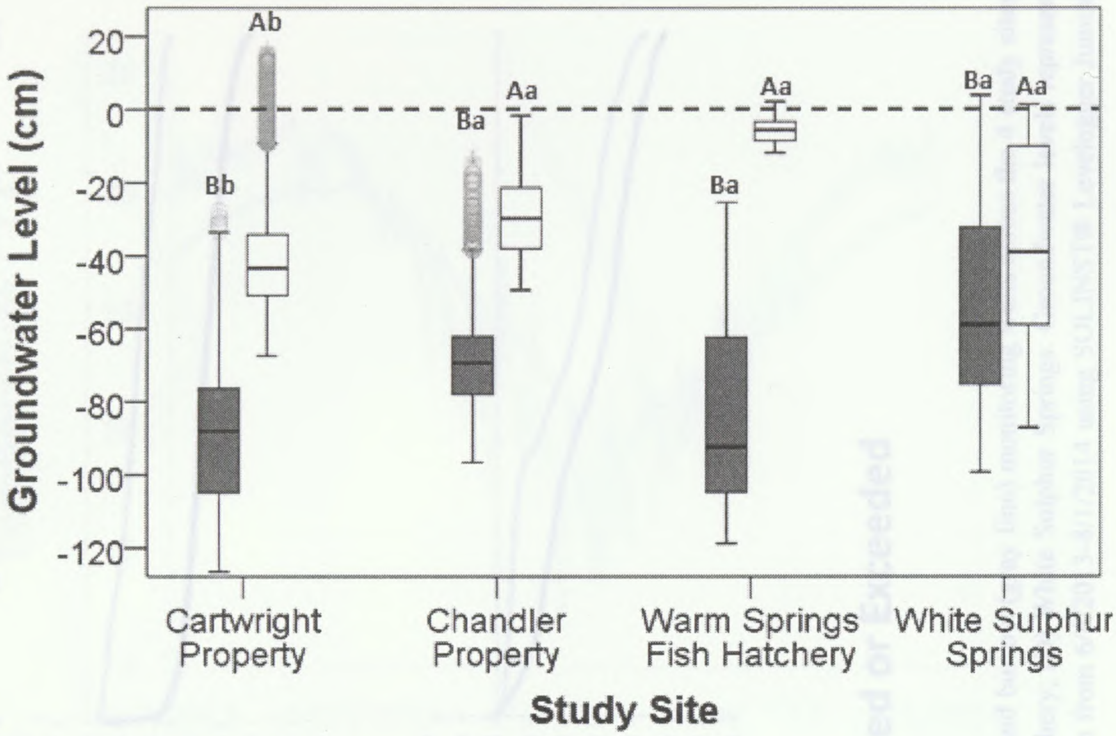

Figure 5. Box plot of groundwater levels between non-burrow (shaded) and burrow (open) wells and across study sites. Dashed line represents groundwater surface. Gray circles represent outliers; stars represent extreme outliers. Measurements were recorded every 30 min from 6/62013-8/1/2014 using SOLINST® Levelogger Juniors (n=161,656). Whiskers represent 10th and 90th percentile; box ends represent 25th and 75th percentiles; dark lines represents 50th percentile. Different letters represent significant statistical differences ($P \leq 0.005$) indicated by Tukey HSD. Treatment differences are represented by capital letters; site differences are represented by lower-case letters.

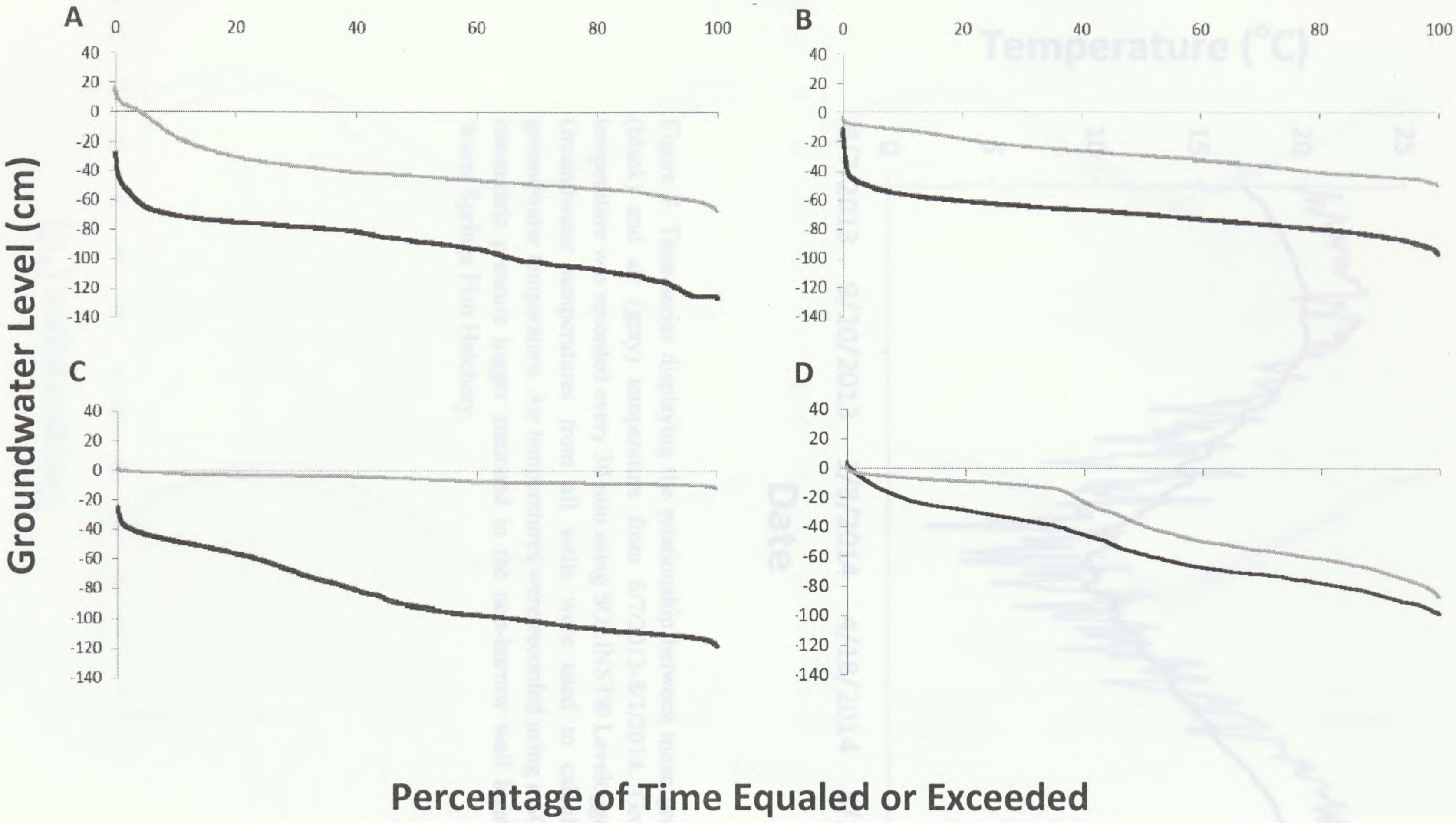

Figure 6. Exceedence plots for groundwater level for non-burrow (black line) and burrow (gray line) monitoring wells across the 4 study sites: (A) Cartwright Property; (B) Chandler Property; (C) Warm Springs Fish Hatchery; (D) White Sulphur Springs. Groundwater levels represent depths below/above ground surface. Measurements were recorded every 30 min from 6/6/2013-8/1/2014 using SOLINST® Levelogger Juniors (n=161,656).

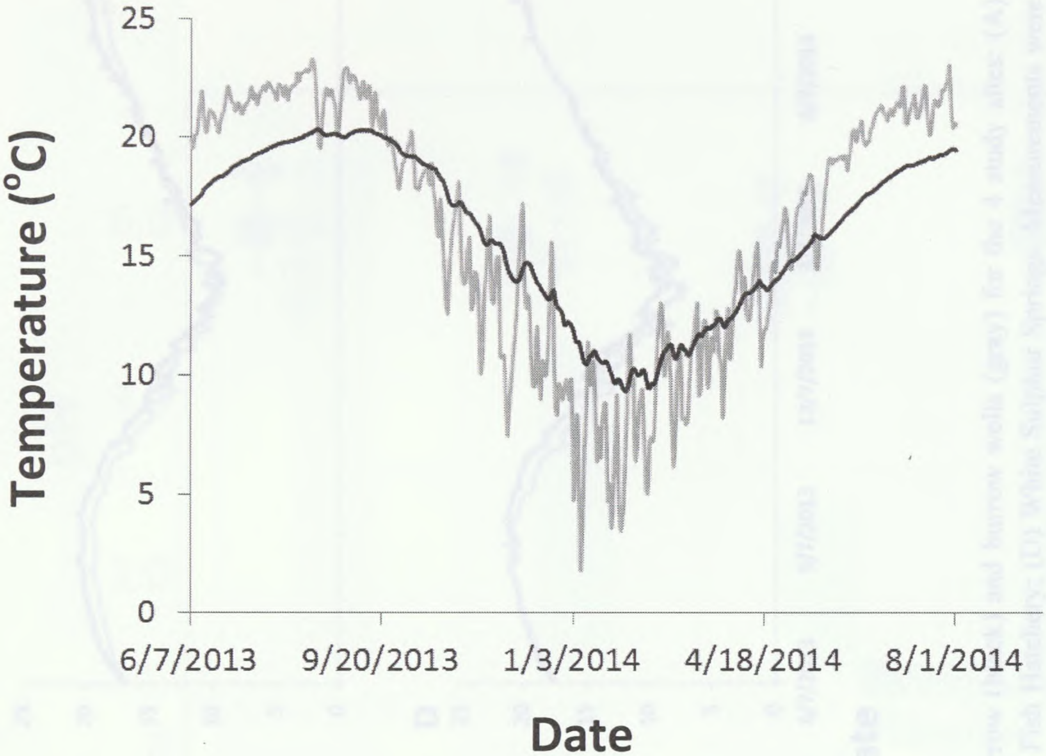

Figure 7. Time-series displaying the relationship between mean groundwater (black) and air (gray) temperature from 6/7/2013-8/1/2014. Groundwater temperature was recorded every 30 min using SOLINST® Levelogger Juniors. Groundwater temperatures from all wells were used to calculate mean groundwater temperature. Air temperatures were recorded using a SOLINST® barometric pressure logger stationed in the non-burrow well located at the Warm Springs Fish Hatchery.

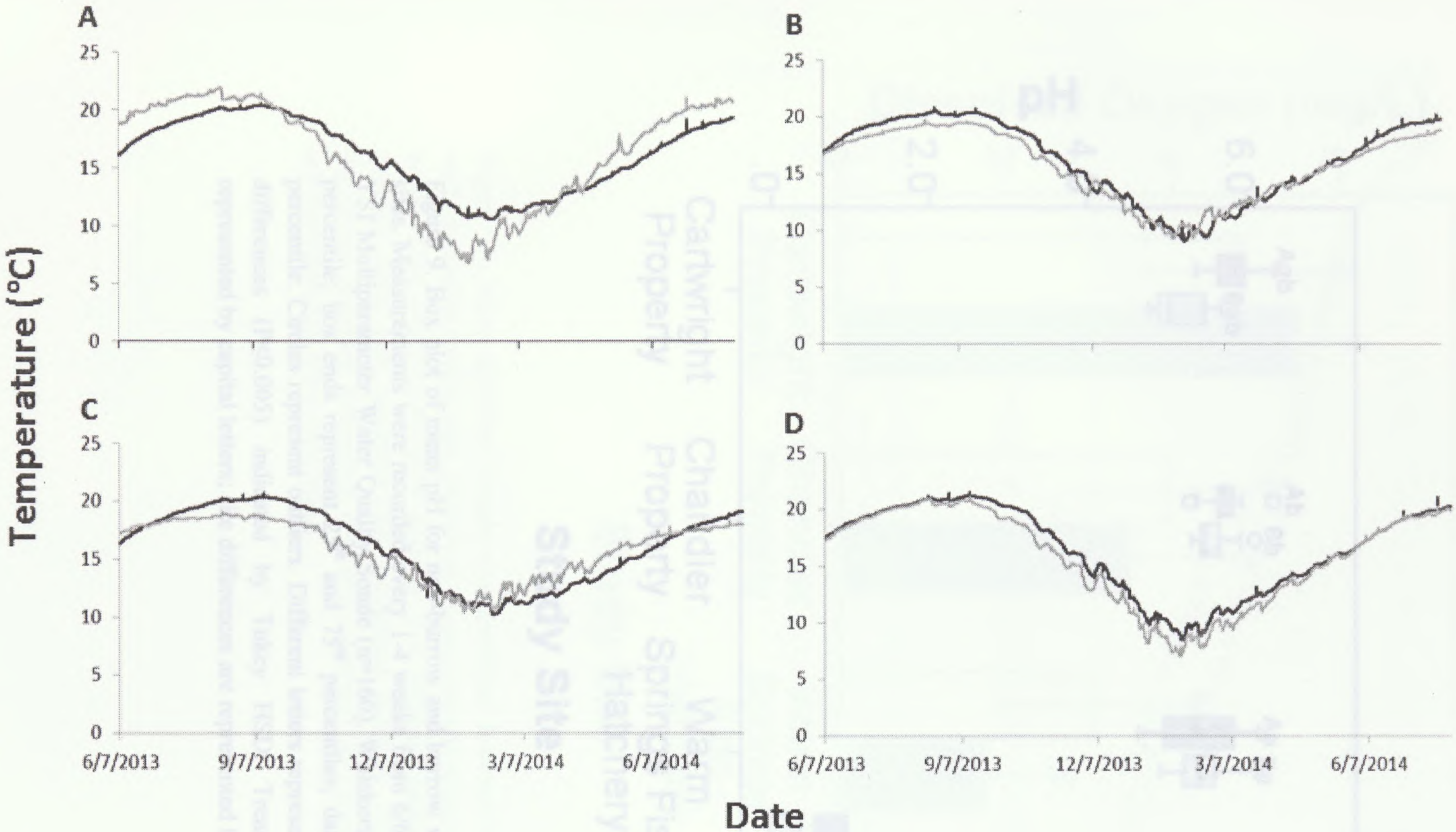

Figure 8. Time-series of groundwater temperatures for non-burrow (black) and burrow wells (gray) for the 4 study sites: (A) Cartwright Property; (B) Chandler Property; (C) Warm Springs Fish Hatchery; (D) White Sulphur Springs. Measurements were recorded every 30 min from 6/6/2013-8/1/2014 using SOLINST® Levelogger Juniors (n=161,656).

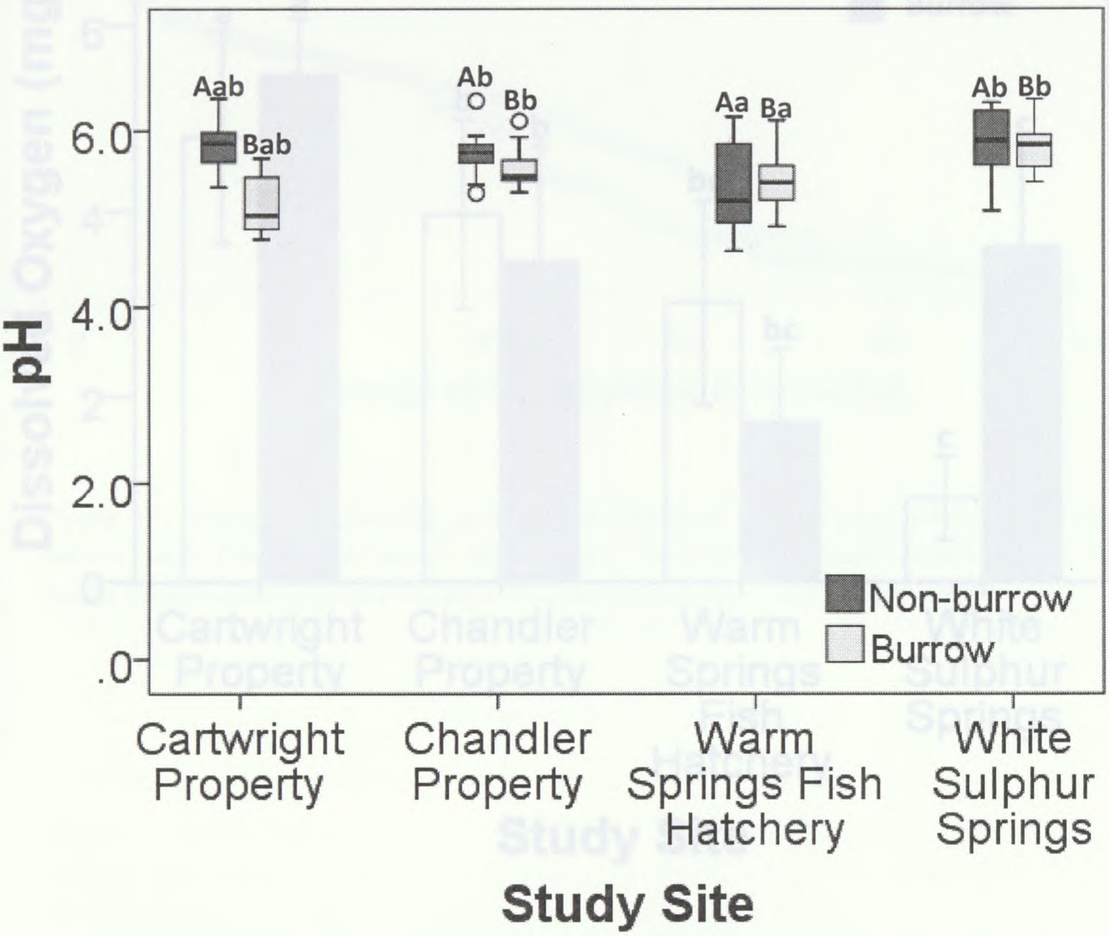

Figure 9. Box plot of mean pH for non-burrow and burrow wells across the 4 study sites. Measurements were recorded every 1-4 weeks from 6/6/2013-8/1/2014 using a YSI Multiparameter Water Quality Sonde (n=160). Whiskers represent 10th and 90th percentile; box ends represent 25th and 75th percentiles; dark lines represents 50th percentile. Circles represent outliers. Different letters represent significant statistical differences ($P \leq 0.005$) indicated by Tukey HSD. Treatment differences are represented by capital letters; site differences are represented by lower-case letters.

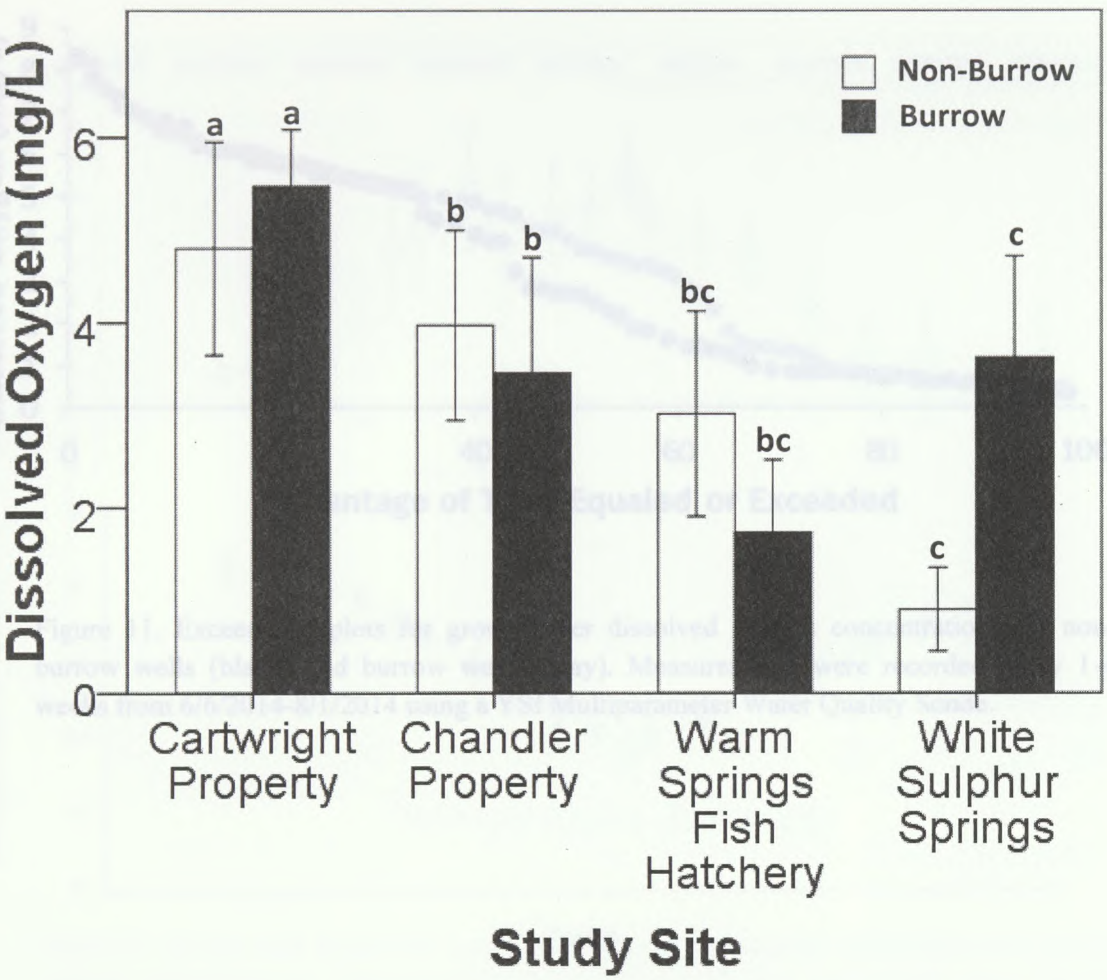

Figure 10. Mean dissolved oxygen concentrations between non-burrow and burrow wells across the 4 study sites. Dissolved oxygen concentrations were recorded every 1-4 weeks from 6/6/2013-8/1/2014 using a YSI Multiparameter Water Quality Sonde (n=152). Error bars represent 95% confidence intervals. Different letters represent significant statistical differences ($P \leq 0.005$) indicated by Tukey HSD. Site differences are represented by lower-case letters.

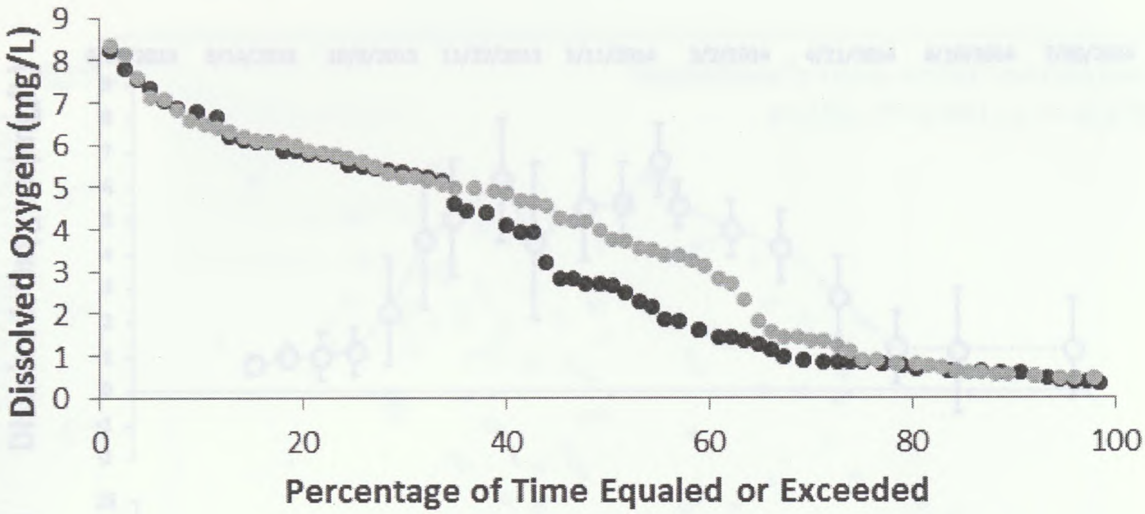

Figure 11. Exceedance plots for groundwater dissolved oxygen concentrations for non-burrow wells (black) and burrow wells (gray). Measurements were recorded every 1-4 weeks from 6/6/2014-8/1/2014 using a YSI Multiparameter Water Quality Sonde.

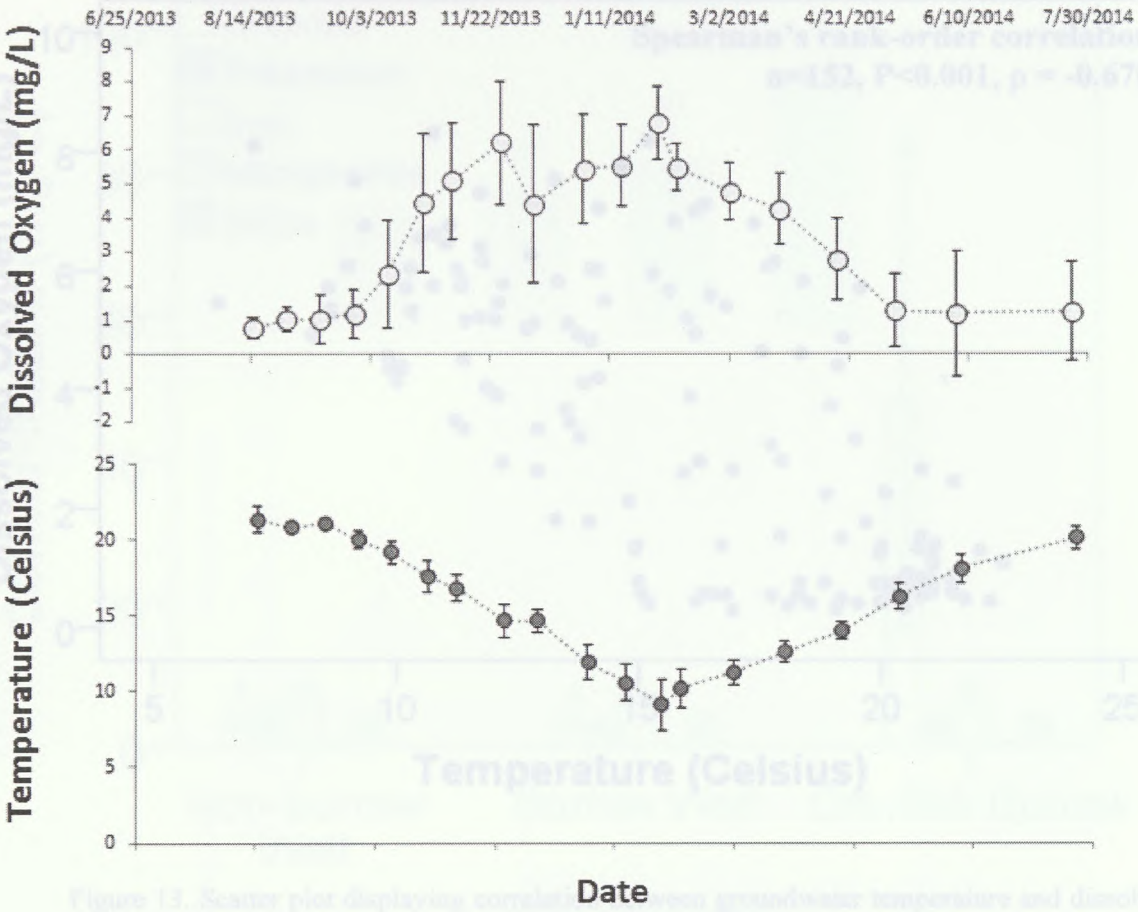

Figure 12. Time-series of groundwater dissolved oxygen concentrations (top) and temperature (bottom). Measurements were recorded every 1-4 weeks from 6/6/2013-8/1/2014. Groundwater temperature and dissolved oxygen concentrations were measured using a YSI Multiparameter Water Quality Sonde (n=152). Error bars represent standard deviations.

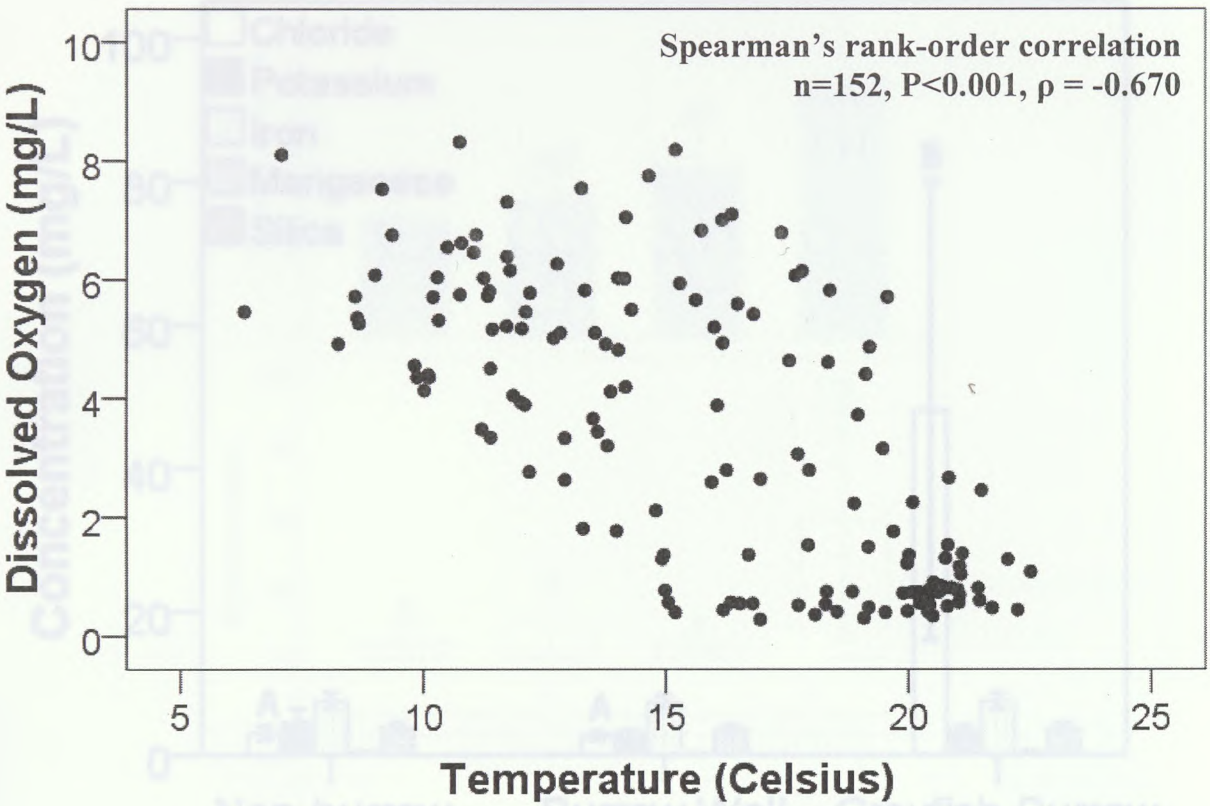

Figure 13. Scatter plot displaying correlation between groundwater temperature and dissolved oxygen concentrations. Measurements were recorded every 1-4 weeks from 6/6/2013-8/1/2014 using a YSI Multiparameter Water Quality Sonde (n=304).

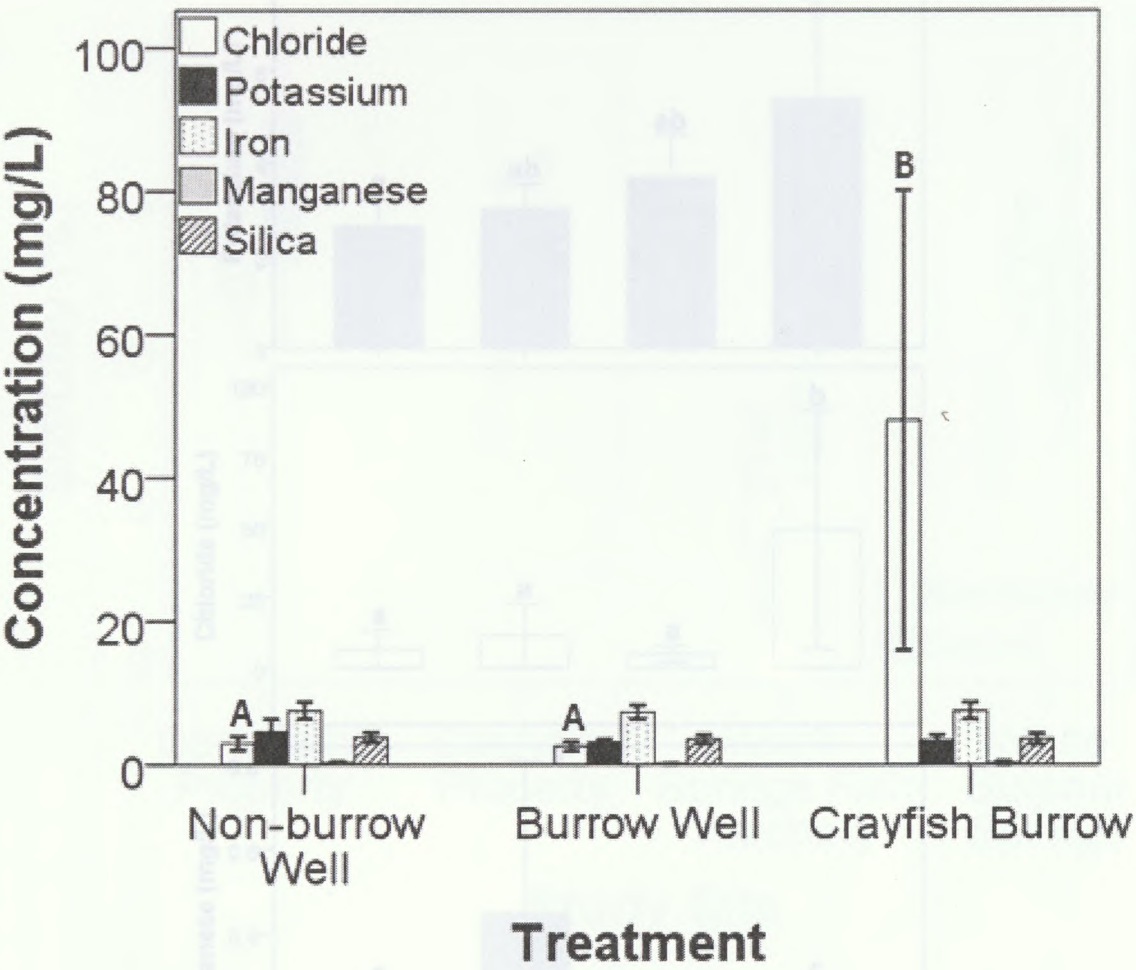

Figure 14. Mean chloride, potassium, iron, manganese, and silica concentrations in groundwater collected from non-burrow wells, burrow wells, and crayfish burrows located at each of the 4 study sites. A total of 10 water quality samples were collected from each well every 1-4 weeks from 1/16/2014-8/1/2014 and analyzed for chloride, potassium, total iron, total manganese, and silica. Crayfish burrow water was collected via burrow excavation. When groundwater levels were near the surface, a siphoning pump and tube were used. Error bars represent 95% confidence intervals. Different letters represent significant statistical differences ($P \leq 0.05$) in chloride concentrations indicated by Tukey HSD.

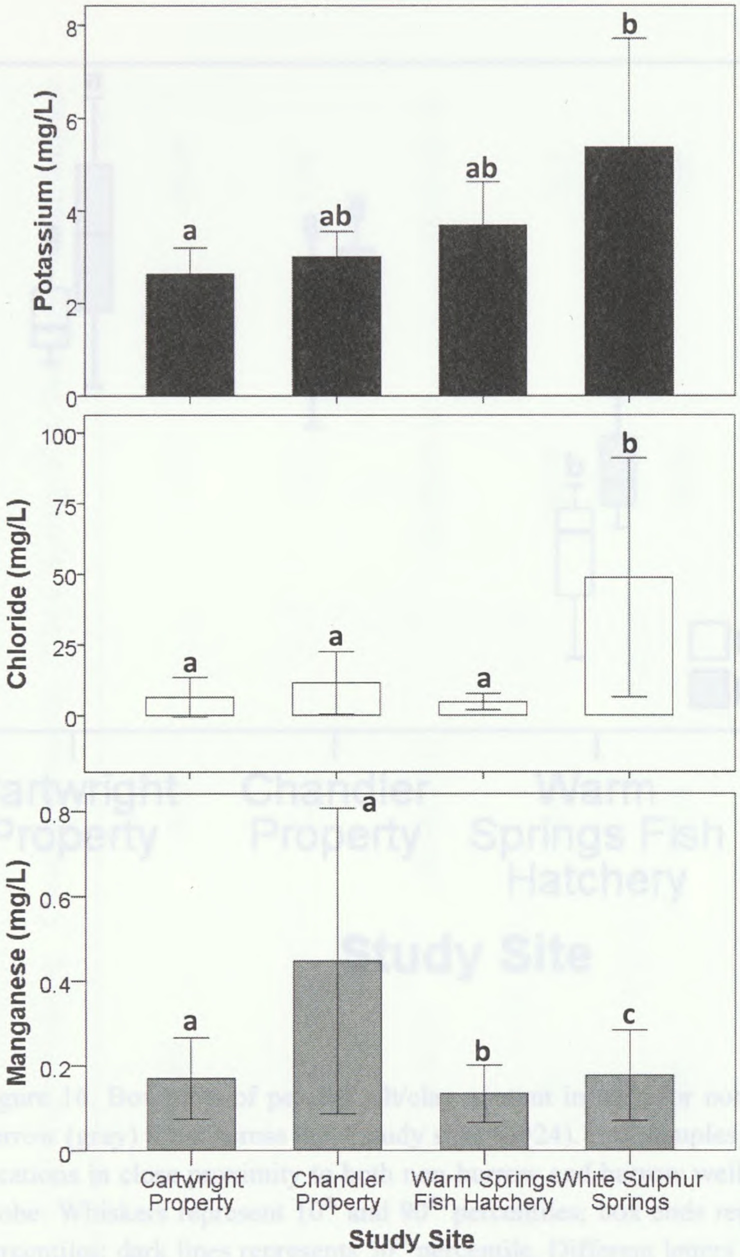

Figure 15. Mean potassium (top), chloride (middle), and manganese (bottom) concentrations in groundwater collected across the 4 study sites. Water samples were collected from each well and burrow every 1-4 weeks from 1/16/2013-8/1/2014. Crayfish burrow water was collected via burrow excavation. When groundwater levels were near the surface, a siphoning pump and tube were used. Error bars represent 95% confidence intervals. Letters represent significant site differences indicated by Tukey HSD ($P \leq 0.05$). Differences in chloride and manganese concentrations across study sites were compared by running a Tukey HSD on ranked values.

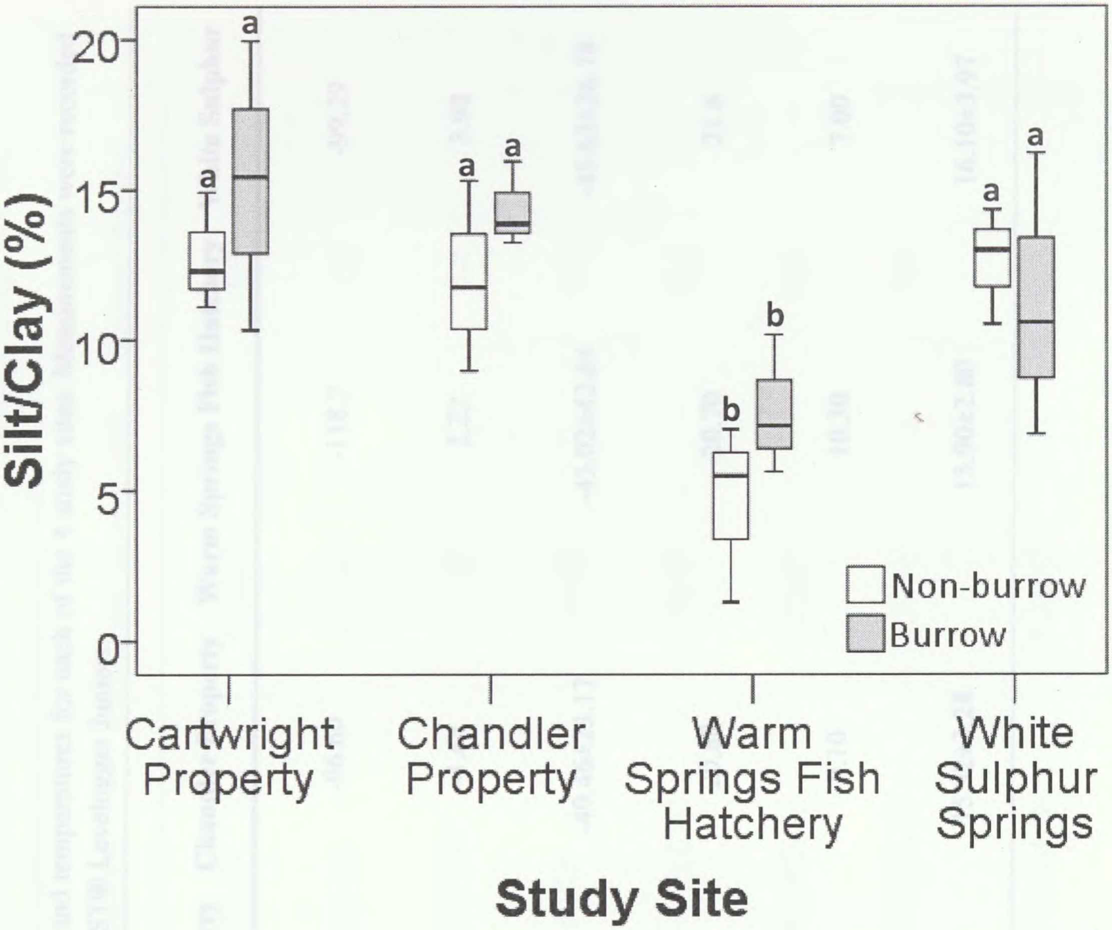

Figure 16. Box plots of percent silt/clay content in soils for non-burrow (clear) and burrow (gray) areas across the 4 study sites (n=24). Soil samples were collected from locations in close proximity to both non-burrow and burrow wells using a 99 cm soil probe. Whiskers represent 10th and 90th percentiles; box ends represent 25th and 75th percentiles; dark lines represents 50th percentile. Different letters represent significant statistical differences ($P \leq 0.005$) indicated by Tukey HSD. Site differences are represented by lower-case letters.

TABLES

Table 1. Max, min, and average groundwater levels and temperatures for each of the 4 study sites. Measurements were recorded every 30 mins from 6/6/2013-8/1/2014 using SOLINST® Levelogger Juniors.

	Cartwright Property	**Chandler Property**	**Warm Springs Fish Hatchery**	**White Sulphur**
Max GW Depth (cm)	-126.60	-96.60	-118.7	-99.29
Min GW Depth (cm)	16.86	-1.64	2.22	3.98
Avg. GW Level (cm)	-65.13±30.22	-49.46±23.17	-45.02±42.64	-45.63±26.78
Max GW Temperature (C^o)	22	20.80	20.70	21.6
Min GW Temperature (C^o)	6.80	9.10	10.30	7.00
Avg. GW Temperature (C^o)	16.05±3.91	15.92±3.28	15.90±2.80	16.10±3.97

±values represent standard deviations

Table 2. Maximum spectra and periods for groundwater levels for monitoring wells. Periods were used to calculate time-scales (in weeks) in which dominant fluctuations occurred. Well ID's ending in C represent non-burrow (control) wells; well ID's ending in E represent burrow (experimental) wells.

Study Site	**Well ID**	**Maximum Spectra (dB)**	**Period (Weeks)**
Cartwright Property	SCL2C	1.7×10^5	6.7
Cartwright Property	SCL1E	8.5×10^4	7.5
Chandler Property	JCLW11C	4.1×10^4	6.0
Chandler Property	W10E	5.3×10^4	12.1
Warm Springs Fish Hatchery	WSF-WELL2C	2.9×10^3	12.1
Warm Springs Fish Hatchery	WSF3E	9.9×10^2	12.1
White Sulphur Springs	WSSPWELL2C	3.3×10^3	8.6
White Sulphur Springs	WSSPWELL1E	3.2×10^3	12.1

Table 3. Chemicals tested for in groundwater and their associated methods.

Chemical	Method	Source Method
Chloride	Mercuric thiocyanate	HACH 8113
Potassium	Tetraphenylborate	HACH 8049
Iron	Phenanthroline	40 CFR 136
Manganese	Periodate oxidation	40 CFR 136
Silica	Heteropoly blue	HACH 8186

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APPENDIX A

PHYSICOCHEMISTRY

Date	Study Site	Well ID	Temperature (°C)	Specific Conductance (µS/cm)	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)
8/15/2013	Cartwright Property	SCL2C	20.62	141	5.83	8.3	0.74
8/29/2013	Cartwright Property	SCL2C	20.44	169	6.01	8.8	0.79
9/12/2013	Cartwright Property	SCL2C	21.05	NA	5.99	13.1	1.17
9/26/2013	Cartwright Property	SCL2C	20.08	247	5.98	8.2	0.74
10/10/2013	Cartwright Property	SCL2C	19.47	142	5.88	34.8	3.15
10/25/2013	Cartwright Property	SCL2C	18.34	149	5.81	48.8	4.6
11/6/2013	Cartwright Property	SCL2C	17.67	50	5.9	63.7	6.06
11/26/2013	Cartwright Property	SCL2C	15.2	44	6.37	81.5	8.18
12/10/2013	Cartwright Property	SCL2C	15.29	41	5.74	59.3	5.93

12/31/2013	Cartwright Property	SCL2C	13.33	31	5.56	55.7	5.81
1/16/2014	Cartwright Property	SCL2C	11.73	25	5.62	67.2	7.3
1/31/2014	Cartwright Property	SCL2C	10.75	27	5.67	75.1	8.31
2/8/2014	Cartwright Property	SCL2C	11.09	31	5.36	61.1	6.75
3/2/2014	Cartwright Property	SCL2C	11.36	31	5.51	53	5.8
3/23/2014	Cartwright Property	SCL2C	12.03	35	5.9	46.2	5.17
4/16/2014	Cartwright Property	SCL2C	13.5	93	5.86	35.1	3.66
5/10/2014	Cartwright Property	SCL2C	14.98	137	6.24	7.7	0.77
6/5/2014	Cartwright Property	SCL2C	17.73	NA	6.31	5.6	0.52
6/13/2014	Cartwright Property	SCL2C	NA	NA	NA	NA	NA
6/30/2014	Cartwright Property	SCL2C	NA	NA	NA	NA	NA
7/23/2014	Cartwright Property	SCL2C	19.89	73	5.62	7.9	0.72
8/1/2014	Cartwright Property	SCL2C	NA	NA	NA	NA	NA

8/15/2013	Cartwright Property	SCL1E	22.52	49	4.94	12.5	1.08
8/29/2013	Cartwright Property	SCL1E	21.11	58	5.56	15.7	1.39
9/12/2013	Cartwright Property	SCL1E	21.43	98	5.56	9.2	0.81
9/26/2013	Cartwright Property	SCL1E	20.01	69	5.24	15	1.36
10/10/2013	Cartwright Property	SCL1E	18.96	49	5.45	40.2	3.72
10/25/2013	Cartwright Property	SCL1E	16.48	43	5.59	57.2	5.58
11/6/2013	Cartwright Property	SCL1E	15.62	38	5.69	56.9	5.65
11/26/2013	Cartwright Property	SCL1E	12.76	54	5.28	59.1	6.26
12/10/2013	Cartwright Property	SCL1E	13.54	62	4.94	49.1	5.1
12/31/2013	Cartwright Property	SCL1E	10.12	38	4.88	38.7	4.35
1/16/2014	Cartwright Property	SCL1E	9.01	36	4.87	52.5	6.07
1/31/2014	Cartwright Property	SCL1E	7.08	40	5.04	66.9	8.1
2/8/2014	Cartwright Property	SCL1E	8.64	37	4.77	46	5.35

3/2/2014	Cartwright Property	SCL1E	10.1	34	4.8	39.3	4.39
3/23/2014	Cartwright Property	SCL1E	11.85	33	5.31	37.5	4.05
4/16/2014	Cartwright Property	SCL1E	13.29	30	4.78	17.3	1.81
5/10/2014	Cartwright Property	SCL1E	16.7	37	4.94	14.1	1.37
6/5/2014	Cartwright Property	SCL1E	18.84	52	5.5	8	0.75
6/13/2014	Cartwright Property	SCL1E	19.65	63	5.67	9.6	0.88
6/30/2014	Cartwright Property	SCL1E	20.7	100	6.23	2.7	0.24
7/23/2014	Cartwright Property	SCL1E	21.5	34	4.89	27.7	2.45
8/1/2014	Cartwright Property	SCL1E	20.98	36	5.12	12.9	1.15
6/6/2013	Chandler Property	JCLW11C	17.35	111	5.5	12.3	1.16
8/15/2013	Chandler Property	JCLW11C	21.05	74	5.63	7.7	0.69
8/29/2013	Chandler Property	JCLW11C	20.66	88	5.88	9.3	0.84
9/12/2013	Chandler Property	JCLW11C	20.88	87	5.75	9.1	0.81

9/26/2013	Chandler Property	W10E	19.97	92	5.75	13.5	1.22
10/10/2013	Chandler Property	JCLW11C	19.17	87	5.7	16.3	1.5
10/25/2013	Chandler Property	JCLW11C	17.54	47	5.49	48.4	4.63
11/6/2013	Chandler Property	JCLW11C	16.81	42	5.84	55.7	5.41
11/26/2013	Chandler Property	JCLW11C	14.65	37	6.34	76.3	7.74
12/10/2013	Chandler Property	JCLW11C	14.29	42	5.82	53.7	5.49
12/31/2013	Chandler Property	JCLW11C	11.79	40	5.76	56.9	6.15
1/16/2014	Chandler Property	JCLW11C	10.79	31	5.68	59.8	6.61
1/31/2014	Chandler Property	JCLW11C	9.15	35	5.66	65.3	7.52
2/8/2014	Chandler Property	JCLW11C	10.2	35	5.39	50.9	5.7
3/2/2014	Chandler Property	JCLW11C	11.33	32	5.85	52.3	5.72
3/23/2014	Chandler Property	JCLW11C	12.82	34	5.89	48.2	5.1
4/16/2014	Chandler Property	JCLW11C	14.96	35	5.29	13.6	1.37

5/10/2014	Chandler Property	JCLW11C	16.18	40	5.41	4.5	0.44
6/5/2014	Chandler Property	JCLW11C	18.29	68	5.64	5.8	0.54
6/13/2014	Chandler Property	JCLW11C	18.95	59	5.6	6.9	0.64
6/30/2014	Chandler Property	JCLW11C	20.06	105	5.92	4.3	0.39
7/23/2014	Chandler Property	JCLW11C	20.23	92	5.94	6.2	0.56
8/1/2014	Chandler Property	JCLW11C	20.38	93	5.64	8.7	0.78
6/6/2013	Chandler Property	W10E	18.05	132	5	6	0.57
8/15/2013	Chandler Property	W10E	NA	54	5.42	4.7	0.41
8/29/2013	Chandler Property	W10E	20.4	58	5.53	7.4	0.67
9/12/2013	Chandler Property	W10E	20.8	59	5.46	5.6	0.5
9/26/2013	Chandler Property	W10E	19.52	59	5.41	4.4	0.4
10/10/2013	Chandler Property	W10E	18.52	54	5.3	4.4	0.41
10/25/2013	Chandler Property	W10E	16.95	51	5.51	27.5	2.64

11/6/2013	Chandler Property	W10E	16.06	42	5.72	39.5	3.88
11/26/2013	Chandler Property	W10E	14.02	37	5.88	46.9	4.81
12/10/2013	Chandler Property	W10E	14.17	38	5.67	40.9	4.19
12/31/2013	Chandler Property	W10E	11.73	37	5.6	58.9	6.38
1/16/2014	Chandler Property	W10E	10.76	32	5.66	51.7	5.74
1/31/2014	Chandler Property	W10E	9.36	37	6.11	58.9	6.75
2/8/2014	Chandler Property	W10E	10.32	35	5.44	47.4	5.31
3/2/2014	Chandler Property	W10E	11.38	33	5.47	30.5	3.34
3/23/2014	Chandler Property	W10E	12.91	35	5.93	24.9	2.63
4/16/2014	Chandler Property	W10E	13.98	36	5.37	17.2	1.77
5/10/2014	Chandler Property	W10E	16.53	49	5.48	5.6	0.55
6/5/2014	Chandler Property	W10E	16.94	61	5.43	2.4	0.28
6/13/2014	Chandler Property	W10E	18.73	50	5.43	4.5	0.42

6/30/2014	Chandler Property	W10E	19.46	54	5.41	4.6	0.42
7/23/2014	Chandler Property	W10E	19.98	54	5.48	4.6	0.42
8/1/2014	Chandler Property	W10E	20.06	48	5.19	3.3	0.3
8/15/2013	Warm Springs Fish Hatchery	WSF-WELL2C	20.65	76	5.98	9	0.8
8/29/2013	Warm Springs Fish Hatchery	WSF-WELL2C	20.51	73	5.2	10	0.9
9/12/2013	Warm Springs Fish Hatchery	WSF-WELL2C	20.76	79	6.08	9.2	0.81
9/26/2013	Warm Springs Fish Hatchery	WSF-WELL2C	20.22	59	5.84	8.2	0.74
10/10/2013	Warm Springs Fish Hatchery	WSF-WELL2C	19.69	75	6.16	19.3	1.76
10/25/2013	Warm Springs Fish Hatchery	WSF-WELL2C	18.38	24	5.66	62	5.81
11/6/2013	Warm Springs Fish Hatchery	WSF-WELL2C	17.81	19	5.46	64.6	6.14
11/26/2013	Warm Springs Fish Hatchery	WSF-WELL2C	16.16	18	5.87	71.2	7
12/10/2013	Warm Springs Fish Hatchery	WSF-WELL2C	15.73	19	5.62	68.6	6.82
12/31/2013	Warm Springs Fish Hatchery	WSF-WELL2C	13.26	24	4.88	72.1	7.53

1/16/2014	Warm Springs Fish Hatchery	WSF-WELL2C	12.11	22	5.03	50.8	5.45
1/31/2014	Warm Springs Fish Hatchery	WSF-WELL2C	11.04	23	5.16	58.6	6.45
2/8/2014	Warm Springs Fish Hatchery	WSF-WELL2C	11.25	23	4.86	54.9	6.02
3/2/2014	Warm Springs Fish Hatchery	WSF-WELL2C	11.38	24	4.76	41.27	4.5
3/23/2014	Warm Springs Fish Hatchery	WSF-WELL2C	12.2	24	5.19	53.9	5.77
4/16/2014	Warm Springs Fish Hatchery	WSF-WELL2C	13.76	24	5.12	47.4	4.91
5/10/2014	Warm Springs Fish Hatchery	WSF-WELL2C	15.05	23	4.83	5.7	0.57
6/5/2014	Warm Springs Fish Hatchery	WSF-WELL2C	16.79	26	4.63	5.7	0.55
6/13/2014	Warm Springs Fish Hatchery	WSF-WELL2C	17.44	27	5.24	5.9	0.56
6/30/2014	Warm Springs Fish Hatchery	WSF-WELL2C	18.25	46	5.88	4.8	0.45
7/23/2014	Warm Springs Fish Hatchery	WSF-WELL2C	19.18	46	5.86	5.4	0.48
8/1/2014	Warm Springs Fish Hatchery	WSF-WELL2C	19.57	32	5.32	10.5	0.97
8/15/2013	Warm Springs Fish Hatchery	WSF3E	21.01	58	5.55	8.8	0.78

8/29/2013	Warm Springs Fish Hatchery	WSF3E	20.81	34	5.75	17.2	1.53
9/12/2013	Warm Springs Fish Hatchery	WSF3E	20.83	32	5.62	29.9	2.66
9/26/2013	Warm Springs Fish Hatchery	WSF3E	18.88	26	5.27	24.1	2.23
10/10/2013	Warm Springs Fish Hatchery	WSF3E	17.95	22	5.06	29.5	2.79
10/25/2013	Warm Springs Fish Hatchery	WSF3E	16	18	5.2	52.6	5.19
11/6/2013	Warm Springs Fish Hatchery	WSF3E	16.17	16	5.41	50.3	4.93
11/26/2013	Warm Springs Fish Hatchery	WSF3E	14.15	16	6.12	58.5	6.01
12/10/2013	Warm Springs Fish Hatchery	WSF3E	14.92	26	5.64	12.9	1.3
12/31/2013	Warm Springs Fish Hatchery	WSF3E	12.18	23	5.45	25.9	2.76
1/16/2014	Warm Springs Fish Hatchery	WSF3E	11.2	19	5.29	31.7	3.48
1/31/2014	Warm Springs Fish Hatchery	WSF3E	10.29	18	5.22	53.1	6.03
2/8/2014	Warm Springs Fish Hatchery	WSF3E	11.72	19	4.94	48	5.21
3/2/2014	Warm Springs Fish Hatchery	WSF3E	12.68	20	5.8	47.2	5.01

3/23/2014	Warm Springs Fish Hatchery	WSF3E	13.86	21	5.58	39.8	4.11
4/16/2014	Warm Springs Fish Hatchery	WSF3E	13.79	20	4.95	31.9	3.2
5/10/2014	Warm Springs Fish Hatchery	WSF3E	17.72	21	5.33	32.1	3.06
6/5/2014	Warm Springs Fish Hatchery	WSF3E	19.57	23	4.91	6	0.57
6/13/2014	Warm Springs Fish Hatchery	WSF3E	19.64	20	5.13	18.1	1.66
6/30/2014	Warm Springs Fish Hatchery	WSF3E	20.67	25	5.69	13.8	1.24
7/23/2014	Warm Springs Fish Hatchery	WSF3E	19.12	29	5.42	4.7	0.44
8/1/2014	Warm Springs Fish Hatchery	WSF3E	19.31	20	5.07	10.8	1
6/6/2013	White Sulphur Springs	WSSPWELL2C	18.2	358	6.5	5.1	0.48
8/15/2013	White Sulphur Springs	WSSPWELL2C	22.25	420	6.29	5.1	0.44
8/29/2013	White Sulphur Springs	WSSPWELL2C	21.46	443	6.28	6.8	0.6
9/12/2013	White Sulphur Springs	WSSPWELL2C	21.72	453	6.22	5.4	0.47
9/26/2013	White Sulphur Springs	WSSPWELL2C	21.04	464	6.23	6.4	0.57

10/10/2013	White Sulphur Springs	WSSPWELL2C	20.43	439	6.26	5.8	0.52
10/25/2013	White Sulphur Springs	WSSPWELL2C	19.08	323	5.09	3.4	0.31
11/6/2013	White Sulphur Springs	WSSPWELL2C	17.93	212	6.02	16.2	1.53
11/26/2013	White Sulphur Springs	WSSPWELL2C	15.94	196	6.08	26.4	2.59
12/10/2013	White Sulphur Springs	WSSPWELL2C	15.19	169	5.89	4	0.4
12/31/2013	White Sulphur Springs	WSSPWELL2C	12.09	131	5.43	36.2	3.89
1/16/2014	White Sulphur Springs	WSSPWELL2C	9.87	98	5.73	38.5	4.35
1/31/2014	White Sulphur Springs	WSSPWELL2C	8.6	96	5.69	49	5.71
2/8/2014	White Sulphur Springs	WSSPWELL2C	9.82	87	5.54	40.2	4.55
3/2/2014	White Sulphur Springs	WSSPWELL2C	11.42	82	5.88	47.4	5.16
3/23/2014	White Sulphur Springs	WSSPWELL2C	12.91	79	6.14	31.5	3.33
4/16/2014	White Sulphur Springs	WSSPWELL2C	14.79	81	5.5	20.8	2.11
5/10/2014	White Sulphur Springs	WSSPWELL2C	16.34	113	5.68	5.8	0.57

6/5/2014	White Sulphur Springs	WSSPWELL2C	18.07	241	5.11	3.8	0.36
6/13/2014	White Sulphur Springs	WSSPWELL2C	18.87	227	6.2	3.8	0.35
6/30/2014	White Sulphur Springs	WSSPWELL2C	19.77	308	6.29	3.7	0.33
7/23/2014	White Sulphur Springs	WSSPWELL2C	20.5	392	6.32	3.8	0.34
8/1/2014	White Sulphur Springs	WSSPWELL2C	20.66	325	6	5.4	0.48
6/6/2013	White Sulphur Springs	WSSPWELL1E	18.79	59	5.88	15.1	1.41
8/15/2013	White Sulphur Springs	WSSPWELL1E	22.05	39	5.49	14.8	1.29
8/29/2013	White Sulphur Springs	WSSPWELL1E	20.76	47	5.67	14.5	1.31
9/12/2013	White Sulphur Springs	WSSPWELL1E	21.08	89	5.74	11.7	1.04
9/26/2013	White Sulphur Springs	WSSPWELL1E	20.09	70	5.61	24.9	2.25
10/10/2013	White Sulphur Springs	WSSPWELL1E	19.2	53	5.97	52.6	4.86
10/25/2013	White Sulphur Springs	WSSPWELL1E	17.38	47	5.94	70.7	6.78
11/6/2013	White Sulphur Springs	WSSPWELL1E	16.36	38	6.36	72.4	7.09

11/26/2013	White Sulphur Springs	WSSPWELL1E	14.18	39	5.86	68.6	7.04
12/10/2013	White Sulphur Springs	WSSPWELL1E	14.01	36	5.84	58.6	6.02
12/31/2013	White Sulphur Springs	WSSPWELL1E	10.5	28	5.98	58.7	6.54
1/16/2014	White Sulphur Springs	WSSPWELL1E	8.68	21	5.86	45.3	5.26
1/31/2014	White Sulphur Springs	WSSPWELL1E	6.32	22	5.54	44.19	5.45
2/8/2014	White Sulphur Springs	WSSPWELL1E	8.25	22	5.64	41.7	4.91
3/2/2014	White Sulphur Springs	WSSPWELL1E	10.02	22	5.57	36.6	4.13
3/23/2014	White Sulphur Springs	WSSPWELL1E	12.01	23	6.25	36.5	3.93
4/16/2014	White Sulphur Springs	WSSPWELL1E	13.59	27	5.42	34.5	3.44
5/10/2014	White Sulphur Springs	WSSPWELL1E	16.25	28	5.86	28.5	2.79
6/5/2014	White Sulphur Springs	WSSPWELL1E	18.31	43	5.51	8	0.75
6/13/2014	White Sulphur Springs	WSSPWELL1E	18.6	62	5.73	3	0.28
6/30/2014	White Sulphur Springs	WSSPWELL1E	20.05	82	5.98	10.1	0.92

7/23/2014	White Sulphur Springs	WSSPWELL1E	20.23	93	5.97	6.4	0.58
8/1/2014	White Sulphur Springs	WSSPWELL1E	20.26	109	5.89	7.5	0.68

APPENDIX B

GROUNDWATER CHEMISTRY

Date Collected	Study Site	Well ID	Chloride (mg/L)	Potassium (mg/L)	Iron (mg/L)	Manganese (mg/L)	Silica (mg/L)
1/16/2014	Cartwright Property	SCL-Burrow	2.9	4.4	6.4	0	2.396
2/8/2014	Cartwright Property	SCL-Burrow	2.2	7.1	6.86	0.1	8.225
3/2/2014	Cartwright Property	SCL-Burrow	12.6	0.1	5.17	0.7	2.477
3/23/2014	Cartwright Property	SCL-Burrow	1.6	4.9	9.44	0.6	1.658
4/16/2014	Cartwright Property	SCL-Burrow	1.2	1.1	6.78	0	0.932
5/10/2014	Cartwright Property	SCL-Burrow	2.1	3.3	10.9	0	2.489
6/13/2014	Cartwright Property	SCL-Burrow	1.5	2.3	6.38	0.1	1.207
6/30/2014	Cartwright Property	SCL-Burrow	14.9	1.8	5.66	0	2.465
7/23/2014	Cartwright Property	SCL-Burrow	104.2	1.9	16.48	0	7.653

8/1/2014	Cartwright Property	SCL-Burrow	0.1	2	1.53	0	3.12
1/16/2014	Cartwright Property	SCL2C	1.5	0.4	0.004	0.1	5.05
2/8/2014	Cartwright Property	SCL2C	1.8	2.7	12.5	0	6.114
3/2/2014	Cartwright Property	SCL2C	1.4	0.5	6.98	0	3.542
3/23/2014	Cartwright Property	SCL2C	1.5	4.2	6.9	0.2	2.004
4/16/2014	Cartwright Property	SCL2C	1.5	3.3	9.9	0.8	5.914
5/10/2014	Cartwright Property	SCL2C	2.2	2.1	11.36	0.6	3.576
6/13/2014	Cartwright Property	SCL2C	1	2.5	11.34	0.1	3.219
6/30/2014	Cartwright Property	SCL2C	13.7	4.5	5.12	0	4.172
7/23/2014	Cartwright Property	SCL2C	1.5	1.7	9.3	0.6	5.826
8/1/2014	Cartwright Property	SCL2C	3.5	4.6	2.81	0	5.102
1/16/2014	Cartwright Property	SCL1E	1.6	2.7	0.31	0	3.386
2/8/2014	Cartwright Property	SCL1E	3.8	1.3	4.38	0.1	2.856

3/2/2014	Cartwright Property	SCL1E	0.1	0.9	10.12	0.6	2.004
3/23/2014	Cartwright Property	SCL1E	0.9	2.3	6.08	0.2	1.928
4/16/2014	Cartwright Property	SCL1E	0.9	2.2	10.34	0	2.538
5/10/2014	Cartwright Property	SCL1E	3.9	2.9	10.28	0	3.506
6/13/2014	Cartwright Property	SCL1E	7.2	2.7	6.01	0.3	0.348
6/30/2014	Cartwright Property	SCL1E	1.3	3.5	6.76	0	5.27
7/23/2014	Cartwright Property	SCL1E	0.8	2.7	7.8	0	3.67
8/1/2014	Cartwright Property	SCL1E	0.5	2.3	1.92	0	4.526
1/16/2014	Chandler Property	JCL-Burrow	2.9	2.5	9.1	0.1	6.174
2/8/2014	Chandler Property	JCL-Burrow	13.4	2.5	4.22	0.1	7.533
3/2/2014	Chandler Property	JCL-Burrow	43.5	0.6	4.69	2.5	1.938
3/23/2014	Chandler Property	JCL-Burrow	4.2	5.2	9.46	0	1.886
4/16/2014	Chandler Property	JCL-Burrow	9.7	2.4	9.92	4.1	3.107

5/10/2014	Chandler Property	JCL-Burrow	4.2	4.4	11.8	0	2.193
6/13/2014	Chandler Property	JCL-Burrow	8.4	4.5	12.64	0	4.868
6/30/2014	Chandler Property	JCL-Burrow	31.6	4.6	4.31	0.2	3.58
7/23/2014	Chandler Property	JCL-Burrow	11.5	2.8	12.63	0	4.974
8/1/2014	Chandler Property	JCL-Burrow	160.5	1.6	10.08	0	1.128
1/16/2014	Chandler Property	JCLW11C	0.7	0.9	3.53	1.9	4.852
2/8/2014	Chandler Property	JCLW11C	2.1	1.6	17.07	0	6.644
3/2/2014	Chandler Property	JCLW11C	2.9	1.3	9.5	0	2.747
3/23/2014	Chandler Property	JCLW11C	2.1	4.4	6.27	0.2	2.772
4/16/2014	Chandler Property	JCLW11C	1.8	2.8	9.62	2.4	3.055
5/10/2014	Chandler Property	JCLW11C	3.8	2	10.76	0	4.678
6/13/2014	Chandler Property	JCLW11C	3	2.3	6.89	0.1	3.51
6/30/2014	Chandler Property	JCLW11C	3.5	1.2	6.8	0	6.587

7/23/2014	Chandler Property	JCLW11C	2.7	4	9.42	0.3	3.455
8/1/2014	Chandler Property	JCLW11C	1.8	3	5.84	0	1.276
1/16/2014	Chandler Property	W10E	1.6	4.7	5.8	0.3	7.408
2/8/2014	Chandler Property	W10E	1.9	1.7	6.51	0	5.648
3/2/2014	Chandler Property	W10E	1.8	1.5	5.08	0	2.598
3/23/2014	Chandler Property	W10E	0.8	3.3	2.89	0.4	1.577
4/16/2014	Chandler Property	W10E	2.3	5	6.67	0	2.947
5/10/2014	Chandler Property	W10E	7.5	4.9	11.52	0.1	8.877
6/13/2014	Chandler Property	W10E	3.6	1.5	4.94	0.2	1.958
6/30/2014	Chandler Property	W10E	3.9	5.1	10.26	0.3	4.412
7/23/2014	Chandler Property	W10E	5.1	4.1	7.92	0.1	2.782
8/1/2014	Chandler Property	W10E	21	3.7	11.66	0.1	1.821
1/16/2014	Warm Springs Fish Hatchery	WSF-Burrow	2.2	3.9	6.04	0	4.036

2/8/2014	Warm Springs Fish Hatchery	WSF-Burrow	7.5	13.5	6.25	0.1	5.56
3/2/2014	Warm Springs Fish Hatchery	WSF-Burrow	6.5	1.9	6.89	0.1	3.017
3/23/2014	Warm Springs Fish Hatchery	WSF-Burrow	2.9	2.9	5.24	0.2	3.172
4/16/2014	Warm Springs Fish Hatchery	WSF-Burrow	3.9	3.6	5.89	0.2	2.6
5/10/2014	Warm Springs Fish Hatchery	WSF-Burrow	33.6	4.6	6.67	0	0.625
6/13/2014	Warm Springs Fish Hatchery	WSF-Burrow	30.7	4.3	3.91	0	1.446
6/30/2014	Warm Springs Fish Hatchery	WSF-Burrow	6.9	8.6	4.05	0	2.271
7/23/2014	Warm Springs Fish Hatchery	WSF-Burrow	2.3	5.2	13.2	0	0.349
8/1/2014	Warm Springs Fish Hatchery	WSF-Burrow	1.8	2.2	3.1	0.1	5.01
1/16/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.5	1.7	0.3	0	3.116
2/8/2014	Warm Springs Fish Hatchery	WSF-WELL2C	2.3	4.8	11.62	0.4	2.907
3/2/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.3	2.4	6.71	0	2.281
3/23/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.2	5.4	4.54	0.5	1.078

4/16/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.9	5.6	10.7	0.2	1.148
5/10/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.8	2.2	8.52	0.3	1.695
6/13/2014	Warm Springs Fish Hatchery	WSF-WELL2C	0.1	4.6	5.41	0	5.854
6/30/2014	Warm Springs Fish Hatchery	WSF-WELL2C	3.1	2.4	5.12	0.5	1.263
7/23/2014	Warm Springs Fish Hatchery	WSF-WELL2C	3	1.6	8.43	0.4	2.559
8/1/2014	Warm Springs Fish Hatchery	WSF-WELL2C	1.3	1.6	3.88	0	3.968
1/16/2014	Warm Springs Fish Hatchery	WSF3E	7.5	2.9	5.72	0.1	3.46
2/8/2014	Warm Springs Fish Hatchery	WSF3E	4.7	4	11.9	0.2	3.53
3/2/2014	Warm Springs Fish Hatchery	WSF3E	2.5	1.9	6.47	0	1.996
3/23/2014	Warm Springs Fish Hatchery	WSF3E	2.2	1.4	7.46	0.1	1.612
4/16/2014	Warm Springs Fish Hatchery	WSF3E	0.1	4.2	3.66	0.6	1.684
5/10/2014	Warm Springs Fish Hatchery	WSF3E	5.2	4.3	8.9	0	2.04
6/13/2014	Warm Springs Fish Hatchery	WSF3E	2.9	1.2	2.6	0	1.937

6/30/2014	Warm Springs Fish Hatchery	WSF3E	5.1	2.2	6.42	0	4.1
7/23/2014	Warm Springs Fish Hatchery	WSF3E	1.7	3.9	9.4	0	4.712
8/1/2014	Warm Springs Fish Hatchery	WSF3E	0.8	1.4	4.7	0	5.734
1/16/2014	White Sulfur Springs	WSSP-Burrow	2.7	2.3	10	0.2	5.996
2/8/2014	White Sulfur Springs	WSSP-Burrow	25.4	5.3	6.68	0.1	9.748
3/2/2014	White Sulfur Springs	WSSP-Burrow	126.6	1.4	2.75	0.8	2.117
3/23/2014	White Sulfur Springs	WSSP-Burrow	9.7	3.2	10.62	0	1.237
4/16/2014	White Sulfur Springs	WSSP-Burrow	1.6	3.9	6.6	0.5	6.464
5/10/2014	White Sulfur Springs	WSSP-Burrow	122	2.1	12.12	0	4.334
6/13/2014	White Sulfur Springs	WSSP-Burrow	417.2	2.7	5.14	0	3.954
6/30/2014	White Sulfur Springs	WSSP-Burrow	15	1.7	6.09	0	3.698
7/23/2014	White Sulfur Springs	WSSP-Burrow	412	0	13.92	0.1	7.431
8/1/2014	White Sulfur Springs	WSSP-Burrow	258.5	0.206	0.48	0	0.497

1/16/2014	White Sulfur Springs	WSSPWELL2C	2.8	5.3	0.26	1.1	4.934
2/8/2014	White Sulfur Springs	WSSPWELL2C	4.3	4.4	12.86	0	9.057
3/2/2014	White Sulfur Springs	WSSPWELL2C	3.8	3.4	9.44	0	6.232
3/23/2014	White Sulfur Springs	WSSPWELL2C	5.4	6.7	3.99	0.6	1.477
4/16/2014	White Sulfur Springs	WSSPWELL2C	3.1	5.4	4.92	0	2.631
5/10/2014	White Sulfur Springs	WSSPWELL2C	10.4	3.9	9.9	0	2.034
6/13/2014	White Sulfur Springs	WSSPWELL2C	6.5	5.5	9.82	0.1	4.854
6/30/2014	White Sulfur Springs	WSSPWELL2C	4	21.5	6.01	0.3	8.751
7/23/2014	White Sulfur Springs	WSSPWELL2C	11.2	31.2	8.16	0	0.059
8/1/2014	White Sulfur Springs	WSSPWELL2C	1.4	12.7	10	0	2.348
1/16/2014	White Sulfur Springs	WSSPWELL1E	1.4	0.9	9.46	0.2	4.79
2/8/2014	White Sulfur Springs	WSSPWELL1E	1.4	3.8	3.8	0	8.128
3/2/2014	White Sulfur Springs	WSSPWELL1E	3	3.4	10.08	0.1	2.967

3/23/2014	White Sulfur Springs	WSSPWELL1E	1.4	4.9	12.44	0.2	2.421
4/16/2014	White Sulfur Springs	WSSPWELL1E	0.8	4.1	10.2	0	4.878
5/10/2014	White Sulfur Springs	WSSPWELL1E	2.7	5.3	10.92	0	1.339
6/13/2014	White Sulfur Springs	WSSPWELL1E	3.3	4.7	9.14	0.3	0.592
6/30/2014	White Sulfur Springs	WSSPWELL1E	2.3	4.6	5.15	0	6.25
7/23/2014	White Sulfur Springs	WSSPWELL1E	1.9	2.6	11.2	0.7	3.249
8/1/2014	White Sulfur Springs	WSSPWELL1E	0.9	3.8	5.82	0	5.696

Well ID ending in E represent burrow locations; well ID ending in C represent non-burrow locations.

APPENDIX C

SOIL TEXTURE

Date Collected	Study Site	Treatment	Grain Size						
			2 mm	1 mm	0.5 mm	0.25 mm	0.125 mm	0.06 mm	<0.06 mm
3/4/2013	Warm Springs Fish Hatchery	Burrow	5.13	7.48	19.29	28.37	24.91	9.20	5.62
3/4/2013	Warm Springs Fish Hatchery	Burrow	8.38	8.03	14.00	26.04	22.95	10.43	10.18
3/4/2013	Warm Springs Fish Hatchery	Burrow	4.67	5.90	13.91	13.91	31.51	22.95	7.15
5/30/2013	Warm Springs Fish Hatchery	Non-Burrow	5.53	7.17	12.63	38.32	21.38	9.49	5.48
5/30/2013	Warm Springs Fish Hatchery	Non-Burrow	8.32	8.99	21.01	37.84	18.02	4.54	1.28
5/30/2013	Warm Springs Fish Hatchery	Non-Burrow	10.52	5.90	13.88	32.87	20.01	9.79	7.04
3/4/2013	Chandler Property	Burrow	2.20	7.30	26.02	23.37	14.79	10.39	15.94
3/4/2013	Chandler Property	Burrow	1.05	6.58	24.40	23.86	19.09	11.78	13.24
3/4/2013	Chandler Property	Burrow	3.42	9.55	26.39	23.13	13.74	9.91	13.87
3/28/2013	Chandler Property	Non-Burrow	2.76	0.30	29.70	29.62	16.75	9.12	11.76
3/28/2013	Chandler Property	Non-Burrow	0.98	3.60	20.32	24.30	22.03	13.47	15.30
3/28/2013	Chandler Property	Non-Burrow	3.61	4.61	21.90	28.38	22.01	10.50	8.98
3/8/2013	White_Sulphur_Springs	Burrow	0.79	4.53	25.85	22.24	17.39	12.98	16.22
3/8/2013	White_Sulphur_Springs	Burrow	3.60	5.82	23.21	25.34	19.32	12.13	10.59
3/8/2013	White_Sulphur_Springs	Burrow	1.95	7.91	26.41	33.54	17.42	5.91	6.87

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SOIL TEXTURE

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