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The Crayfishes of West Virginia's Southwestern Coalfields Region with an Emphasis on the Life History of Cambarus theepiensis

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The Crayfishes of West Virginia's Southwestern Coalfields Region with an Emphasis on the Life History of *Cambarus theepiensis*

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Chapter I:

Introduction

Crayfish Conservation in the Coalfields of West Virginia

Background, Taxonomy, and Environmental Roles

Crayfish, also known as crawdads or mudbugs, are one of the largest and most important freshwater benthic macroinvertebrates in waterways across the globe (Taylor and Schuster, 2004; Taylor et al., 2007). They are members of the subphylum Crustacea in the Class Malacostraca as well as the Order Decapoda, along with related crabs, shrimps, and lobsters. Marine lobsters and freshwater crayfish form the infraorder Astacidea, and are further divided into three families. North American and European crayfishes belong to the family Astacidae and Cambaridae under the Superfamily Astacoidea, while the southern hemisphere's family Parastacidae falls under the Superfamily Parastacoidea, which contains all crayfish from South America, Madagascar, and Australasia (Hobbs, 1974).

Within ecosystems, crayfish serve as an important food sources. Fish, hellbenders, owls, queen snakes, turtles, and raccoons all have diets high in crayfish (Roell and Orth, 1993; Lodge and Hill, 1994; Dorn and Mittelbach, 1999; Swecker 2012). Crayfish also serve a role more complex than a food source, as keystone species in streams and wetlands in which they occur (Momot 1995, Dorn and Mittelbach, 1999, Whiteledge and Rabeni, 1997). Crayfish function as opportunistic omnivores as well as detritivores, feeding on algae, macrophytes, macroinvertebrates, fallen leaves, and dead or decaying organic matter in lentic (Chambers et al., 1990; Lodge et al., 1998; Momot, 1995; Swecker, 2012), lotic (Huryn and Wallace, 1987; Charlebois and Lamberti, 1996; Whitledge and Rabeni, 1997; Swecker, 2012), and semi terrestrial (Loughman, 2010) habitats.

Crayfish are not only important for their role in food webs but also in their role as ecosystem engineers. Within lentic and lotic waterways, crayfish are responsible for overturn as well as creation of new microhabitats via burrows and shallow depressions under stones that other macroinvertebrates depend on. Burrowing crayfish are particularly important in terrestrial habitats such as marshes, swamps, floodplains, wet fields, and seeps due to their creation of habitats that animals within these environs have coevolved to depend on. Some examples of crayfish obligate taxa include *Sisturus catenatus* (Rafinesque, 1818) the Massasauga Rattlesnake, *Lithobates areolatus* (Baird and Girard, 1852) the Crawfish Frog, and *Somatochlora hineana* Williamson, 1931 the Hine's Emerald Dragonfly (Phillips et al., 1999; Ernst and Ernst, 2003; Pintor and Soluk, 2006). Burrows also aerate soils, preventing compaction (Welch et al., 2008).

Crayfish are the third most endangered faunal group in North America with 43% ranked as being imperiled (Taylor et al. 2007), and the third most endangered faunal group in the world (Cordeiro, 2010) behind freshwater snails and freshwater mussels (Strayer, 2008). Reasons for imperilment include, but are not limited to: extractive industry, land use, water pollution, limited geographic range, disease and introduction of invasive species (Jezarinac et al., 1995; Taylor et al., 2007). North America holds the highest diversity of crayfish species worldwide with 363 species as of 2007 (Taylor et al., 2007). Of these 363 species, a large portion occurs within the Appalachian Mountains of Eastern North America. Abundant forests with a highly diverse range of habitats combined with millions of years of evolutionary seclusion through a variety of geographic barriers has led to this area becoming a hotspot of biological diversity for many groups of organisms including salamanders, fish, insects, plants, and crayfish through allopatric speciation (Hobbs, 1969; Parker and Roane, 1969).

Land Use and Anthropogenic Activities within the Study Area

Diversity of plant life, particularly trees, combined with a vast expanse of forest along the Appalachians naturally grasped the attention of logging companies. Starting in the early 1800's and continuing to the present, Appalachian forests were and are heavily logged. West Virginia received its heaviest logging pressure from 1850- 1920 (Lewis, 1998). Vast tracts of forests were cut as railroads began to open the interior of the state to outside commerce (Lewis, 1998). At one point, West Virginia was one of the leading producers of timber, exporting 15 billion board feet of lumber in its peak production year of 1910 (Lewis, 1998). White Oak (*Quercus alba*), Black Walnut (*Juglans nigra*), Red Spruce (*Picea rubens*), and many other mesophytic trees and conifers were sought after for their prized wood to be used for planks, building materials, and furniture (Lewis, 1998). Outside of plank and lumber production, other timber related industries began to flourish including tanneries which utilized bark from West Virginia's wealth of Eastern Hemlock trees (*Tsuga canadensis*) to make their tannins (Lewis, 1998; Michael, 2002). West Virginia's Southwestern Coalfields most notorious lumbermen were the Hatfields of West Virginia, a family of timberers and colliers widely known for their bloody feud with the McCoy family of Kentucky along the banks of the Tug River during the late 1800's (Riddel, 2008).

While an economic boom took place in the state at this time, it was not without consequence. Clear cutting large tracts of forest lead to heavy siltation inputs into streams and decreased riparian buffer zones (Lewis 1998). Although not noted or known at the time, today it is documented that this leads to increased embeddedness of stream substrates and decreased temperature buffering for streams, resulting in increased water temperature (Hauer and Lamberti, 1996; Johnson and Jones, 2000). In addition to these factors, it is also noted that lumber mills were built downstream of towns due to black water resulting from milling processes, which made water unfit for consumption by humans (Lewis, 1998). This black water, in addition to containing contaminants from the milling process, also contained increased tannins within the water from trees such as hemlocks. Tannins increase acidity, resulting in very acidic water through leachates of humic materials contained within organic material of fallen and milled trees (Thurman, 1985). While high alpine streams and some coastal plain swamp systems have species evolved to live within such acidic waters, it is unlikely West Virginian streams found within the Appalachian plateau and organisms living therein were able to fully cope with such a drastic shift in acidity.

In addition to timber, southwestern West Virginia's landscape contained another highly sought after resource in coal buried beneath the forested mountains. Coal was initially discovered within West Virginia as early as 1742 (Lewis, 1998). Although a valuable resource, it was mostly used to heat homes or sparingly for blacksmithing until the early 1800's when it became a common fuel for furnaces, heating, and powering steamboats and trains (WVGES, 2004). Coal production continued to grow throughout the state until the outbreak of the Civil War in 1861 when coal mines along the Kanawha River Valley closed due to blockades and destruction of locks and dams which prevented shipping (WVGES, 2004). Following the Civil War, coal mining continued to increase throughout West Virginia, and peeked in 1947 when production reached 173.6 million tons of coal (WVGES, 2004). Coal production has continued throughout West Virginia, particularly in the area of emphasis for this study. In recent decades, extreme surface mining in the form of mountaintop removal has begun to replace traditional deep mining methods. While deep mining involved extracting coal from subterranean coal veins and removing coal slowly, mountaintop removal allows for relatively quick acquisition of coal by removing the top layers of a mountain which then fills an adjacent valley. This process allows for large tracks of coal located beneath the Earth to be removed and at an expedited rate by huge excavation machines.

Both deep and surface mining have played a role in the current health of West Virginian streams. Deep mining requires outward production of effluent known as mine water. This water is frequently saturated with salts and heavy metals, particularly iron, which leads to what is referred to as acid mine drainage (Moore et al., 1991). Acid Mine drainage occurs when the iron in mine water effluent oxidizes, forming yellow boy, giving the water a yellowish to deep rust orange color. Conductivity, pH, and salinity levels increase and eventually alter the biota of the associated stream. Fish are heavily affected by heavy metal accumulation, particularly aluminum, within epithelial tissue of their gills, which leads to decreased oxygen uptake and decreased survivability (Youson and Neville, 1987). Sensitive species of invertebrates, specficially EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera) and unionid mussels begin to die off due to conductance issues (Hartman, 2005; Pond et al. 2008). As higher levels of heavy metals and salts are reached, cascades can take place throughout the stream system until little to no life can survive within the stream, eventually leading to possible extirpation of crayfish through direct effects of physiochemical interactions or lack of food resources directly caused by these interactions (Gallaway, 1991).

Mountaintop removal poses its own unique set of environmental problems. Associated valley fills bury headwater streams which feed larger streams and tie directly into biota and energy inputs in water located downstream of impacted sites (Pond et al., 2008). This siltation causes embededness within effected streams and leads to decreased habitat within the streambed as the substrate's matrix is filled in by silt. This causes decreased habitat for crayfish and other macroinvertebrates, and can lead to localized extirpations due to lack of habitat for some species.

Stream input processes aforementioned in mining and timber also take place through additional land use, particularly during road construction. Blasting and bulldozing can create large amounts of siltation, often laden with heavy amounts of metals, salts, and chemicals from equipment maintenance and operation, or from blasting. In accordance with the Clean Water Act, all agencies practicing in land use are required to take steps in order to prevent byproducts from these processes from entering nearby streams (Clean Water Act of 1972). Preemptive measures to prevent siltation of streams include implementing silt fences along stream banks within the construction site. Silt fences along with creation of settlement ponds where overland flow is channeled catch fine particulate matter and allow it to settle. While these measures do aid in preventing large siltation inputs into nearby streams, failures of these measures can occur.

Justification of Study Efforts

All previously mentioned factors could have severely impacted Appalachia's crayfish fauna throughout West Virginia and possibly lead to declines in, or extirpations of, species before a baseline list of species present in West Virginia was documented by Raymond Jezarinac in 1995. Within West Virginia is a geographic region known as West Virginia's Southwestern Coalfields. The area is considered separate from West Virginia's interior coal basin, situated within the Kanawha drainage system.   The Southwestern Coalfields have received few focused efforts towards crayfish fauna documentation despite housing *Cambarus veteranus*, the state's most imperiled crayfish species (Jezarinac et al., 1995). Mining and extractive industry still plays a heavy role within the region as many coal mines are currently in operation. Due to continued impacts of extractive industries, large future land alteration projects, and very little data on crayfish within the region, targeted sampling during the ongoing West Virginia state crayfish survey was focused in the region from 2009-2012. Analysis of these data to date has resulted in description of two new species of crayfish in the genus *Cambarus* (Loughman et al., 2013; Loughman et al. *in press*) and a life history description for one of these species, *Cambarus theepiensis*. Life history data for *C. theepiensis* as well as knowledge of all known crayfishes located within the study area is presented within this thesis.

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Appendix: Chapter I

Chapter I: Supplemental Figure

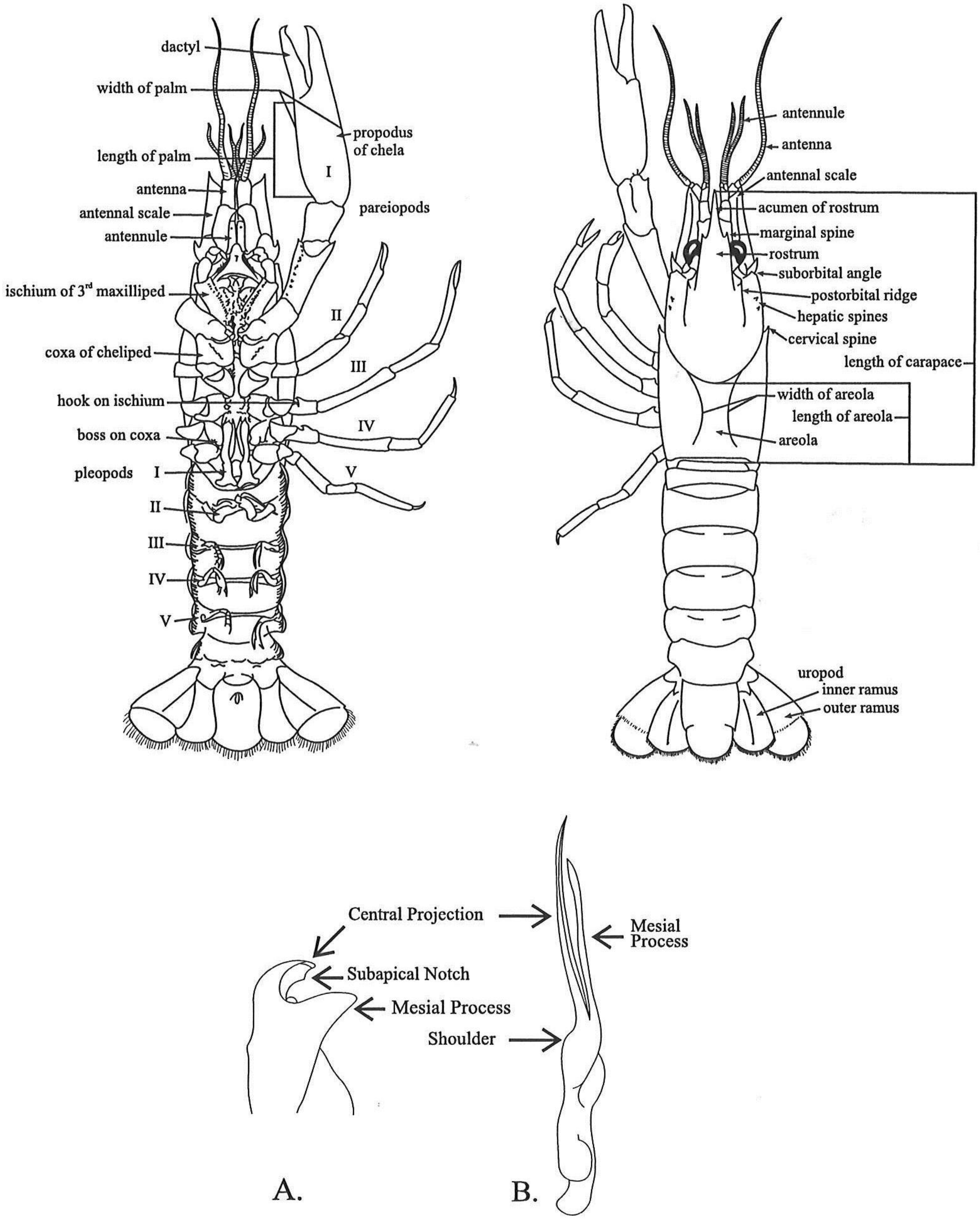

Figure 1.1: Schematic diagram of a generalized male crayfish illustrating characters mentioned for species description and morphometrics in Chapters 2 and 3. **A.** Represents a *Cambarus* gonopod. **B.** represents a *Orconectes* gonopod. Taken from Hobbs 1989.

Chapter II:

Life History of *Cambarus theepiensis,* a newly described crayfish from the coalfields of Southwestern West Virginia and Eastern Kentucky

Abstract

Crayfish are the third most endangered faunal group in the world behind freshwater snails and unionid mussels. A better understanding of each species' life history is vital in order to aid in crayfish conservation; however, little to no life history information is available for most crayfish. Recently, an undescribed species of crayfish, *Cambarus theepiensis*, was discovered in the Cumberland Mountains of West Virginia and Eastern Kentucky. In conjunction with the species' scientific description, life history data was collected from May 2012 through April 2013 from two sites located within the Twelvepole watershed of southwestern West Virginia. Animals were collected monthly for one continuous year to determine the annual life history of this species. With the exception of ten females retained monthly for dissection and gonadal development analysis, all animals were returned to streams following demographic and morphometric analysis. Mature females showed signs of glair development in early May. Egg extrusion was noted in early June, with first through third stage instars occurring in July and concluding in August, before becoming free living juveniles by late August /September. Evidence for possible overwintering with young was also found as a mature female with free living juveniles was collected in early April. Mature female total carapace length (TCL) ranged from 29.7-52.4mm. Evidence was found supporting the hypothesis that form change also occurs in female cambarid crayfishes in *C. theepiensis*. Form I males were collected throughout the year, but reached their highest densities May through August. Form I male TCL ranged from

30.6-50.6mm. Size cohorts determined through histograms indicate six size cohorts existed between both streams following introduction of young of the year (YOY) in late summer. *Cambarus theepiensis* appears to have life history characteristics similar to previous *Cambarus* species whose life histories have been determined.

Introduction

Crayfish play vital roles in aquatic and terrestrial ecosystems, and function at almost every trophic level within food webs (Crocker and Barr, 1968; Chambers et al., 1990; Hanson et al., 1990; MacIsaac, 1994). Due to their many uses and functions, crayfish are often viewed as keystone species within the ecosystems they inhabit (Momot et al., 1978; Creed, 1994; Momot, 1995; Rabeni et al., 1995; Simberloff, 1998; Swecker, 2012; DiStefano et al., 2013). Crayfish are currently in decline, and are listed as the third most imperiled faunal group globally behind freshwater snails and Unionid mussels (NatureServe, 2010). Despite their importance, both economically and ecologically, and their continued imperilment, relatively little biological information is known on crayfish when compared to other taxa groups. New species are discovered yearly, and of the known 360+ North American taxa, less than 15% have had life history studies conducted on them (DiStefano et al., 2013).

In 2010, during ongoing collecting and documentation of crayfish species in West Virginia by Loughman and Welsh, a new species of crayfish, *Cambarus theepiensis* Loughman et al. 2013 (Coalfields Crayfish), was discovered within the Twelvepole watershed in southwestern West Virginia southeast of Huntington, WV. Upon discovery, further targeted investigations began with the aim of documenting the species range to augment *C. theepiensis* formal scientific description. In 2013, Loughman et al. noted *C. theepienesis* appears to be endemic to the junction of the Cumberland Mountains and the Appalachian Plateau in eastern Kentucky and southwest West Virginia. Within this area, *C. theepiensis* inhabits the Big and Little Sandy basins, Levisa Fork, Twelvepole, and the Guyandotte River basins as well as their tributaries (Loughman et al. 2013a). Despite this relatively small geographic range, *C. theepiensis* appears stable within streams it inhabits; however, ever increasing extractive industry, land use, and invasive species of crayfish potentially threaten *C. theepiensis* (D. A. Foltz II & Z. J. Loughman, personal observation).

Due to these possible threats and zero previous conservation oriented knowledge specific to *C. theepiensis*, any knowledge of habitat requirements and utilization, crayfish associates, and life history could prove critical to *C. theepiensis* in the future should conservation efforts become warranted. Because this information was unknown at the time, a life history study of *C. theepiensis* was initiated alongside formal description of the species. Our goal in regards to the life history study was to provide a sound understanding of the species' annual life cycle, growth, and habitat utilization.

Materials and Methods

Site Selection

Wadeable streams located within the lower Guyandotte and Twelvepole drainages were sampled and ranked based on habitat, ease of access, and most importantly, abundance of *C. theepiensis*. Roughly 40 streams were sampled and abundance determined by catch per unit effort (CPUE) for five seine hauls (# crayfish collected/ # seine hauls). Most streams scored <. 2

and many streams scored 0, however; two streams scored above 1.0 and were determined to be adequate study sites.

Study Sites

We conducted our study in two streams, Butler Adkins Branch and Right Fork Miller's Creek, in the mid Twelvepole Drainage basin, Wayne County, West Virginia (Figures 2.1 & 2.2). Both streams flow into Beech Fork Lake located in Beech Fork State Park, Wayne County, West Virginia. Butler Adkins Branch is a small, well shaded second order stream averaging <1.5 m wide and is characterized by deeply incised banks and high sinuosity due to previous land use and alteration, as well as multiple high water events. Stream banks were composed predominantly of hardpan/ clay with intermittent slab boulders and cobbles. Stream substrates were primarily large cobbles with occasional slab boulders. Riffles were the dominant habitat of both streams, of which several precede deep (some in excess of 2.5 m) pools. Deep pools always contained course woody debris. One 10 m section was supported on the right descending bank with rip rap and large slabs in order to stabilize the nearby road that runs parallel to the stream.

Right Fork Miller's Creek is a 3rd order stream averaging 3 m wide and is partially shaded. It is further characterized by naturally cut banks running through sandstone and shale, with the descending left bank covered in herbaceous vegetation. Both banks were lined with cobble, gravel, small slabs, and occasional bedrock glides. Riffles predominated in stream macrohabitats, followed by occasional 0.25 – 1.25 meter deep pools. Long, shallow runs are common throughout the study reach. A hard-surface road crossing was present at the middle of this site and during the last three months of sampling, the lower 15 m portion of the site was dredged and modified by local farmers to act as drinking water for livestock during low winter flows.

Butler Adkins branch ceased flow once during the study and was often at low flow while Right Fork Miller's Creek never stopped flowing but did reach low flows during late summer and late winter. Both study sites crayfish associates included *Cambarus bartonii cavatus* Hay, 1902 and *Orconectes sanbornii* (Faxon, 1884). *Cambarus thomai* Jezerinac, 1993 and *Cambarus dubius* Faxon, 1884 were found nearby burrowing in stream floodplains, but were not collected or observed in the stream bed proper. Lastly, *Orconectes rusticus* (Girard, 1852) was noted directly downstream of both sites near their confluence with Beech Fork Lake, but was not collected at either site during the current effort.

Life History

Sampling occurred monthly from May 2012 through April 2013 within the 1st through 9th of each month. Seining using a 2.44 x 1.22 m seine was the primary method of collection allowing for CPUE (Catch per unit effort) calculation. Additionally, 30 seine hauls were broken into subsets of 10 hauls per riffle, run, and pool allowing for calculation of habitat preference. Seines were placed downstream of best available habitat (slabs, rocks, roots, etc.), angled back at roughly 40 degree angle, and held by one collector as a second collector flipped, disturbed, and kicked the stream substrate, pushing crayfishes downstream into the net. Crayfish were placed into holding vessels and retained for identification and morphometric analysis.

Hand collecting was the primary method used during periods of low or no flow, and also served as a supplemental method during all months. Seining was typically conducted from 8 am – 3pm and took place until 10 seine hauls per each habitat type were completed at the site. A goal of 50 crayfish per site per month was set at the beginning of the study but became difficult to unattainable during fall and winter months. During the month of September 2012, no crayfish were collected at Butler Adkins branch due to complete drying of the stream bed.

Captured crayfish were taken to the nearest stream bank where species determination occurred prior to being measured using Pittsburgh 6" Digital Calipers. Morphometrics acquired for all specimens included total carapace length (TCL, from the terminating point of the rostrum to the posterior most portion of the cephalothorax), palm length (PaL, from the most distal portion of the palm to the most proximal portion of the palm on the chelae), propodus length (PrL, from the most distal portion of the propodus to the most proximal portion of the propodus on the chelae), abdominal length(AbL, from the anterior most portion of the abdomen to the posterior most portion of the telson) and abdominal width (AbW, the measurement from the two widest points on the most anterior tergal plate of the abdomen). All measurements were recorded to the nearest 0.1 mm. Once measured, all captured crayfish were returned alive to the reach they were collected from.

Additionally, molt state (judged by strength, cleanliness, and traction of the exoskeleton), male reproductive state (sexually mature Form I or sexually immature Form II), and female reproductive state (Reproductively active females exhibit pre-glair/ glair in their glair glands, elongated pleopods, or eggs/hatchlings/ or young of the year (YOY) attached to their pleopods) were determined for each crayfish. Ovigerous females (having eggs, hatchlings, or YOY attached to their pleopods) were retained in clear plastic tubs filled with water and the number of eggs/hatchlings/ YOY was counted to best approximation without removing or damaging the eggs.

Physiochemical

Water temperature was recorded monthly at each site (with the exception of months when low flow/ dry bed prevented collection) using a YSI model 6920 V2 Data sonde. Collection of pH, conductivity and % Dissolved Oxygen was originally planned, but during the month of July sensors for these metrics were damaged, and cost and repair turnaround made collection of these parameters untenable.

Gomedic development analysis

In addition to monthly monitoring of both sites, monthly collection and vouchering of 10 sexually mature female *C. theepiensis* also occurred monthly. Collections ran from May 2012 through October 2012 when large *C. theepiensis* appeared to retreat into their burrows making further collections extremely arduous. Collections of females took place in neighboring creeks within the Twelvepole drainage or from extreme downstream sections of streams studied as collection of females directly from study sites could severely influence numbers of females and young obtained during later months of study. Female *C. theepiensis* were placed into 75% EtOH onsite immediately following capture in order to halt gomedic development and preserve specimens. If females were harboring eggs or instars, they were carefully removed, counted, measured, and placed into vials filled with 75% EtOH which was then retained with their mother for later analysis.

Preserved females were dissected for retrieval of gomedic material. This was performed by carefully severing the visceral lining connecting the carapace to the abdomen using a dissecting probe, after which the carapace was then carefully lifted anteriorly, revealing the viscera of each specimen. Once completed, the stomach was carefully removed and saved for future analysis for a separate study.

Ovaries were removed following the stomach, and placed under a Leica stereomicroscope. Once under the microscope, the focus was adjusted until a clear image of the ovaries was obtained before being captured. From the captured image, widths of 10 eggs per female were measured to the nearest 0.001 mm and inputted into a database to determine mean egg size per month.

Statistical analysis

In order to determine sexually mature male and female *C. theepiensis* lower limit TCL maturity "thresholds" were established using the smallest individuals of each sex to display sexual maturity (Jones and Eversole, 2011). Form I gonopods for males and glair, eggs, instars, or elongated pleopods for females were the characters used to determine sexual maturity (Payne and Price, 1983; DiStefano et al., 2013). Analysis was structured to focus only on adult specimens of similar size with female TCL >= 29.0mm; and Male TCL >= 30.0mm. This minimum-size restriction was based on the TCL of the smallest observed sexually mature female and male specimen, respectively. The constrained male and female characteristics displayed homoscedasticity between variable groups, and were found suitable for comparison via analysis of covariance [ANCOVA]. Comparisons of PrL (propodus length) and AbW (abdominal width), utilizing ANCOVA, were performed between Form I and Form II conspecifics of each sex via SPSS v20 (IBM, 2011). This allowed for better comparison of allometric sexual morphs at the same size while helping eliminate possible disparity caused by comparing large juveniles to sexually mature adults. Additionally, any individuals with double regenerated chelae, double missing chelae, damaged and/ or mutated abdomens, carapaces, or chelae which would not allow for an elucidated comparison of the morphs and would detract from the data were eliminated from the data set.

In addition to statistical comparison of sexual morphs for males and females, comparison of monthly and overall ratios of males vs. females was tested using chi-square. Mean, range, and standard error were also computed for all morphs for the measurements of TCL (total carapace length), PaL (palmer length), PrL (propodus length), AbL (abdominal length), and AbW (abdominal width). Mean and standard deviation of internal size of eggs for female *C. theepiensis* were calculated monthly for the year of collections. Lastly, Microsoft Excel (Microsoft Office, 2010) was used to create monthly histograms for the year of sampling in order to analyze size classes. Separation of classes by the lowest point between two parabolic shapes (DiStefano et al., 2013) was agreed upon by three observers.

Additionally, efforts were made to quantify the average size of *C. theepiensis* dwelling within riffle, run, pool, and riparian (bank) habitats by equally sampling each habitat type at each site monthly. This was done as accurate determination of crayfish population size or ageclass structure requires sampling of available stream habitat types proportionally due to unequal distribution throughout habitat types (Muck et al., 2002; Distefano et al., 2013).

Results

Due to no noticeable difference in size classes, sex ratio, molting, and reproductive events from Butler Adkins Branch or Right Fork Miller's Creek along with the very close proximity of both populations to each other, data from both sites were pooled to give a more robust and comprehensive view of *C. theepiensis'* life history within the Twelvepole Drainage. Inter- species CPUE rates are presented in Figure 2.3.

Reproductive Cycle

Most male *C. theepiensis* collected throughout the year were Form II (86.2% of all males pooled over 12 months); however, Form I males were collected every month of the year, and reached their peak occurrence (out of all males collected) October through January. Form I males reached their highest counts in April through July. Peaks during October 2012 – January 2013 should be cautiously interpreted as very small sample sizes were obtained during these months relative to May-August of 2012 and April of 2013.

Like male *C. theepiensis*, most females collected throughout the year were also Form II (81.0% of all females). Form I females were collected from all months of the year, and reached a peak occurrence (out of all females collected) in September and October; their highest counts occurred in April-June and again in August. Like males, the peak in January should be cautiously interpreted due to small sample sizes. Presence of glair was noted during May 2012 and was followed by eggs in June 2012, before disappearing in July 2012 with the onset of instars (Fig. 2.4).

Monthly presence of glair, eggs, instars, and free living young of the year at all Twelvepole sites is presented in Table 2.1. After appearance of instars, active glair glands were not observed in female *C. theepiensis* until February 2013, after which glair continued to be observed through April 2013 when sampling ended. Presence of glair reached its highest peak in March 2013. Only nine females with eggs/instars were collected at both sites during the scope of monthly collections, with five ovigerous females collected in June 2012 ($\bar{X}$ =16 °C) and one female with eggs and two females with instars collected in July 2012 ($\bar{X}$ =22 °C). During August 2012 ($\bar{X}$ =23 °C) a female was observed with instars but retreated into a burrow under an immovable slab partially extending from the bank, and was unable to be retrieved for morphometric analysis. Additionally, one female with young molting to fourth instar stage was collected during April 2013 ($\bar{X}$ =6 °C). Nine additional ovigerous females carrying eggs or instars were collected during monthly collections of females for internal ova analysis outside the primary study sites, with three ovigerous females collected in June 2012 ($\bar{X}$ =19 °C) and one female with eggs and 3 females with instars collected in July 2012 ($\bar{X}$ =23 °C). During September of 2012 ($\bar{X}$ =21 °C) a female was collected with seven young that had just molted into free living instars. Lastly, during October of 2012 ($\bar{X}$ =15°C) a large female with 18 instars that had just molted to free living juveniles was found dead after a failed molt. Neonates were observed feeding on their mother.

Free living young of the year *C. theepiensis* were first observed in July of 2012. Total carapace length of YOY was 4.0-6.0 mm in July and increased monthly (Fig. 2.6). Recruitment of YOY appeared to be complete by November of 2012; however, this should be interpreted cautiously due to low sample size during colder months. No large synchronous molts for *C. theepiensis* were observed (Fig. 2.4).

Gomedic development

Form I females ranged from 29.7 – 52.4 mm TCL (Table 2.2). Laboratory and field ($\bar{X}$ =113, n= 10) egg counts ranged between 13 to 210 attached eggs on female pleopods. Only four of ten females were considered to have full complements of eggs. Six of ten were collected during egg extrusion and were measured and returned to the stream to prevent offsetting the number of YOY recorded during later months. A weak positive relationship occurred (r^2= 0.73) between TCL and egg number. Four Form I female *C. theepiensis* collected for internal ova analysis had attached eggs which were removed and measured. Mean external egg diameter ranged from 2.5- 2.8 mm and showed no correlation to female's TCL (r^2 = 0.08).

Monthly internal egg analysis was conducted from May 2012- October 2012. Collections were not possible and were abandoned after October as mature sized female *C. theepiensis* outside of the study sites became extremely arduous to collect. Mean internal egg size in May 2012 started at 0.93mm and grew to 2.65mm by June 2012 before severely declining to 0.42 mm in July 2012. After July 2012 it slowly began to increase and had reached 0.94 mm before collections and analysis were halted in October 2012 (Fig. 2.5).

Sex ratios

Sex ratios in *C. theepiensis* were skewed towards females with a total (M:F) ratio of 0.655:1 for both sites combined (X^2= 35.217, p <.0001) showing statistical significance. July was the only month males outnumbered females (1.079:1). During the months of June (0.771:1), August (0.879:1), and January (0.818:1) ratios approached 1:1, but during all other months, ratios remained 0.557:1 or less.

Form analyses

The assumption of homogeneity of regression coefficients was valid for both sexes, regarding PrL and AbW, co-varied by TCL. No significant difference was detected between Form I and Form II male AbW adjusted for TCL (F = 0.012, P = 0.912), though Form I and Form II female AbW adjusted for TCL showed highly significant difference (F = 14.327, P < 0.001). Significance was also detected between Form I and Form II of both sexes with regard to PrL adjusted for TCL, with Form I and Form II males (F = 55.902, P < 0.001), and also with Form I and Form II females (F = 7.747, P = 0.006).

Size at maturity

Male *C. theepiensis* were able to reproduce in their 4th size cohort (with inclusion of the YOY cohort (Figure 2.6). The smallest Form I male TCL 30.6 mm, while the largest TCL was 50.6 mm. Female *C. theepiensis* also appear to reach sexual maturity in their 4th size cohort (Figure 2.6). Dissected specimens showed oocyte development from spring through early fall of 2013; however dissections indicated that some females could possibly become ovigerous during the fall and overwinter with their young. Collections in April appeared to support this, as a sexually mature female was collected with several instars, but further investigation is warranted as this was only noted in a single female. The smallest sexually mature female TCL was 29.7mm while the largest sexually mature female measured 52.4mm.

Size class structure and habitat utilization

Cambarus theepiensis TCL ranged from 4.8-52.5 mm within the Twelvepole drainage (Table 2.2) during the sampling year of May 2012- April 2013. Monthly histograms depicting TCL- frequency for *C. theepiensis* (Figure. 2.6) were standardized in order to show accurate frequencies per month. Prior to introduction of the 2012 young of the year (YOY) size class, the 2011 YOY class showed a wider distribution of TCL than the young of the year cohort introduced in 2012 (Figure 2.6). August served as the best indicator of size classes with six visible peaks including 2012 YOY which ranged from 5-8 mm. Overall habitat utilization for the year of May 2012 – April 2013 showed average TCL (mm) ± SD for *C. theepiensis* found in the riparian (bank) habitat was 29.68 ± 9.25 while pool habitat was 28.67 ± 13.0, riffle habitat was 15.65 ± 7.3, and run habitat was 21.77 ± 13.45.

Damage and mutations

Among Form I male crayfish (n= 12), 30.8% exhibited damage to the body or regenerated/ missing chelae while 26.6% Form II male crayfish (n=75) exhibited damage. Among Form I female crayfish (n=38), 40.9% exhibited damage to the body or regenerated/ missing chelae while 18.7% of all Form II female crayfish (n= 74) exhibited such damage. Few Form I male crayfish (n=1), 2.5% exhibited mutations (inverted jointing, odd curvature of limbs, or multiple projections from the point of either the dactyl or propodus) while 1.4% of all Form II male crayfish (n=4) exhibited such mutations. In regards to female crayfish and mutations, 1.1% of all Form I female crayfish (n=1) exhibited such mutations while 0.3% of all Form II females exhibited such mutations.

Discussion

Reproductive cycle

Timing of *C. theepiensis'* reproductive events seemed to occur between early March and May, given both Form I males and Form I females' general behavior shifted at this time. Beginning in September and continuing through winter, captures declined with a decrease in water temperature. Sampling vigor and techniques remained constant throughout this season. Upon closer inspection, burrows under slabs adjacent to the bank, as well as under mid-channel slabs contained *C. theepiensis*. From September through November, when bank and mid channel slabs were overturned, a pair of antennae or chelae could often be scene at a burrow entrance. Beginning in December, zero crayfish were observed at burrow entrances until March of 2013. Beginning in March, with the rise of water temperature, sexually mature males and females reappeared in substantial numbers throughout both streams.

Form I male behavior during March and April was of particular interest, given they were often observed cruising in open water along the substrate. This contrasted sharply with observations during previous months, when observation of large *C. theepiensis* moving through stream channels was a rare occurrence. During the month of April 2013, all large male *C. theepiensis* observed moving during sampling periods were followed until they took refuge under, in all cases, large slab boulders. All males followed were determined to be Form I, and all large slab boulders utilized as cover contained one to three glaired female *C. theepiensis*. These observations were replicated following sampling events upstream of both sites and are similar to what Loughman et al. (2013b) observed in *Cambarus chasmodactylus* James 1966, another large tertiary burrowing crayfish. Sexual activity however, was not observed.

During May-July of 2012, ovigerous *C. theepiensis* appeared to choose one of two strategies regarding habitat selection for extrusion of eggs and carrying of instars. Ovigerous females often were found secluded in bank burrows under large slabs. Burrows and seclusional areas chosen were much more difficult to reach than typical bank habitat utilized by crayfish. Flipping and removal of multiple large boulders and slabs before excavating laterally into the bank was the only way to obtain these animals. Low detection of females with eggs and instars could be attributed to this behavioral strategy by ovigerous females. The less frequently used strategy by ovigerous *C. theepiensis* was to take cover under large slabs mid-channel that were embedded in a manner so that only a single entrance was open. Additionally, females secluding themselves in this manner were often found with Form I male *C. theepiensis*. This behavior has been noted in other *Cambarus* species in WV (Loughman, 2013); however, it remains unclear as to whether this is a form of parental care, mate guarding, or simply a non-beneficial sharing of habitat.

Females with glair began to appear in February and were present in the population through June, disappearing with the onset of ovigerous females in June-July. Eggs hatched to instars July-August, with instars transitioning to free living young of the year August-November.

Evidence for female *C. theepiensis* overwintering with young was documented when a female was collected in April of 2013 from a burrow under a large slab boulder with freshly molted, free living neonates still on her pleopods. Previous investigators theorized that egg extrusion was linked to seasonal water temperature (Aiken, 1969; Distefano et al. 2013), which also appears to be the case in *C. theepiensis*.

While simultaneous collection of data from streams was conducted (Corey, 1988; Distefano et al., 2002; Distefano et al. 2013), no differences in reproductive events were recorded from either stream. This however is most likely due to both streams falling within the same general geographic proximity of each other. Sampling of *C. theepiensis* from other streams within its range may yield variance. Evidence for this already exists as Loughman et al. (2013) reported collecting females with eggs in April 2012 from Hood Creek in Lawrence County, Kentucky. While this could be attributed purely to dependence on temperature regimes because the winter of 2011 in the area was extremely mild, further investigation is warranted.

Gomedic development

Internal gonadal analysis of female *C. theepiensis* suggests that Form I females' eggs for the following year begin development in mid-June through July, and develop slowly throughout proceeding months. While collections for dissections ceased in October, mean egg diameter suggests that eggs have already reached a plateau in growth given the mean diameter for October was 0.01 mm greater than mean egg diameter in early May. This may be a developmental strategy as *C. theepiensis* activity and physiological functions slow in response to winter, if not halt during colder winter months. Many specimens collected in the winter months were stationed under slabs mid channel and showed little to no response when collected. From May to June however, following potential mating, mean egg diameter rapidly increases to roughly three times the mean egg diameter in May. Eggs swell to such a size that they take on a rhomboidal shape internally due to limited space, and turn bright red when preserved in ethanol instead of the usual white to light yellow color prior to fertilization.

Of particular note was a female in July who still retained one egg from the 2012 year, but was already in the process of developing eggs for 2013. The egg was easily distinguished due to its large size and bright red coloration compared to the small white eggs surrounding it, appeared in the process of being sclerotized and reabsorbed as a hard white substance was present over a fair portion of the egg.

Form analyses

Form alteration in male *C. theepiensis* occurred much like that of other *Cambarus* species (Hamr and Berrill, 1985; Corey, 1990; Jones and Eversole, 2011), with Form I males possessing larger chelae in relation to their Form II conspecifics (Hamr and Berrill, 1985; Jones and Eversole, 2011). Transition to Form II was documented when a Form I male captured downstream during the month of July was retained in a laboratory setting, and molted to Form II during the month of September. Although form alteration of male crayfish is well documented, form alteration of female crayfish is fairly new (Wetzel, 2002). First documented by Wetzel (2002) in five *Orconectes* species, and again by Jones and Eversole (2011) in

Cambarus elkensis Jezerinac and Stocker, 1993. While Form I vs. Form II is easily distinguishable in males due to observable differences in gonopod structure, obvious external structures used to differentiate between Forms I and II do not exist in females, and differences are determined by calculation of meristic ratios (Wetzel, 2002). Form I female *C. theepiensis* have longer chelae based on propodus vs. TBL ratio and much wider pleons based on AbW vs. TBL ratio. Both of these results confer with those obtained by Jones and Eversole (2011) for *Cambarus elkensis*. Both Wetzel (2002) and Jones and Eversole (2011) noted that Form I females have noticeably different abdominal widths when compared to Form II female conspecifics. This held true for *C. theepiensis* as well. The abdominal terga appeared wider in comparison to abdomen and overall body length. Additionally, pleopods were much longer and wider in comparison to Form II female conspecifics.

Due to the larger size of *Cambarus* species in general, it was possible to visually examine adult females in late summer to fall months and determine if the crayfish had raised young previously based on pleopod size. Remnants of glair used to secure the eggs, which appeared to brown and blacken over time as well as retain a globular, semi-adhesive feel months after instars had been released to streams were also visible during these months. While statistically significant differences for female *C. theepiensis* were shown, a more puzzling question is presented. Do Form I females change back to Form II individuals like their male conspecifics, or do they remain Form I and only continue to molt to allow for growth?

Few freshly molted Form I *C. theepiensis* were found during spring months, and were usually among the smaller size class of mature females. It was not uncommon to find encrusted females clearly exhibiting glair, and later even with exuded eggs or attached instars (Fig. 2.7). Furthermore, discovery of a female with attached young in October after a failed molt aids in elucidation of the previously stated question, as the exuvia was located nearby and could clearly be determined as Form I due the presence of relatively wide pleons and large chelae size, but the freshly molted female also possessed widened pleons and very large chelae. These observations would appear to suggest Form I *C. theepiensis* females maintain Form I state once it is achieved; however, without mark recapture and closer observation to test this hypothesis, the question remains unanswered. Further research could yield interesting results and may be important to understanding reproduction of *C. theepiensis* as well as other species of the Genus *Cambarus*.

Size class structure and habitat utilization

The populations of *C. theepiensis* from Butler Adkins Branch and Right Fork of Miller's Creek had an average TCL of 22.8 mm (n=811). Loughman et al. (2013) did not report a mean TCL for all specimens collected for description of the species and instead listed means of each demographic state; however, upon request, access to the data was granted, and an average TCL of 23.9 mm (n= 1279) was calculated for specimens used in description of the species. The two averages are quite close to each other, and mean TCL size for *C. theepiensis* within Twelvepole appears to fall in line with data obtained by Loughman et al. (2013a) across *C. theepiensis'* range. The slightly smaller mean is most likely explained by a smaller number of YOY collected by Loughman et al. (2013).

Data regarding habitat utilization in relation to TCL appears to show smaller *C. theepiensis* utilizing shallow riffles more often, while larger *C. theepiensis* utilize the protective cover of large slab boulders found along banks and in deeper pools. Runs appear to function as the equally utilized habitat between demographics. Quantifying of size vs. microhabitat types such as gravel/cobble vs. boulder vs. woody debris/ leaf packs could give more insight into size class structure as different sizes of *C. theepiensis* appeared to have particular microhabitat preferences, but these results were not quantified during this study due to difficulties involved discerning such data as many of these microhabitats overlap within stream channels.. Estimates of five to six size cohorts of *C. theepiensis* conforms to that of other large, stream dwelling *Cambarus* species such as *C. b. cavatus*, *C. elkensis*, and *C. robustus* (Hamr and Berrill, 1985; Corey, 1990; Jones and Eversole, 2011).

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Appendix: Chapter II

Chapter II: Life History of *Cambarus theepiensis* tables

Table 2.1: Monthly occurrence of glair, eggs, instars, and free living juveniles for mature female *Cambarus theepiensis* within Twelvepole watershed for the sampling year of May 2012 – April 2013.

Month	Glair	Eggs	Instars	Fl. Juveniles
May 2012	X			
Jun 2012	X	X		
Jul 2012		X	X	X
Aug 2012			X	X
Sept 2012				X
Oct 2012				X
Nov 2012				X
Dec 2012				
Jan 2013				
Feb 2013	X			
Mar 2013	X			
Apr 2013	X			X

Table 2.2: Mean ± standard error and range (mm) for total carapace length (TCL), palmar length (PaL), propodus length (PrL), abdominal length (AbL), and abdominal width (AbW) for Form I & II male and female *Cambarus theepiensis* collected from the Twelvepole watershed during the sampling year of May 2012- April 2013.

Gender Class	**Form II ♀**	**Form I ♀**	**Form II ♂**	**Form I ♂**
n	**397**	**93**	**282**	**39**
Mean TCL ± SE (mm)	16.1 ± 0.9 (4.8 - 43.3)	41.3 ± 0.5 (29.7 - 52.4)	23.6 ± 0.7 (5.2-52.5)	41.6 ± 0.7 (30.6 - 50.6)
Mean PaL ± SE (mm)	3.2 ± 0.1 (1.0 - 10.2)	9.2 ± 0.2 (5.7 - 12.6)	5.0 ± 0.2 (0.9 - 12.9)	10.6 ± 0.3 (7.1 - 16.4)
Mean PrL ± SE (mm)	9.8 ± 0.4 (2.6 - 36.1)	33.6 ± 0.6 (21.9 - 47.9)	17.0 ± 0.7 (2.8 - 51.2)	40 ± 1.2 (22.0 - 58.0)
Mean AbL ± SE (mm)	17.1 ± 0.5 (5.1 - 45.1)	40.5 ± 0.4 (30.9 - 48.9)	23.9 ± 0.6 (5.4 - 45.3)	38.9 ± 0.7 (31.5 - 50.8)
Mean AbW ± SE (mm)	6.7 ± 0.2 (1.4 - 21.1)	20.0 ± 0.3 (14.3 - 26.3)	9.9 ± 0.3 (1.5 - 21.7)	18.1 ± 0.4 (12.7 - 21.9)

Chapter II: Life History of *Cambarus theepiensis* figures

Figure 2.1: Butler Adkins Branch, Wayne County (Twelvepole basin) (Site #1) June 2012.

Figure 2.2: Left Fork Miller's Branch, Wayne County (Twelvepole basin) (Site #2) June 2012.

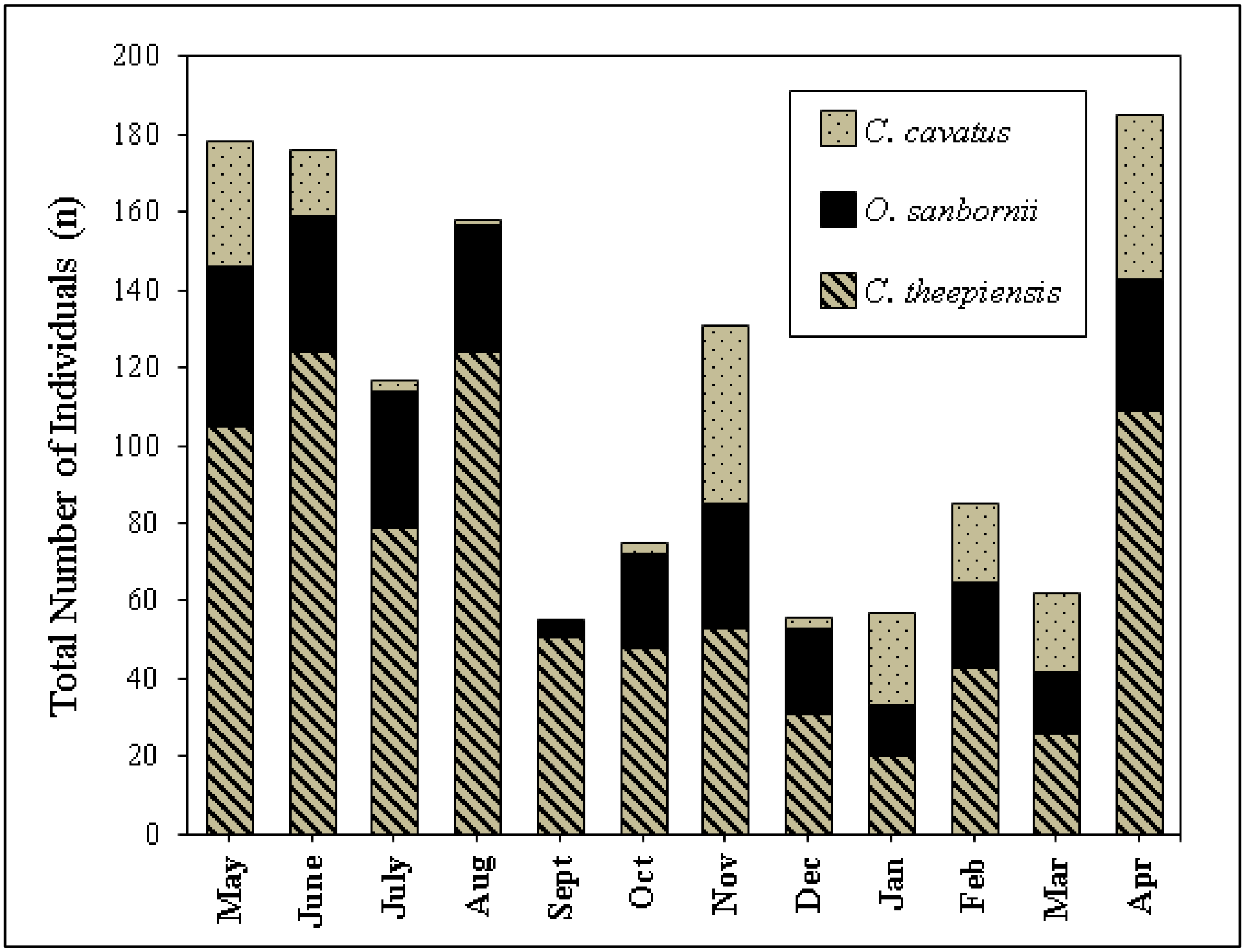

Figure 2.3: Pooled monthly numbers of Crayfish species collected from Butler Adkins Branch and Left Fork Miller's Branch.

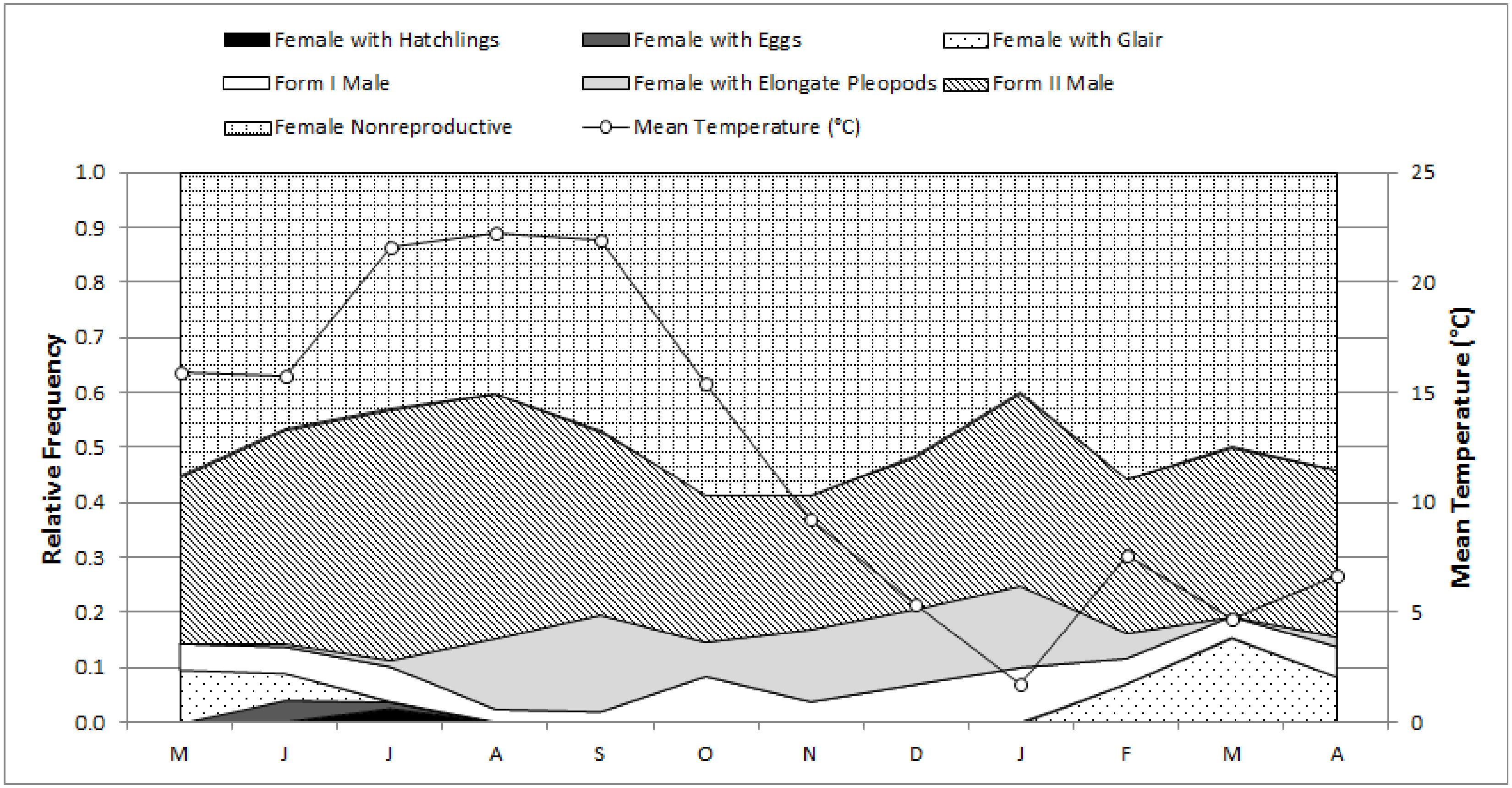

Figure 2.4: Monthly reproductive state of *C. theepiensis* for the year of May 2012- April 2013 in Twelvepole watershed. Percentage occurrence is the frequency at which *C. theepiensis* were collected in noted sexual state relative to all *C. theepiensis* collected during the indicated monthly sampling event. Form 1 crayfish were crayfish that exhibited Form 1 gonapods for males, and elongated pleopods for females. Glair, eggs, and instars are shown separately for females in order to show the limited time these reproductive signs were shown.

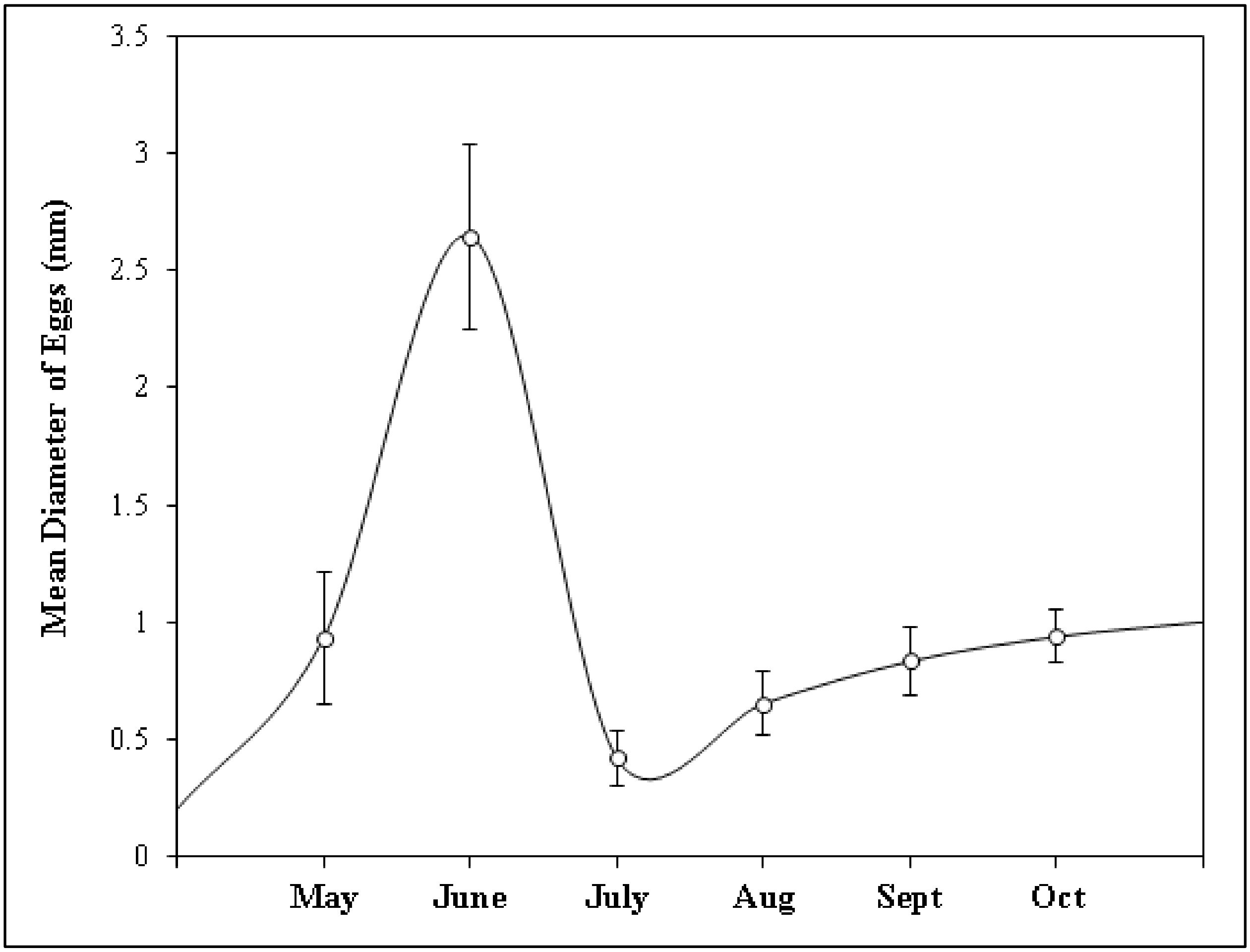

Figure 2.5: Mean internal egg diameter and standard deviation from May – October 2012 for mature female *Cambarus theepiensis* with curve showing action potential of the reproductive process.

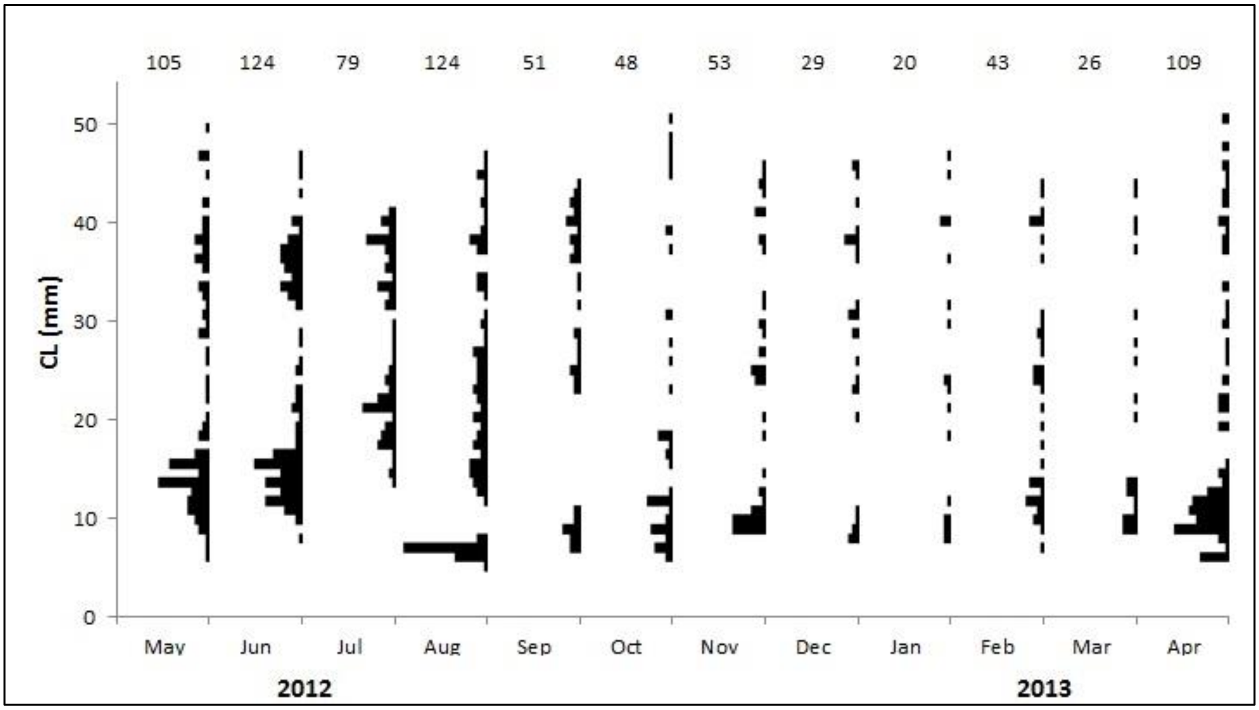

Figure 2.6: Frequency Histogram plots by total carapace length (TCL) for *Cambarus theepiensis* sampled from the upper Twelvepole watershed during May 2012- April 2013. Monthly sample numbers are listed above each histogram.

Figure 2.7: Female *Cambarus theepiensis* with attached instars collected during additional sampling for ova dissections.

Chapter III:

Crayfishes of West Virginia's Southwestern Coalfields: Zoogeography, Taxonomy, and Conservation

Abstract

West Virginia's crayfish fauna currently stands at 25 known species with several still undescribed taxa. Previous statewide surveys efforts have been conducted across all providences, and in recent years more focused efforts have taken place in lotic, lentic, and semi terrestrial habitats throughout the state. Surveying efforts were conducted from 2009-2013 within West Virginia's Southwestern Coalfields. Focus within the area was warranted in response to relative under sampling of region as well as reported declines of *Cambarus veteranus*, a species having a limited geographic range and known only from a small portion of WV, KY, and VA. *Cambarus (P.) theepiensis* and *Cambarus (C.) hatfieldi. Cambarus (C.) bartonii cavatus, Cambarus (J.) dubius, Cambarus (T.) thomai, Cambarus (P.) veteranus, Orconectes (C.) cristavarius, Orconectes (P.) rusticus, Orconectes (C.) sanbornii,* and *Orconectes (G.) virilis* were documented during the survey. *Cambarus veteranus* was found at only one of fourteen known historic sites within the Upper Guyandotte despite increased survey efforts at these sites. Two new localities for *C. veteranus* were found within the Tug Fork system, documenting the species there for the first time in West Virginia. With only three known locations remaining within West Virginia and documented and continued habitat loss and destruction throughout its range, further investigation focusing solely on *C. veteranus* as well as increased conservation efforts for the species are warranted and recommended.

Introduction

Despite significantly increased survey efforts, conservational awareness, and implemented conservation efforts in recent years, crayfish remain one of the most endangered fauna groups in North America and the world (Taylor et al., 1996; Schuster, 1997; Taylor et al., 1999; Taylor and Schuster, 2004; Taylor et al. 2007; Loughman and Simon, 2011, Loughman and Welsh, 2013). Aside from high levels endemism and continued expansion of invasive species (Daniels et al., 2001; Hobbs et al., 1989; Lodge et al., 2000a,b) crayfish species are also vulnerable to surrounding land alteration and use, stream channelization, habitat shifts, and cumulative or compounding effects of aforementioned threats (Loughman and Welsh, 2013).

Like many other faunal groups occurring within the Appalachians, crayfish diversity reaches high levels within North America in the Appalachian Mountains (Hobbs, 1969; Taylor and Schuster, 2005). West Virginia rests in the heart of Appalachia geographically, and while it doesn't reach the levels of diversity associated with the crayfish fauna of it neighbor Kentucky (Taylor and Schuster, 2004) or Georgia, (Hobbs, 1981; Skelton, 2010) it does encompass a fairly diverse range of species (Jezerinac et al., 1995; Loughman and Welsh, 2010; Loughman and Welsh, 2013). Documentation of West Virginia crayfishes was first undertaken by Faxon (1914) and later treated by Necombe (1929), and Jezarinac et al. (1995). As of November, 2013, 25 formally described crayfish species were reported from within West Virginia (Loughman and Welsh, 2013) with additional species currently undergoing taxonomic description.

The area known as the Southwestern Coalfields of West Virginia was chosen as the focal point of the survey due to its small yet varied geographic breadth which has received little focused effort in previous years along with its geographic remoteness, particularly in regard to the upper reaches of the Tug Fork and Upper Guyandotte drainages. Additionally, this portion of West Virginia has received heavy and continuing anthropogenic pressures in the form of extractive industries such as timber and coal, land and stream alteration, and straight piping. Due to the aforementioned remoteness, lack or survey efforts, and continuing anthropogenic pressures, West Virginia's Southwestern Coalfields were determined to be worthy of detailed regional investigation and documentation of crayfish fauna located therein.

Focus within this study area was further warranted primarily due to the fact that this region contains the only known populations of *Cambarus veteranus*, a species of crayfish considered for federal listing due to severe habitat loss and alteration throughout its small known range within Virginia, Kentucky, and West Virginia as well as documented loss of the species at historic localities (Jones et al., 2010; Loughman and Welsh, 2010; Loughman and Welsh, 2013). *C. veteranus* appears sensitive to habitat alteration, particularly removal of large slab boulders and heavy siltation resulting in increased embededness (Loughman, 2013).

Habitats within West Virginia's Southwestern Coalfields

West Virginia's Southwestern Coalfields is a broad geographic area spanning the southern portion of the state where the Appalachian Plateau region of West Virginia meets the Cumberland Mountains region of Eastern Kentucky. The region, as its namesake states, relies predominantly on coal mining centered within the region. Within this region six HUC 8 drainage basins occur: Upper Guyandotte, Lower Guyandotte, Twelvepole, Tug Fork, the Big

Sandy River, and Raccoon-Symmes (Figure 3.1). All basins ultimately drain to the Ohio River. Of particular note is the Raccoon-Symmes drainage which is the portion of the Middle Ohio Watershed located on West Virginia's side of the Ohio River. Although this drainage area is considered its own drainage basin, only treatment of Fourpole Creek, a small drainage located between the Lower Guyandotte and Twelvepole Creek, is covered in this study. This was done as the upper Raccoon-Symmes drainage is not located within the coalfields and has already received adequate documentation (Loughman and Simon, 2011). Lotic habitat within these areas varies from high gradient mountain headwater streams in the Tug Fork and Upper Guyandotte to large, lower gradient streams along the Ohio River Floodplain in Twelvepole and the Lower Guyandotte. Additionally lentic and semi terrestrial habitats such as backwaters, impoundments, ephemeral pools, wet fields, roadside ditches, and upland seeps are also utilized by burrowing crayfish in the coalfields. Due to the variety of lotic, lentic, and semi terrestrial habitat within the region, crayfish diversity is high.

Materials and Methods

Study Area

Streams within the Southwestern Coalfields represent some of the state's most anthropogenically disturbed and altered habitats. The area also represents one of the most biologically under sampled areas in West Virginia due in part to remoteness of the area. Lower gradient drainages emptying into the Ohio River main stem include Fourpole, Twelvepole, the

Lower Guyandotte, and the Big Sandy rivers. Higher gradient streams are located within Tug Fork (Figure 3.2), particularly the headwater reaches, and the Upper Guyandotte. Habitats contained therein range from wet mountain forest dominated by hemlock (*Tsuga Canadensis,* L.) and mountain-laurel (*Kalmia latifolia*, L.) to floodplain habitat dominated by silver maple (*Acer saccharinum*, Marsh), red maple (*Acer rubrum*, L.), and cottonwood (*Populus deltoids*, L.). Urban and suburban streams are also present in the lower drainages of the area and are often impacted by channelization, inclusion of rip-rap, and occasional straight piping along with other anthropogenic influences.

Collection methods

Both lotic and lentic habitats as well as semi terrestrial habitats were surveyed during the study; however, the majority of sampling targeted streams. Seine hauls were performed in streams throughout the study area as the main collection method; however, supplemental sampling via minnow traps, nocturnal searches, and baited lines was used to sample burrowing crayfish as well as crayfish found in lentic waters throughout the survey area such as Beech Fork Lake. During periods of low flow, hand collecting and excavation were also utilized for both epigean and burrowing species. Specifics for each method are detailed below.

Burrowing crayfish collecting methods

Excavation and baited line during nocturnal searches were the primary collecting methods for burrowing crayfish during the study. Excavation was focused on burrows determined to be active by the presence of freshly moved mud or a chimney. Burrows were excavated to the resting chamber of the crayfish through use of hands and trowels if tenable. Excavations ranged from shallow 0.25 m deep burrows in soft sediment adjacent to standing water to burrows > 1.0 m deep through rocky, root laden soils along hillsides.

Due to the arduous nature of excavation, often resulting in failed capture, baited lines (Loughman et al. in press) utilizing a section of night crawler on a hook tethered to the collector's hand by fishing line were used during nocturnal searches. These searches were employed in order to collect *Cambarus dubius* and *Cambarus thomai*, two species of primary burrowers; however, collecting of *Cambarus b. cavatus*, a secondary burrowing species, was not uncommon, particularly when within <= 5 m of a stream bank. Nocturnal searches were often separate sampling events from stream sampling or targeted excavation as nocturnal searches rely on predatory and exploratory activity of burrowing species after nightfall while stream sampling and burrow excavation are much better suited to daylight hours due to better visibility.

Stream dwelling crayfish collecting methods

Seine hauls were the primary method used for sampling stream reaches for epigean crayfish. Seines were angled backward at 45 degrees downstream of suitable habitat such as leaf packs, slab boulders, and boulder/cobble clusters within riffles, runs, and pools of streams where depth did not prevent sampling. Next, the substrate was disturbed through use of flipping and sweep kicking downstream. This method dislodge the disturbed crayfish from their cover and allowed the current to carry them downstream into the net where they were collected and placed into holding vessels until specimens were preserved in 80% ethanol as vouchers. At each stream sampling site, a minimum of five seine haul efforts (one seine haul represents one seining effort) to a maximum of 10 seine haul efforts were performed based on most suitable habitat.

Additional sampling of large leaf packs were performed through use of dip nets. Leaves were kicked and scooped into nets before being spread out on a flat surface, often a nearby bank, and sifted through. Collected crayfish were placed into collecting vessels and were later retained and vouchered in the same manner as seine sampled crayfish. Remaining leaf litter was scraped back into the net and returned to the water upstream to allow for further utilization by benthic communities present at the site. While all life stages were collected from leaf packs and they do appear to play a significant role as stated by Loughman and Simon (2011), sampling of leaf packs in stream reaches within this study area yielded a higher number of YOY and smaller individuals, making this sampling particularly important for the study of earlier life stages of stream dwelling species.

Data collection

After each sampling event per site surveyed within the study area, field data sheets and jar labels were completed (Loughman and Simon, 2011). Vouchered crayfish were placed into 80% ethanol and were later transferred to fresh 70% ethanol for storage after morphometric analysis, species identification, sexual morph, and physical maladies and/or mutations were documented. Morphometrics were measured using digital calipers. Only total carapace length

(TCL) is reported hereafter. Sexual stage of each individual crayfish per site was determined, (Hobbs, 1981) and any female crayfish bearing eggs or instars were placed in separate jars following counting and measurements of offspring. Jars containing ovigerous females and offspring were given an identical label to that of the original collection site and maintained separately to aid in ease of access for life history studies. Physical maladies including scars, missing limbs, regenerated chelae, damage to body, etc. along with mutations such as inverted joint articulation and multiple terminal dactyl or propodal points were noted for each individual crayfish (Loughman and Simon, 2011). Museum accession numbers refer to specimens housed within the West Liberty University Astacology Collection at West Liberty University (WLU), West Liberty, West Virginia.

Conservation Ranks

Conservation ranks listed hereafter were determined in accordance with conservation ranking criteria set forth by Nature Serve (Masters et al., 2009; Loughman and Simon, 2011; Loughman and Welsh, 2013)

Explanation of Species Accounts

Accounts for species of crayfish collected thus far within West Virginia's Coalfields are provided. Descriptions based on morphometrics and color in life along with natural history, habitat, distribution within the study area, and conservation for each species are discussed. Synonymies, adapted and updated from previous works (Jezerinac et al., 1995; Loughman and Simon, 2011) are provided at the beginning of each species account in order to give a list of previous works involving the species presented. A description of each individual subsection provided for each species can be found below.

Diagnosis and Color in Life

The diagnosis section describes morphological characters per species treated within the study area. All known characters and measurements pertaining to said characters that aid in identification of the species in question are included. Additionally, any coloration, color patterns, or unique geographic morphs for the species in question are discussed. In order to avoid taxonomic confusion, diagnoses for all species are taken from Loughman and Simon (2011), Loughman et al. (2013), and Loughman et al. (*in press*).

Morphometrics

Morphometric data pertaining to animals collected within the study area is provided. Total carapace length (TCL) is stated for the largest Form I and Form II male along with the largest female of each species. Tables containing the number, range, and standard deviation of TCL for females and Forms I & II males for each species are also provided.

Distribution

Distribution for each species occurring in West Virginia's Southwestern Coalfields are discussed in comparison with previous sampling efforts of Jezarinac et al. (1995). Maps showing distribution of each species throughout the study area as well as individual basins contained therein are provided and represent only the sampling efforts of this survey and not those of historic sampling events.

Natural History and Habitat

Observations of habitat preference and utilization, burrowing classification and ability, and commensal crayfish species within the area of study are stated for each species and are compared alongside observations of previous research if the given species within the study area was found to have behaviors or preferences differing from populations of the same species outside the study area. For stream dwelling species, utilization of lentic and lotic habitats and microhabitats contained therein are noted. For burrowing species, burrow morphology, usage, and relation to nearby water is stated. Any observed seasonal shifts, in regards to habitat usage or behavior are also stated in this section.

Conservation Status

Current conservation standing as well potential anthropogenic, environmental, and invasive mechanisms for imperilment in regards to each species are stated and discussed in accordance with Masters et al. (2009) and Loughman and Simon (2011). If warranted by current data, observations, and knowledge; recommendations for future monitoring and conservation efforts are listed for each species.

Results and Species Accounts

Genus *Cambarus*

Monthly documentation of sexual morphs for all *Cambarus* species within the study area are provided in Table 3.1.

***Cambarus (Cambarus) bartonii cavatus* Hay, 1902** - Appalachian Brook Crayfish

Cambarus bartonii cavatus Hay, 1902. Faxon, 1914. Taylor et al. 1996.

Cambarus (Bartonius) bartonii.Ortmann, 1905b.

Cambarus (Cambarus) bartoni cavatus.Ortmann, 1931.

Cambarus (Cambarus) bartonii cavatus. Fowler, 1912. Hobbs, 1969. 1974, 1989. Jezarinac et al., 1995 Taylor and Schuster.2004. Loughman and Simon, 2011; Taylor and Schuster, 2004.Taylor et al., 2007.

Diagnosis. Rostrum broad and excavated, margins thickened and parallel, terminating in gentle angle cephalically to form acumen ending in a single upturned tubercle; postorbital ridges short and reduced, cephalic margin with reduced tubercle; cephalothorax dorsoventrally flattened in profile, anterior portion weakly vaulted; 4-6 punctations across narrowest region of areola; branchiostegal region moderately punctate, with small tubercles; chelae subtriangular; mesial surface of palm consisting of two rows of addpressed tubercles; palm free of tubercles; two subpalmar tubercles present; first form gonopods contiguous at base, with 2 terminal elements bent 90° to base; central projection with distinct subapical notch; total length of central projection equal to mesial process length; mesial process bulbous, truncating distally; second form gonopod non-corneous and blunt; annulus ventralis rhomboid in shape, embedded shallowly in sternum and movable

Color in Life. Carapace dorsally beige, olive, or tan fading to lighter colors or cream ventrally; rostrum margins chestnut brown to brown; chelae olive green to brown; dactyl and palmer tubercles cream or yellow; pereiopods cream, gray, light green, or tan; abdominal terga dorsally brown or beige, outlined in gray; ventral surfaces semi-translucent cream or white (Figure 3.3).

Distribution. *Cambarus b. cavatus* ranges from Eastern Indiana east across Ohio and into Western West Virginia down through the Western most potion of Virginia to Kentucky, Tennessee, and the Northwestern portion of Georgia (Jezarinac et al. 1995; Taylor and Schuster 2004). Within the study area *C. b. cavatus* is found within all drainages except for the Tug Fork, and is the only secondary burrower found within the study area (Figure 3.4).

Morphometrics. *Cambarus b. cavatus* is a moderate sized crayfish. Mean TCL was 13.5 mm (n= 318, SE= .50 mm). The largest individual was a female with a TCL of 47.8 mm collected from Butler Adkins Branch in Wayne County. The largest form I male had a TCL of 40.3 mm and was also collected from Butler Adkins Branch. The largest form II male had a TCL of 39.0 mm and was collected from Miller Creek in Wayne County. Morphometric data for *C. b. cavatus* is presented in Table 3.2.

Habitat and natural history. *Cambarus b. cavatus* can be found throughout a wide variety of habitats including seeps, roadside ditches, creek embankments along larger order streams, and within smaller order streams under rocks and slabs. Taylor and Schuster (2004) noted *C. b. cavtus'* fondness for small headwater streams and Jezarinac et al. (1995) documented burrows in headwater creeks, ditches, and springs, as well as underneath large slab boulders along the banks of larger creeks. Both observations hold true within our study area. High numbers of *C. b. cavatus* were collected in 1st and 2nd order streams throughout the study area. In Butler Adkins Branch within the Twelvepole watershed in Wayne County, *C. b. cavatus* was noted to occur within the stream proper during periods of flow but would burrow diagonally or vertically within the stream bed or banks during periods of low flow and drought to avoid desiccation. *C. b. cavtus'* ability to burrow and not directly utilize surface waters is likely a key factor for it avoiding extirpation by *Orconectes rusticus* within Fourpole Creek. Crayfish associates in the study area include *C. dubius*, *C. hatfieldi, C. theepiensis, C. thomai, C. veteranus, O. cristivarius, O. rusticus, O. sanbornii*, and *O. virilis*.

Conservation status within study area. *Cambarus b. cavatus* populations within the southwestern coalfields appear stable and do not currently warrant special attention.

Cambarus (Cambarus) hatfieldi* Loughman *in press- Tug Valley Crayfish

Cambarus bartoni sciotensis Rhoades, 1944a.

Cambarus sciotensis Holt, 1954. Taylor and Schuster, 2005.

Cambarus bartonii sciotensis Hobbs, 1955.

Cambarus (Cambarus) sciotensis Hobbs, 1969, Taylor and Shuster 2004.

Cambarus (Cambarus) hatfieldi Loughman et al. *in press.* Loughman and Welsh 2013.

Diagnsosis. Rostrum broad; margins reduced, subparallel, terminating cephalically in a gentle angle to form acumen; anterior region of rostrum excavated; acumen consisting of a single upturned spiniform tubercle; postorbital ridges truncated, cephalic margin with weak tubercle; cephalothorax oval shaped and slightly dorsoventrally flattened in profile; 2-3 punctations across narrowest region of areola; branchiostegal region moderately punctate, with small tubercles; chelae broad and robust; mesial surface of palm consisting of two rows of defined tubercles; first row with 5-8 rounded tubercles; second with 3-4 tubercles; two prominent subpalmar tubercles present; first form gonopods contiguous at base, with 2 terminal elements bent 90° to the base; central projection with shallow subapical notch; total length of central projection equal to mesial process length; second form gonopod non-corneous and blunt; mesial process bulbous, truncating distally; annulus ventralis rhomboid in shape, embedded shallowly in sternum and movable.

Color in life. Carapace dorsally orange to pinkish brown fading to lighter colors or cream along ventral margins; rostrum margins orange to red- orange; chelae dorsally green, olive, orange, or brown with tints of light green or olive; palmer and dactyl tubercles yellow to reddish brown; pereiopods mesially brown to olive fading in color to cream both proximally and distally; abdominal terga brown to chestnut or olive fading on outer tergal margins to light brown or light green; ventral surfaces semi-translucent cream or white (Figure 3.5).

Distribution. *Cambarus hatfieldi* has a very limited range within Kentucky, West Virginia, and Virginia and is endemic to the Tug Fork Drainage (Loughman et al., *in press*). It is common within the upper reaches of the drainage; populations decrease in the lower reaches where rocky substrates give way to fine gravels and sands (Loughman et al. In Press; Figure 3.6).

Morphometrics. *Cambarus hatfieldi* is a moderate- large sized crayfish. Mean TCL was 24.6 mm (n= 344, SE=.41mm). The largest individual was a female with a TCL of 42.6 mm collected from Horse Creek, McDowell County. The largest form I male had a TCL of 39.5 mm and was also collected from Horse Creek. The largest form II male had a TCL of 39.3 mm and was collected from Jacobs Creek, McDowell County. Morphometric data for *C. hatfieldi* is presented in Table 3.3.

Habitat and natural history. *Cambarus hatfieldi* inhabits 2nd through 5th order streams within the Tug Fork basin. Preferred habitat includes cobbles, boulders, and large slabs (Loughman et al., *in press*). Leaf packs and course woody debris are also utilized. Relatively little else about the species biology is known as it was only recently described; further study of *C. hatfieldi* is warranted. Crayfish associates in the study area include *C. b. cavatus, C. dubius, C thomai, C. veteranus, and O. cristivarius.*

Conservation status within study area. *C. hatfieldi* populations within the Tug Fork appear stable but due to its limited geographic range and only recent description. *Cambarus hatfieldi* is considered threatened by the WVDNR. Further investigation of the species is warranted.

Cambarus (Jugicambarus) dubius Faxon, 1884- Upland Burrowing Crayfish

Cambarus dubius Faxon, 1884. Taylor and Schuster, 2004.

Cambarus carolinus dubius Faxon, 1914.

Cambarus (*Jugicambarus*) *dubius* Hobbs and Bouchard, 1973. Taylor and Shuster, 2004. Loughman and Simon, 2011. Loughman and Welsh 2013.

Diagnosis. Rostrum short and broad, margins converging to form acumen terminating in single reduced, upturned tubercle; postorbital ridges reduced, rarely terminating in small tubercle; cephalothorax dorsolaterally compressed in profile and vaulted; areola obliterated; branchiostegal region devoid of tubercles; chelae robust and diamond shaped; mesial surface of palm distinct single row of cristiform tubercles; first form male gonopods contiguous, with 2 terminal elements bent 90° to the shaft; central projection truncated distally and lacking sub-apical notch; total length of central projection equal to mesial process length; mesial process short, truncating distally; second form gonopod non-corneous and blunt; annulus ventralis rhomboid in shape with deep "S" shaped sinus, embedded shallowly in sternum, and movable.

Color in life. Extremely variable throughout its range. Two morphs persist within the study area while a third morph exists just north of the study area. Each morph is described in its entirety below with the third morph included should future collections yield specimens within the study area (Figure 3.7).

Orange clawed blue body morph (Kanawha morph)- Carapace dorsally deep blue to purple lightening in color ventrally; rostrum margins brick-bright orange; chelae brick- bright orange, occasionally with deep color to blue edging on the palmer region near brick-bright orange dactyl and palmer tubercles; pereiopods cream colored ventrally with dorsal regions brick-bright orange distally with more proximal dorsal portions fading into deep blue to purple; abdominal terga dorsally deep blue to purple fading to brick- bright orange on outer tergal edges; ventral surfaces semi- translucent cream or white

Orange clawed black body morph (Halloween morph) - Carapace dorsally black lightening in color or fading out to orange ventrally; rostrum margins orange; chelae orange, occasionally with some black on the proximal ergions of the chelae; pereipods ventrally and proximally cream colored fading into orange distally with black occasionally occurring on pereipodal joints; abdominal terga dorsally black fading to orange on outer tergal edges; ventral surfaces semi-translucent cream or white.

Blue morph - Carapace dorsally deep blue fading to bright blue and cream ventrally; rostrum margins deep blue; chelae deep blue with white - cream tipped dactyl and palmer tubercles; pereiopods ventrally and proximally cream colored fading to bright blue distally; abdominal terga dorsally deep blue fading to bright blue on outer tergal edges; ventral surfaces semi-translucent cream or white.

Distribution. *Cambarus dubius* is found throughout the Appalachian plateau. Taylor and Schuster (2004) noted that it occurred from Western PA southward to Tennessee on the westward side of the Appalachian Mountains. Numerous color morphs exist throughout its range. Jezerinac et al. (1995) and Dewees (1972) noted that due to geographic seclusion and small morphometric differences, *C. dubius* is likely a species complex. The blue morph, as described above, occurs directly north of the study area, but future studies could yield specimens within the northern portion of the study area. The Kanawha morph occurs from Charleston to Huntington, WV and westward into Kentucky as well as eastward towards Beckley, WV where it appears to intergrade with the Halloween morph which occurs throughout southernmost portions of WV (Figure 3.8).

Morphometrics. *Cambarus dubius* is a medium sized primary burrowing crayfish located within the study area. Morphometric data for this species during the study is unavailable as records are from casual observation only. Jezerinac et al. (1995) recorded multiple individuals within the southwestern coalfields and noted the largest specimen recorded from Wayne County was a female with a TCL of 36.1 mm. The largest Form I male recorded had a TCL of 37.9 mm but no locality data for the specimen was listed.

Habitat and natural history. Despite physical and geographic differences, *C. dubius* appears to prefer the same habitat throughout its range including hillside seeps, wet fields, roadside ditches, and high creek banks. Taylor and Schuster (2004) and Loughman and Foltz (personal observations) noted *C. dubius* as being particularly difficult to collect due to their tendency to burrow through rocky soils, making excavation problematic. Crayfish associates in the study area include *C. b. cavatus* and *C. thomai*.

Conservation status within study area. *Cambarus dubius* populations within the study area appear stable, although sporadic. Further monitoring may be warranted in order to better assess and understand populations of *C. dubius* in the coalfields region.

Cambarus (Puncticambarus) theepiensis **Loughman et al., 2013**- Coalfields Crayfish

Cambarus robustus Girard, 1852. Hagen, 1870. Crocker and Barr, 1968. Taylor et al., 1996. Taylor et al., 2007.

Cambarus Bartonii robustus.– Faxon, 1885.

Cambarus bartonii robustus.– Faxon, 1890.

Cambarus (*Bartonius*) *bartoni robustus.*– Ortmann, 1905.

Cambarus (*Cambarus*) *bartonii robustus*.– Ortmann, 1931.

Cambarus (*Puncticambarus*) *robustus*. – Hobbs, 1969, 1974, 1989. Lawton and Tarter, 1982. Jezerinac et al., 1995. Taylor and Schuster, 2004.

Cambarus (Cambarus) sciotensis.– Loughman et al. 2009. Jones et al., 2010.

Cambarus (Puncticambarus) theepiensis. – Loughman et al., 2013. Loughman and Welsh 2013.

Diagnsosis. Rostrum broad, margins thickened and parallel, terminating in gentle angle cephalically to form acumen terminating in a single upturned spiniform tubercle; postorbital ridges prominent, cephalic margin with reduced tubercle; cephalothorax dorsoventrally flattened in profile, anterior portion weakly vaulted; 5-7 punctations across narrowest region of areola; branchiostegal region moderately punctate, with small tubercles; chelae robust; mesial surface of palm consisting of two rows of defined tubercles; first row with 7-9 rounded tubercles; second with 5-7 smaller tubercles; palm free of tubercles; three prominent subpalmar tubercles present; first form gonopods contiguous at base, with 2 terminal elements bent 90° to base; central projection with distinct subapical notch; total length of central projection equal to mesial process length; mesial process bulbous, truncating distally; second form gonopod non-corneous and blunt; annulus ventralis rhomboid in shape, embedded shallowly in sternum and movable.

Color in life. Carapace dorsally brown to chestnut fading to lighter colors or cream along ventral margins; rostrum margins orange to bright brown, occasionally red; chelae dorsally brown-orange to olive with tints of light green; palmer and dactyl tubercles yellow to orange; pereiopods mesially brown to green fading in color or to cream both proximally and distally; abdominal terga brown to chestnut fading on outer tergal margins to light brown or light green; ventral surfaces semi-translucent cream or white (Figure 3.9).

Distribution. *Cambarus theepiensis* has a small distribution and is limited to Southwestern West Virginia and Eastern Kentucky (Loughman et al. 2013). It is found within all drainages within the study area with the exception of Fourpole Creek. Habitat within Fourpole is suitable, and *C. theepiensis* likely occurred within the drainage, however, it has since been extirpated by *Orconectes rusticus* within the lower reaches of the drainage. Relict populations of *C. theepiensis* may still persist in the upper headwaters of Fourpole Creek (Figure 3.10).

Morphometrics. *Cambarus theepiensis* is a moderate-large sized crayfish. Mean TCL was 23.9 mm (n= 1279, SE = 0.33 mm). The largest individual collected was a form II male with a TCL of 52.5 mm collected from Butler Adkins Branch in Wayne County. The largest form I male had a TCL of 50.6 mm and was taken from Right Fork Miller's Creek in Wayne County. The largest female collected had a TCL of 48.8 mm and was also taken from Right Fork Miller's Creek. Morphometric data for *C. theepiensis* is presented in Table 3.4.

Habitat and natural history. *Cambarus theepiensis* inhabits 2nd through 5th order streams within the study area. Preferred microhabitats include large slab boulders, both in the stream and lining the bank. Leafs packs, boulders, large cobble, and hardpan burrows are also utilized by the species, however, an extreme preference for slab boulders, particularly in large individuals, seems to be prevalent throughout its range. During months of drawdown and winter inactivity, it was not uncommon to overturn slab boulders and observe *C. theepiensis* resting at the entrance to a burrow leading into the substrate. These burrows were common both mid channel and along the banks under slab boulders during these months.

It is also worth noting that *C. theepiensis* appears to be a keystone species within its habitat during periods of extreme draw-down. At the Butler/ Adkins branch located within the Twelvepole watershed through the months of July and September 2012 during periods of drought, large slab boulders were targeted and searched for *C. theepiensis*. Almost every large slab boulder examined during these months had between one and nine amphibians (*Desmognathus monticola* Dunn, 1916, *Eurycea cirrigera* (Green, 1830), *Lithobates clamitans melanota* (Latreille, 1801), *Lithobates palustrus* (Leconte, 1825), and *Pseudacris crucifer* (Wied-Neuwied, 1838) resting at the moist exposed portion of the burrow, often in groups of mixed species. All amphibians noted appeared to be utilizing water contained within the burrows to prevent desiccation. Crayfish associates in the study area include *C. b. cavatus, C. veteranus, O. cristivarius, O. sanbornii,* and *O. rusticus*

Conservation status within study area. *Cambarus theepiensis* appears to be stable within the study area and no further monitoring is warranted for the species at this time.

Cambarus (Puncticambarus) veteranus **Faxon, 1914**- Big Sandy Crayfish

Cambarus bartonii veteranus Faxon, 1914.

Cambarus bartoni veteranus Ortmann, 1931.

Cambarus (Cambarus) montanus veteranus Ortmann, 1931.

Cambarus veteranus Hobbs, 1955. Taylor and Schuster, 2004.

Cambarus (Puncticambarus) veteranus Hobbs, 1969. Loughman and Welsh, 2010. Loughman 2013. Loughman and Welsh 2013.

Diagnsosis. Rostrum narrow with convergent, slightly concave margins converging to form acumen terminating in single upturned tubercle; postorbital ridges terminate cephalically in acute spine; cephalothorax dorsolaterally compressed in profile; areola 3.2-5.4 times longer than wide; branchiostegal spine acute to weakly developed; cervical spine strong; chelae robust, smooth and diamond shaped; mesial surface of palm with two rows of distinct tubercles; mesialmost row consisting of 5-8 rounded tubercles; dorsolateral row consisting of 5-9 rounded tubercles; subpalmar tubercles absent; first form male gonopods contiguous, with 2 terminal elements bent 90° to the shaft; central projection tapering distally bearing prominent sub-apical notch; total length of central projection equal to mesial process length; mesial process inflated at base, truncating distally; second form gonopod non-corneous and blunt; annulus ventralis asymmetrical and ovoid in shape with deep “S” shaped sinus, embedded shallowly in sternum, and movable.

Color in life. Carapace dorsally dark green, brown, or dark teal fading to lighter tones ventrally; rostrum margins red to deep crimson; chelae dark green to deep teal with yellow to orangedactyl and palmer tubercles on larger individuals with a large crimson tubercle near dactyl insertion; pereiopods white to cream colored proximally fading into green or teal distally with joints highlighted in dull red to bright crimson; abdomen terga dorsally dark green, brown or dark teal outlined in cream to white; ventral surfaces semi- translucent cream or white; antennae usually brown but occasionally red to bright crimson (Figure 3.11).

Distribution. *Cambarus veteranus* has a very limited range and is restricted to a small area covering eastern Kentucky, western Virginia, and Southwestern West Virginia. Its entire range within WV is located within the Upper Guyandotte and Tug River basins, and is confined to our area of study only. Historically, specimens were found in the Bluestone and Upper Guyandotte rivers in West Virginia. A survey by Jones et. al (2010) concluded that *C. veteranus* was likely extirpated from WV. Shortly after, Loughman (2013) resurveyed all known WV historical localities and reported *C. veteranus* did appear to be extirpated from all historical locales with the exception of Pinnacle Creek where three specimens were collected. Additionally, Loughman (2013) reported finding *C. veteranus* within the Tug Fork Drainage, a newly reported population for the species. Subsequent analyses of specimens contained at United States National museum showed that the record from the Bluestone was erroneous, eliminating one of the historical locales for the species within West Virginia (Z. J. Loughman personal communication). Currently, Pinnacle Creek (Figure 3.12), a tributary feeding the Upper Guyandotte main stem (Figure 3.13), along with Dry Fork, and the Tug Fork mainstem are the only known streams to contain extant populations of *C. veteranus* within WV (Figure 3.14).

Morphometrics. *Cambarus veteranus* is a moderate sized crayfish. Mean TCL was 33.9 mm (n=6, SE=2.92mm). The largest individual was a form I male with a TCL of 39.4 mm collected from Tug Fork in McDowell County. The largest form II male had a TCL of 34.9 mm and was also collected from Tug Fork. The largest female had a TCL of 37.5 and was also collected from Tug Fork. Morphometric data for *C. veteranus* is presented in Table 3.5.

Habitat and natural history. *Cambarus veteranus* is found in large streams (3rd order or >) with fast moving current and large, flat slab boulders located throughout the stream with a underlying substrate of cobble and sand (Jezerinac et al. 1995; Taylor and Shuster 2004; Loughman and Welsh 2013). Upon discovery of the species within the Tug Fork near the confluence of Horse Creek with the mainstem, Loughman and Foltz (unpublished data) noted relatively high abundance for *C. veteranus* in water < 10 ft from the bank during a spring high water event. Subsequent searches by Foltz during summer months at the same site yielded no *C. veteranus.* Collection events in the winter, again, yielded numbers of *C. veteranus*. This data resulted in two hypotheses for *C. veteranus* within the Tug Fork Drainage presented by Loughman (2013).

First, *C. veteranus* prefers cool, highly oxygenated waters associated with montane streams and high flow, therefore, during cooler months and/ or periods of high flow, *C. veteranus* can be found in more shallow, exposed margins of the Tug Fork, while during warm months and/or periods of low flow, *C. veteranus* retreats to the thalwegs of the stream (Loughman, 2013). This would explain inability to detect the species during summer months as the center of the Tug Fork is deep and fast moving. The second hypothesis is that *C. veteranus* moves into more shallow water during these months for biological reasons, possibly breeding, providing the young an environment free of larger predators located within the stream while still containing adequate cover, flow, and dissolved oxygen (Loughman, 2013). This hypothesis appears to be supported as female *C. veteranus* in berry were collected during spring sampling by Loughman and Foltz (unpublished data). Crayfish associates in the study area include *C. hatfieldi, C. theepiensis, O. cristavarius,* and *O. sanbornii*.

Conservation status within study area. *C. veteranus* requires immediate assessment and protection within the study area. Since Jezarinac's surveys (1995) the Bluestone population has been determined to be erroneous and populations at 15 of the remaining 16 historic locations have become extirpated due to apparent mining pressures within the area as well as stream channelization and removal of slab boulders from some streams (Loughman and Welsh, 2010). Populations at the remaining historic location of Pinnacle creek appear to be in severe decline. The newly discovered population within the Tug Fork also appears in relatively low numbers and is threatened by extractive industry and other anthropogenic effects; particularly sedimentation as *C. veteranus* appears to be negatively affected by highly embedded streams (Loughman, 2013; Loughman and Welsh, 2013). Current conservation rankings list *C. veteranus* as a G3 and S1 species; however, declines have been listed from both Kentucky and Virginia as well (Loughman, 2013), suggesting a range wide survey effort and possible reclassification is warranted.

***Cambarus (Tubericambarus) thomai* Jezarinac, 1993**- Little Brown Mudbug

Cambarus diogenes Girard 1852. Williamson 1899. Ortmann 1905. Newcombe 1929. Rhoades 1944a, 1944b.

Cambarus diogenes diogenes Hay 1899. Marlow 1960.

Cambarus (Bartonius) diogenes Ortmann 1906. Turner 1926.

Cambarus (Lacunicambarus) diogenes diogenes Hobbs 1969. Bouchard 1972, 1975, Lawton 1979. Thoma and Jezarinac 1982. Jezarinac and Thoma 1984. Jezarinac 1985.

Cambarus (Lacunicambarus) diogenes Jezarinac 1985. Hobbs 1989.

Cambarus (Tubericambarus) thomai Jezarinac 1993..Jezarinac et al. 1995. Taylor and Schuster 2004. Loughman 2010. Loughman and Welsh 2013.

Cambarus thomai Taylor et al. 1996. Taylor et al. 2007.

Diagnsosis. Rostrum slightly broad, margins converging to form acumen terminating in single reduced, upturned tubercle; postorbital ridges reduced, rarely terminating in small tubercle; cephalothorax dorsolaterally compressed in profile and vaulted; areola obliterated; branchiostegal region devoid of tubercles; chelae robust and diamond shaped; mesial surface of palm with disorganized prominent tubercles, mesialmost tubercles serrate; basiodactyl row consisting of 5-9 reduced rounded tubercles; first form male gonopods contiguous, with 2 terminal elements bent 90° to the shaft; central projection truncated distally and lacking sub-apical notch; total length of central projection equal to mesial process length; mesial process short, truncating distally; second form gonopod non-corneous and blunt; annulus ventralis rhomboid in shape with deep "S" shaped sinus, embedded shallowly in sternum, and movable.

Color in life. Carapace dorsally brown, green, olive, light blue, and bluish gray fading to cream colored ventral margins; rostral margins yellow, brown, or orange; chelae highly variable dorsally and can be brown, green, olive, light blue, or bluish gray often with vermiculations and fading to cream laterally; tips of dactly and propodus often accented in bright orange to red; pereiopods mesially brown, green, olive, light blue, or bluish gray fading to cream both proximally and distally; abdominal terga brown, green, olive, light blue, or bluish gray fading on outer tergal margins to cream; vental surfaces semi-translucent cream or white but can be a semi-translucent blue, particularly in bluish individuals (Figure 3.15).

Distribution. *Cambarus thomai* has a wide distribution within the United States. It occurs from Southwestern Pennsylvania and West Virginia northward across Ohio and into Michigan south through Illinois, Kentucky, Tennessee, Mississippi, Alabama, and Western Georgia (Jezarinac, 1993; Jezarinac et al., 1995; Taylor and Schuster, 2004; Loughman, 2010; Loughman and Simon, 2011). The distribution appears to be focused around the Ohio River and its major tributaries. Loughman (2010) documented an apparent introduction of the species into Western Maryland around the northern reaches of Deep Creek Lake; however, means of the introduction remain speculative. Within the study area, *C. thomai* reaches its highest densities along the Ohio River Floodplain and lowland areas surrounding Beech Fork Lake. Jezarinac et al. (1995) noted a specimen in the Upper Guyandotte drainage but our surveys did not detect specimens eastward of Beech Fork State Park (Figure 3.16).

Morphometrics. *Cambarus thomai* is the largest primary burrowing crayfish located within the study area. Morphometric data for this species during the study is unavailable as records are from casual observation only.

Habitat and natural history. *Cambarus thomai* was frequently observed and noted along the Ohio River and its corresponding floodplain but was not observed any further eastward than Beech Fork State Park in this study. Wet fields, roadside ditches, wooded marshes, swamps, bottomland forests, low lying seeps, and stream banks are habitats utilized by *C. thomai* within the study area (Loughman and Simon, 2011). Crayfish associates in the study area include *C. b. cavatus,* and *C. dubius.*

Conservation status within study area. *Cambarus thomai* populations within the study area appear stable, particularly along the Ohio River Floodplain. No further monitoring is warranted at this time.

Genus *Orconectes*

Monthly documentation of sexual morphs for all *Orconectes* species within the study area are provided in Table 3.6.

***Orconectes* (*Crockerinus*) *sanbornii* Faxon, 1884**– Sanborn's Crayfish

Cambarus sanbornii Faxon 1884.

Cambarus propinquus sanbornii Faxon 1885.

*Cambarus propinquus*var. *sanbornii* Underwood 1886.

Cambarus propinquus var. *sanbornii* Osborn and Williamson 1898.

Cambarus propinquus sanbornii Faxon 1898.

Cambarus propinquus sanborni Ortmann 1905b.

Cambarus (*Faxonius*) *propinquus sanbornii* Ortmann 1906.

Cambarus obscures sanbornii Ortmann 1906.

Faxonius sanbornii Creaser 1933a.

Faxonius (*Faxonius*) *sanbornii* Creaser 1933b.

Orconectes propinquus sanbornii Hobbs 1942a. Fitzpatrick 1963.

Orconectes (*Orconectes*) *propinquus sanbornii* Hobbs 1942b.

Faxonius sanborni sanborni Creaser 1962.

Orconectes sanbornii sanbornii Fitzpatrick 1967.

Orconectes sanbornii Stevenson 1967. Taylor et al. 1996. Taylor and Schuster 2004. Taylor et al. 2007.

Orconectes sanbornii sanbornii Hobbs 1974.

Orconectes (*Crockerinus*) *sanbornii sanbornii* Fitzpatrick 1987. Hobbs 1989.

Orconectes (*Crockerinus*) *sanbornii* Jezerinac et al. 1995. Taylor and Schuster 2004. Loughman and Simon 2011. Loughman and Welsh 2013.

Diagnsosis. Rostrum with slightly converging margins, not thickened, with marginal spines or tubercles; median carina absent. Cephalothorax ovoid, slightly, dorsoventrally compressed, without setae. Areola 3.4-9.3 times longer than wide, comprising 31-37% of TCL, with 2-3 rows of punctations across narrowest region; cervical groove interrupted just above cervical spine; lacking hepatic spines; suborbital angle obsolete. Antennal scale about 1.5 times as long as wide; basiopodite spine of antenna well developed; ischiopodite of antenna without spine.

Chelae smooth, broad and robust, length 85% of TCL; mesial surface of palm with two well-developed rows of tubercles; mesial most row consisting of 7-11 tubercles; dorsolateral row with 7-11; lateral margin of propodus smooth, dorsal surfaces of both dactyl and fixed finger of propodus with weak dorsolateral ridges; some elongate setae at base of fixed finger. First form male gonopods short, comprising 30% of TCL, with two terminal elements about subequal length; corneous central projection comprising 16% of pleopod length, tapering distally to point; mesial process non-corneous, spatulate, partially surrounding central projection; cephalic base of central projection sloping, without right angle shoulder. Form two male gonopod non-corneous, blunt, shoulder not prominent or absent. Female annulus ventralis deeply embedded in sternum, moveable, wider than long, cephalolateral prominences flattened; fossa and sulcus shallow; sinus straight.

Color in life. Carapace dorsally brown to tan fading to lighter tones ventrally; brown to chestnut U- shaped saddle running anteriorly on carapace from insertion of abdomen; chelae brown to tan with cream colored dactyl and palmer tubercles; large tubercle at insertion point of dactyl orange; chelae tips orange occasionally accompanied by a thin black band; perieopods mesially brown to tan, fading to cream coloration both proximally and distally; abdominal terga usually show a deep brown mesial stripe outlined in brown to tan with another darker brown outline before fading back to brown and then to cream laterally; ventral surfaces semi-translucent cream or white (Figure 3.17).

Distribution. *Orconectes sanbornii* can commonly be found in streams throughout the Middle Ohio River Drainage located within Ohio, Kentucky, and West Virginia (Loughman and Simon

2011; Taylor and Schuster 2004). *Orconectes sanbornii* occurs in all basins within the study area with the exception of the Tug Fork (Figure 3.18). In the Upper Guyandotte it appears *Orconectes cristivarius* outcompetes *O. sanbornii*, as *O. sanbornii* was only found at two locations within the drainage. While the two species appear very similar*, O. sanbornii* can be differentiated by the lack of a median carina on the rostrum while the carina is present on *O. cristivarius*. As the Guyandotte River reaches lower elevations, populations of *O. cristavarius* appear to gradually fade and give way to *O. sanbornii*. *Orconectes sanbornii* is found at only a single site in the upper reaches of Fourpole Creek. Jezerinac et al. (1995) collected *O. sanbornii* during their survey in the basin; however, it has since been further displaced through competitive exclusion by *Orconectes rusticus* (Loughman and Simon, 2011).

Morphometrics. *Orconectes sanbornii* is a small-moderate sized crayfish. Mean TCL was 17.5 mm (n= 1112, SE=.22 mm). The largest individual was a form I male with a TCL of 44.0 mm collected from Millstone Creek, Mason County. The largest form II male had a TCL of 36.3 mm and was collected from Buffalo Creek, Wayne County. The largest female had a TCL of 38.5 mm and was collected from Beech Fork, Wayne County. Morphometrics data for *O. sanbornii* is presented in Table 3.7.

Habitat and natural history. *Orconectes sanbornii* inhabits small- to large streams throughout the study area. Microhabitats include slab boulders, boulders, large cobble, leaf packs, submerged roots and logs, and any other object creating an interstitial space for the crayfish. Like Loughman and Simon (2011), we noted use of interstitial spaces throughout watersheds and a tendency to travel in the open often during daylight hours. Much like *O. cristavarius,* within smaller streams with rip- rap and high nutrient input (cow pasture runoff, straight pipes, etc.) very high numbers of *O. sanbornii* could be observed. Synchronized molting occurred during March and April and mating pairs were witnessed copulating mid-channel on top of small cobble during April of 2013 at Butler Adkins Branch in Wayne County. An ovigerous female was collected in May 2012 and another in April of 2013 at the same site. Both females were found secluded under slab boulders in pools. Crayfish associates in the study area include *C. b. cavatus, C. hatfieldi, C. theepiensis, C. veteranus, O. cristivarius, O. rusticus,* and *O. virilis*.

Conservation status within study area. *Orconectes sanbornii* appear to be stable within the study area and no further monitoring is warranted for the species at this time.

***Orconectes* (*Gremicambarus*) *virilis* (Hagen, 1870)** - Northern Crayfish

Cambarus virilis Hagen 1870.

Cambarus wisonsinensis Bundy 1876.

Cambarus debilis Bundy 1876.

Cambarus cousii Streets 1877.

Cambarus cousei Faxon 1885.

Cambarus wisconsiensis Harris 1900.

Cambarus cousei Harris 1903.

Cambarus viridis Moenkhaus 1904.

Cambarus (*Faxonius*) *virilis* Ortmann 1905b. Creaser 1931.

Faxonius virilis Creaser 1933a, 1962.

Faxonius (*Faxonius*) *virilis* Creaser 1933b

Orconectes virilis Hobbs 1942a, 1972, 1974.Fitzpatrick 1963. Page 1985. Pflieger 1987, 1996. Taylor et al. 1996. Taylor et al. 2007.

Orconectes (Orconectes) virilis Hobbs 1942b.

Orconectes (Gremicambarus) virilis Fitzpatrick 1987. Hobbs and Jass 1988. Hobbs 1989. Jezerinac et al. 1995.Loughman 2010. Loughman and Simon 2011. Loughman and Welsh 2013.

Diagnsosis.

Rostrum with straight margins, not thickened or possessing spines or tubercles; median carina absent; postorbital ridges terminating cephalically with spine or tubercle. Branchiostegal spine reduced; hepatic spine absent. Cephalothorax oval shaped and slightly dorsoventrally flattened in profile; without setae; suborbital angle obsolete. Areola 7.1–19.0 times longer than wide, comprising 34–39% of TCL, with 1–2 rows of punctations across narrowest region. Chelae smooth, broad and robust; mesial surface of palm with two rows of defined tubercles; first row with 6–8 rounded tubercles; second with 5–8 tubercles; lateral margin of propodus smooth; dorsal surfaces of both dactyl and fixed finger of propodus with prominent well developed longitudinal ridges; elongate plumose setae at base of fixed finger of propodus. First form male gonopods long, comprising 42% of TCL, with 2 terminal elements, both bent and curving at about 30° to the base; central projection corneous, comprising 24% of gonopod length, cephalic base without shoulder. Form two male gonopod noncorneus, gently curving caudally; mesial process subequal in length to central projection, blunt. Female annulus ventralis rhomboid, fossa large, sulcus wide, cephalolateral prominences weak, sinus only evident on caudal surface.

Color in life. Carapace dorsally brown or olive fading to lighter tones ventrally before becoming outlined by a dark stripe along the ventral margin of the carapace; cephalic portion of carapace usually dark brown; rostral margins dark brown; chelae emerald green fading into dark green distally; dactyl and palmer tubercles cream colored to yellow or orange; perieopods mesially emerald lightening in coloration both proximally and distally; abdominal terga dorsally brown to olive; ventral surfaces semi- translucent cream or white (Figure 3.19).

Distribution. Originally native to the Midwest and Central Canada (Pflieger, 1996), *Orconectes virilis* has been widely introduced. Reasons for introduction include both aquaculture and the bait industry, due to its rapid proliferation and large size. Due to this, its range has rapidly expanded and it is now found throughout the United States with some populations completely displacing native species through competitive exclusion or hybridization (Loughman and Welsh, 2010). Jezarinac et al. (1995) noted that *O. virilis* was first recorded in the state in 1970 from the New River drainage and that populations were also found in the Kanawha and Potomac Rivers. *O. virilis* was only recorded from a single site within the study area located on Fourpole Creek within the Raccoon-Symmes drainage (Figure 3.20). In the time from Jezerinac's survey to the ongoing survey by Loughman and Welsh, *O. virilis* has become established almost across the state.

Morphometrics. *Orconectes virilis* is a large sized crayfish and is the largest invasive crayfish in both the study area and the state. No morphometric data for *Orconectes virilis* within the study area is available as its presence is only known from Fourpole Creek based off a single discarded chelae found in a deep pool. Loughman and Simon (2011) noted *O. virilis* morphometrics from Mason County, located immediately north of the study area. Mean TCL for their study was 42.8 mm (n=22, SE=6.11 mm) with the largest individual collected being a form I male with a TCL of 52.4 mm. The largest female collected had a TCL of 43.3 mm. Both individuals collected were from Krodel Park Lake in Mason County.

Habitat and natural history. *O. virilis* inhabits a wide variety of habitats but appears to prefer warm, slow moving waters. In its natural range, Pflieger (1996) noted that it occurs in streams, ponds, and sloughs with fertile, turbid water and utilizes a variety of cover objects from slabs to woody debris as well as occasional horizontal burrows into mud and clay banks. This appears to hold true in West Virginia as the species seems especially effective at proliferation when introduced to disturbed streams with woody litoral zones and clay or mud banks (Loughman et al., 2013). Although Pflieger (1996) stated that *O. virilis* does well in ponds with low numbers of predatory fish, *O. virilis* appears to thrive in ponds and reservoirs within WV despite high numbers of predatory fish. In fact, *O. virilis* found in these areas often reach enormous sizes of >6" TBL. Crayfish associates in the study area include *C. b. cavatus,* and *O. rusticus.*

Conservation status within study area. *Orconectes virilis* populations within the study area require monitoring. *O. virilis* has successfully caused dramatic declines and extirpation of several species of crayfish within the mid-Atlantic region (Jezarinac et al., 1995; Killian et al., 2010; Loughman and Welsh, 2010; Swecker et al., 2010; Loughman and Simon, 2011). *Orconectes virilis* was only noted within the lower reaches of Fourpole Creek within the study area and appears to be outcompeted within the drainage by another invasive, *Orconectes rusticus,* which has fully extirpated all native tertiary crayfish within the Fourpole drainage.

***Orconectes (Procericambarus) cristavarius* Taylor, 2000**- Spiny Stream Crayfish

Cambarus spinosus Bundy, 1877.

Cambarus (Faxonius) spinosus Ortmann, 1905.

Cambarus (faxonius) juvenilis Ortmann, 1931.

Orconectes spinosus Hobbs, 1944.

Orconectes (Procericambarus) spinosus Fitzpatrick, 1987.

Orconectes cristavarius Taylor and Schuster 2004.

Orconectes (Procericambarus) cristavarius Taylor, 2000. Loughman and Welsh 2013.

Diagnsosis. Rostrum with straight slightly converging margins; ditisnct spines; median carina present; postorbital ridges terminating cephalically with spine or tubercle. Branchiostegal spine reduced; hepatic spine absent. Cephalothorax oval shaped and slightly dorsoventrally flattened in profile; without setae; suborbital angle obsolete. Areola 6.0 - 8.0 times longer than wide, with 1-2 rows of punctations across narrowest region. Chelae smooth and elongate; mesial surface of palm with two rows of defined tubercles; first row with 7-11 rounded tubercles; second with 5-8 tubercles; lateral margin of propodus smooth; dorsal surfaces of both dactyl and fixed finger of propodus with prominent well developed longitudinal ridges. First form male gonopods long, comprising >40% of TCL, with 2 terminal elements, both bent and curving at about 30° to the base; cephalic base with shoulder. Form two male gonopod noncorneus, gently curving caudally; mesial process subequal in length to central projection, blunt. Female annulus ventralis rhomboid, fossa large, sulcus wide, cephalolateral prominences weak, sinus only evident on caudal surface.

Color in life. Carapace dorsally brown, tan, or olive speckled with small dark maculations and fading to lighter tones ventrally; brown to chestnut U- shaped saddle running anteriorly on carapace from insertion of abdomen; chelae brown, tan, or olive speckled with small dark maculations with cream colored dactyl and palmer tubercles; large tubercle at insertion point of dactyl orange to crimson; chelae tips orange to crimson outlined by a thin black band; perieopods mesially brown, tan or olive fading to cream coloration both proximally and distally; abdominal terga dorsally brown, tan, or olive occasionally speckled with small dark maculations; terga outlined in orange to crimson; first abdominal terga usually contains a dark dorsal bar; ventral surfaces semi- translucent cream or white (Figure 3.21)

Distribution. *Orconectes cristavarius* is noted by Taylor and Schuster (2004) as being a fairly wide ranging species occurring within Eastern Kentucky into Southern West Virginia and south into Western Virginia and North Carolina. Within the study area it is found within the Big Sandy, Tug, Twelve Pole, Upper Guyandotte, and Lower Guyandotte drainages (Figure 3.22).

Morphometrics. *Orconectes cristivarius* is a small-moderate sized crayfish. Mean TCL was 19.3 mm (n=653, SE=.29mm). The largest individual was a female with a TCL of 35.7 mm collected from Briar Creek, Wyoming County. The largest form I male had a TCL of 35.3 mm and was also collected from Briar Creek. The largest from II male had a TCL of 34.0 mm and was collected from Elklick Branch, Logan County. Morphometric data for *O. cristivarius* is presented in Table 3.8.

Habitat and natural history. Taylor and Schuster (2004) stated that *O. cristavarius* occurs in all stream types within its range except for large rivers. Like Taylor and Schuster, our collections also indicated *O. cristavarius* appears to prefer moderate stream orders, particularly those with large amounts of gravel, cobble, and boulders. Leaf packs, rip-rap, root masses, and submerged logs also served as habitat for *O. cristavarius*. Essentially, any habitat that offered high habitat heterogeneity with an abundance of small interstitial spaces seemed to yield higher numbers of *O. cristavarius*. We were able to collect *O. cristavarius* from large mainstems; however, their numbers were lower than numbers of *O. cristavarius* collected in smaller streams and the species usually only occurred within the shallow peripheries in larger streams. *Orconectes cristavarius* along with *O. sanbornii* appear better suited for certain anthropogenic effects than some of their less tolerant cambarid associates. Within the study area, smaller streams with rip-rap and high nutrient input (cow pasture runoff, straight pipes, etc.) held very high numbers of *O. cristavarius* or *O. sanbornii* depending on the drainage. *Orconectes cristivarius* numbers were so prolific at some sites that walking to the stream would disturb hundreds of individuals. Crayfish associates in the study area include *C. b. cavatus, C. hatfieldii, C. theepiensis, C. veteranus, O. rusticus,* and *O. sanbornii*.

Conservation status within study area. *Orconectes cristavarius* appear to be stable within the study area and no further monitoring is warranted for the species at this time.

***Orconectes* (*Procericambarus*) *rusticus* Girard, 1852**– Rusty Crayfish

Cambarus rusticus Girard 1852.Faxon 1885.

Cambarus juvenilis Hagen 1870.

Cambarus (*Faxonius*) *rusticus* Ortmann 1905b.

Faxonius rusticus Williamson 1907. Creaser 1933a.

Cambarus (*Faxonius*) *rusticus rusticus* Ortmann 1931.

Cambarus (*Faxonius*) *juvenilis* Ortmann 1931:84.

Faxonius (*Faxonius*) *rusticus rusticus* Creaser 1933b.

Orconectes rusticus rusticus Hobbs 1942a. Fitzpatrick 1963.

Orconectes (*Orconectes*) *juvenilis* Hobbs 1942b.

Orconectes rusticus Pennak 1953. Hobbs 1972. Page 1985. Taylor et al. 1996. Taylor and Schuster 2007. Taylor et al. 2007.

Procambarus rusticus Huner 1978.

Orconectes (*Procericambarus*) *rusticus*. Fitzpatrick 1987. Hobbs and Jass 1988. Hobbs 1989. Jezerinac et al. 1995. Taylor 2000. Taylor and Schuster 2004. Loughman and Simon 2011. Loughman and Welsh 2013.

Diagnsosis. Rostrum with concave margins, not thickened, with spines or tubercles; median carina absent; mandible with smooth cutting edge. Cephalothorax oval, slightly dorsoventrally compressed in profile, Areola 4.6-19.4 times longer than wide, comprising 36-39% of TCL, with 2-3 rows of punctations across narrowest region; branchiostegal spine poorly developed; suborbital angle obsolete or poorly developed. Chelae robust; mesial surface of palm with two rows of defined tubercles, first row with 5-9 tubercles; second row with 4-9 tubercles tubercles of smaller diameter. First form male gonopods long, comprising 26% of TCL, with 2 straight terminal elements; central projection comprising 56% of gonopod length; well-developed shoulder at cephalic base of central projection. Second form male gonopod non-corneous, straight, mesial process slightly subequal in length to central projection, blunt, shoulder not evident. Annulus ventralis rhomboid in shape, fossa moderately large, cephlolateral prominences well developed, trough narrow, sinus evident on caudal surface.

Color in life. Carapace dorsally beige, brown, or olive fading to lighter tones ventrally; large rust colored patch on posterior lateral portion of carapace; chelae variable ranging from brown, green, grey, purple, and light blue; cream colored dactyl and palmer tubercles; distal tips of chelae orange to crimson followed proximally by a black band; perieopods mesially brown or olive fading to light brown or teal coloration both proximally and distally; abdominal terga dorsally beige or brown; ventral surfaces semi- translucent cream or white (Figure 3.23).

Distribution. *Orconectes rusticus* is described by Taylor (2000) as being native to the lower middle Ohio River drainage in central Kentucky ranging up through western Ohio, and into central Indiana and southeastern Michigan (Taylor and Schuster 2004; Loughman and Simon, 2011). Due to bait bucket introductions, it is now established across the east coast, particularly in the New England States, and populations within the Ohio River have expanded into Pennsylvania and West Virginia, while populations have also steadily expanded into the Midwest. Lawton (1979) first noted *O. rusticus* from Fourpole Creek and Jezerinac et al. (1995) noted introductions into Twelvepole through way of Beech Fork Reservoir (Figure 3.24).

Currently, *O. rusticus* has remained in these basins and has yet to be documented in other basins within the study area although its range has expanded throughout the state of WV since Jezarinac's survey (Loughman and Welsh, 2013).

Morphometrics. *Orconectes rusticus* is a small-moderate sized crayfish. Mean TCL was 17.4mm (n=29, SE=1.69mm). The largest individual was a form I male with a TCL of 40.7 mm collected from Fourpole Creek in Cabell County. The largest form II male had a TCL of 15.4 mm and was also collected from Fourpole Creek. The largest female had a TCL of 33.2 mm and was also collected from Fourpole Creek. Morphometric data for *O. rusticus* is presented in Table 3.9.

Habitat and natural history. Within its native range, *O. rusticus* was noted in all types of streams, rivers, and lakes while utilizing a wide variety of microhabitats (Taylor and Schuster 2004); however, within the study area, *O. rusticus* appears to be confined to areas of low flow with disturbed or altered habitat, often coupled with introduced cover objects (rip-rap) and mud or silt bottoms. *Orconectes rusticus* within the study area appear to only be able to hold these extremely disturbed areas, and was not found at Left Fork Miller's Creek or Butler Adkins Branch despite both streams feeding directly into Beech Fork reservoir where high numbers of *O. rusticus* have been noted along the shoreline. While it has displaced native crayfish populations within Fourpole Creek (a highly altered stream) it appears unable to move into tributaries with higher flow and more natural habitat within both the Fourpole and Twelvepole drainage. Further investigation into what is acting as a barrier to these crayfish is warranted due to *O. rusticus'* invasive nature and disastrous effects it has had and continues to pose on other native populations of crayfish. Crayfish associates in the study area include *C. b. cavatus, C. theepiensis, O. rusticus, O. sanbornii,* and *O. virilis*.

Conservation status within study area. *Orconectes rusticus* populations within the study area warrant further monitoring as it is a known highly destructive invasive species of crayfish (Jezerinac et al., 1995; Taylor and Shuster, 2004; Loughman and Simon, 2011). It has completely extirpated all native tertiary crayfish from the lower reaches of Fourpole Creek and now shares the creek with *Orconectes virilis*, another invasive crayfish. It also is common within the Twelvepole watershed, particularly in areas of low flow such as Beech Fork Lake. It does however, appear to be unable to hold populations within the headwaters of both Fourpole and Twelvepole. Reasons for this could be inability to deal with severe drawdown noted within these creeks during periods of drought or inability to cope with high flow gradient during periods of flow.

Discussion

HUC8 Watershed Faunas

Six basins compose West Virginia's Southwestern Coalfields region (Figure 3.1). From north to south they are the Raccoon- Symmes, Lower Guyandotte, Twelvepole, Big Sandy, Tug Fork, and Upper Guyandotte. As mentioned in the introduction, Fourpole Creek was the only stream within the Raccoon-Symmes drainage treated in this survey due to its unique inclusion within the geographic area in relation to the rest of the drainage. Each basin is referred to as an 8-digit watershed (HUC) in geographic mapping terms by federal agencies. A synopsis of each basin's epigean and burrowing crayfish fauna is provided along with current conservation rankings (Figure 3.10).

Fourpole

Five crayfish species were encountered within Fourpole Creek (Table 3.10). *Cambarus b. cavatus* is a secondary burrower and occurs primarily as a burrowing species in the mid reaches of Fourpole Creek; however, it can be found as lotic taxa in the upper reaches of Fourpole. Interspecific competition with *Orconectes rusticus*, an invasive found throughout the mid and lower reaches of the stream appear to prevent it from utilizing stream habitats as it does in the upper reaches where *O. rusticus* is not found. *Orconectes rusticus* flourishes in the mid and lower reaches of Fourpole where it has apparently displaced all native tertiary burrowing species that previously occurred in the lower reaches of the drainage. *Orconectes virilis* is known in the watershed from a single excised chelae found in a deeper pool near Ritter Park, Huntington, WV. Without further targeted sampling, it is impossible to say whether an established population of *O. virilis* occurs in the drainage or if the chelae belonged to a sole surviving remnant of a bait bucket introduction.

Orconectes sanbornii persists at a single location in the upper headwaters of Fourpole. It has been completely displaced from the lower reaches of the drainage by *Orconectes rusticus*.

Cambarus dubius is found along wetted seeps and hillsides throughout the basin and can be readily encountered during night hikes through Ritter Park in Huntington, Cabell County, West Virginia.

Lower Guyandotte

Six native species of crayfish comprise the fauna of the Lower Guyandotte drainage (Table 3.10). Both *Cambarus dubius* and *Cambarus thomai*, primary burrowing species, can be found sparsely throughout the basin. *Cambarus thomai* appears to prefer the floodplains of the Lower Guyandotte along with wetted ditches. *Cambarus dubius* utilizes the same habitat within the basin but can also be found in seeps, and usually further from a flowing source of water than *C. thomai*. *Cambarus b. cavatus* is the only secondary burrower found within the basin and does not appear to have any interspecific competition with the primary burrowers, *C. dubius*, and *C. thomai*; however, it does appear to compete with *C. theepiensis*, particularly in streams > 2^{nd} order where *C. theepiensis* appears to maintain cover mid-channel while *C. b. cavatus* is usually found near or in the banks of streams. Much like in the headwaters of Fourpole Creek*, C. b. cavatus* is found throughout the stream in streams < 3^{rd} order.

Orconectes sanbornii and *O. cristavarius* both occur within the Lower Guyandotte. Despite similar appearance, and overlapping niches within the drainage, *O. sanbornii* appears competitively exclude *O. cristavarius* as *O. cristavarius* was only collected from three sites in the upper portion of the basin while *O. sanbornii* was prolific throughout lower portions of the drainage. Both species exhibit interspecific competition with *C. theepiensis*; however, it does not appear to competitively exclude any of the species from habitat within the creek. Different microhabitat utilization and ecological functions within streams of this basin may explain this.

Twelvepole

Seven species of crayfish occur within Twelvepole (Table 3.10). *Orconectes rusticus* is prolific throughout the area of Beech Fork Lake and low gradient tributaries feeding into its backwaters; however, the species appears to be confined to the lake and unable to spread as no new localities for the species within the drainage have been found since Jezarinac et al. (1995) noted its presence within the lake proper. Reasons for such a well-documented invasive inability to spread throughout the drainage remain unclear but high gradient flows coupled with severe draw down in many of Twelvepole's headwaters may play a part.

Cambarus thomai and *Cambarus dubius* are the only two species of primary burrowers to occur within the Twelvepole drainage. Unlike their slightly varied habitat preferences within the Lower Guyandotte drainage, both species utilize the same habitat within this drainage, often with interspecific burrow colonies. *Cambarus b. cavatus* is again present and displays interspecific competition with *Cambarus theepiensis*, utilizing burrows and riparian habitats in streams > 2nd order while utilizing the stream proper in streams <3rd order.

Cambarus theepiensis, O*rconectes sanbornii,* and *Orconectes cristavarius* were the only native tertiary burrowing species present within the drainage. Despite these species utilizing all habitats within the streams of the basin, very different microhabitat preferences were noticed. Both *Orconectes sanbornii* and *Orconectes cristavarius* appeared to favor gravel/cobble habitat, riparian habitats, and woody littoral zones while *C. theepiensis* showed a strong preference for boulders and large slab boulders. *O. cristavarius* was collected at only a single site from Twelvepole while *O. sanbornii* is found throughout the drainage. Ovigerous female *C.*

theepiensis with in the basin also appeared to show a strong preference for bank habitat, often utilizing burrows created under large slab boulders within the banks.

Big Sandy

Five native species of crayfish occur within the Big Sandy Drainage (Table 3.10). *Cambarus thomai* is the only primary burrowing species located within the basin and was found in a single bottomland forest near the confluence with the Ohio River. Both *O. sanbornii* and *O. cristavarius* were found within the basin, but unlike the Lower Guyandotte, relative abundance of each species is reversed with *O. sanbornii* known from a single site in lower reaches of the basin while *O. cristavarius* is prolific throughout. Reasons for the inverse proliferations of these species within the two drainages remains a mystery, as both basins have seemingly identical habitat. C*ambarus theepiensis* and *C. b. cavatus* also occur within the basin and maintain the same interspecific competition mentioned in the previous drainage discussions.

Tug Fork

Five native species of crayfish were documented within the Tug Fork drainage (Table 3.10). *Cambarus dubius* was the only reported species of primary burrowing crayfish and appeared to prefer riparian forests in the drainage. Both *C. theepiensis* and *C. hatfieldi* were present within the Tug Fork, with *C. theepiensis* confined to the lower reaches while *C. hatfieldi* was prolific throughout the upper portion of the system. *Cambarus hatfieldi* appears to completely displace *C. b. cavatus* within the Tug Fork as *C. hatfieldi* was noted to occupy headwater streams throughout the drainage, while occupying riparian and bank habitats along the mainstem, (D. A. Foltz II, personal observation) a niche *C. b. cavatus* occupies in all other drainages throughout the study area. *Orconectes cristavarius* was present throughout the basin, but appeared to reach higher densities in the upper reaches of the basin. Clear, slow flowing runs with cobble habitat appeared to be the preferred microhabitat for the species within the drainage.

Cambarus veteranus was documented within the drainage during surveying efforts despite no known occurrence of the species within the drainage during prior surveys. This was most likely due to inability to sample the deeper sections of the large main stem of the Tug as *C. veteranus* were collected under large slabs during the spring months with high flow but appeared to retreat to the deeper sections of the streams during low flow (Z. J. Loughman and D. A. Foltz II, personal observation). Interspecific competition between *C. veteranus* and *C. hatfieldi* could occur as both appear to prefer large slab boulders as cover objects within the streams they inhabit; however, along the mainstem, *C. hatfieldi* appears displaced to riparian and bank habitats while *C. veteranus* appears to dominate large slab boulder habitat found within deeper reaches of the channel (D. A. Foltz II, personal observation).

Upper Guyandotte

The Upper Guyandotte produced seven species of crayfish during survey efforts within the basin (Table 3.10). *Cambarus thomai* and *C. dubius* both occur within the basin. *Cambarus thomai* was found at only one location. *Cambarus dubius* appears to favor riparian forests within this basin and the Halloween morph replaces the Kanawha morph found in previously mentioned basins within the study region. *Orconectes cristavarius* was present throughout the basin and appeared to prefer clear water streams over cobble substrates mixed with boulders, although it could be found in most habitats throughout the streams within the basin. *Orconectes sanbornii* also appeared to favor the same habitat as *O. cristavarius*; however, *O sanbornii* was only collected from two sites within the basin and appears displaced by *O. cristavarius.*

Cambarus b. cavatus and *C. theepiensis* also occur within the Upper Guyandotte and again appear to maintain an interspecific competition dynamic confining *C. b. cavatus* to bank and burrow habitats in streams > 2nd order while streams < 3rd order contain *C. b. cavatus* throughout. *Cambarus veteranus*, despite previous historic records throughout the basin, was found only at Pinnacle Creek, a known historic location. At the site, *C. veteranus* was only encountered under the largest slab boulders within the fastest flowing sections of the stream. Destruction and alteration of habitat at all other historic locations appears to have severely limited the species distribution throughout the drainage (Loughman and Welsh, 2013).

Conservation concerns for West Virginia's Southwestern Coalfields crayfish populations

Several potential sources of imperilment exist for native crayfish species within West Virginia's Southwestern Coalfields. Although invasive species are present within Twelvepole and Fourpole, two lower basins with Ohio River confluences, emigration from these areas seems unlikely as no new populations of *O. rusticus* have been documented since Jezarinac et al. (1995). These areas due continue to pose a threat as possible source location for bait bucket introductions as fishing, particularly around Beech Fork Lake, is very popular within the area. Established invasive populations just outside of the study area also pose threats as source populations. Krodel Park Lake, Point Pleasant, Mason County, West Virginia lies just north of the study area and Loughman and Simon (2011) expressed concern that the population of *O. virilis* within the lake would serve as a source population for potential future invasions as flooding of the lake has already carried *O. virilis* into nearby wetlands.

Habitat destruction and alterations throughout the study area also pose an ever present issue to crayfish populations, particularly those more sensitive to disturbance (*Cambarus veteranus)*. The geographic region of the study area has undergone years of anthropogenic impacts ranging from small scale land use to heavy logging and mining operations. Areas of the Upper Guyandotte where *C. veteranus* was originally established have undergone drastic changes since the time of Jezarinac's study alone. Many of the streams *C. veteranus* was originally collected from were noted to contain large slab boulders and fast flowing riffles. Most of these streams are now devoid of slabs and boulders. In some cases, these streams lack anything more than cobble as stream channelization and "recreational bulldozing" have completely altered stream morphology and biological integrity (Loughman and Welsh, 2013). Pinnacle creek remains as the lone bastion for *C. veteranus* within the Upper Guyandotte. While Pinnacle creek still contains large slabs and fast flowing riffles; however, populations of *C. veteranus* within are very low. Possible reasons for decline within the stream are extractive industry and adverse water quality as coal fines and increased sedimentation were present throughout the reach of Pinnacle creek surveyed. Similar threats to *C. veteranus* exist within Tug Fork; however, populations of *C. veteranus* within the basin appear slightly higher than those of Pinnacle Creek. Possible reasons for this despite similar land use and water quality issues could be the added width and depth offered within the Tug Fork, providing *C. veteranus* with fast flowing water and large cover objects with decreased sedimentation even during summer draw down.

Alteration of habitat occurs throughout the study area on variable scale. Other epigean species within the area seem better suited to toleration than *C. veteranus*, as *Cambarus theepiensis* and *Cambarus hatfieldi* can be found in adverse conditions, although usually in much lower numbers. Some species such as those of *Orconectes sanbornii* and *Orconectes cristivarius* even appear to flourish in some disturbed habitats, particularly those with heightened nutrient inputs such as straight piping or agricultural runoff. Both species of *Orconectes* were observed in abnormally high densities in streams containing these sources of nutrient input (D. A. Foltz and Z. J. Loughman, personal observation).

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Appendix: Chapter III

Chapter III: Crayfishes of the Coalfields tables

Table 3.1: Seasonal data for West Virginia's Southwestern Coalfields *Cambarus* species

Species	J	F	M	A	M	Jn	J	A	S	O	N	D
Cambarus b. cavatus												
Male 1				x		x	x		x	x	x	
Male 2			x	x	x	x	x		x	x		
Female			x	x	x	x	x		x	x		
Ovigerous Female							x	x				
Cambarus dubius												
Male 1					x	x	x	x	x	x		
Male 2				x	x	x	x	x	x	x		
Female				x	x	x	x	x	x	x		
Ovigerous Female					x	x	x					
Cambarus hatfieldi												
Male 1			x	x		x	x	x	x			
Male 2			x	x		x	x	x	x	x		
Female			x	x		x	x	x	x	x		
Ovigerous Female							x	x				
Cambarus theepiensis												
Male 1	x	x	x	x	x	x	x	x	x	x	x	x
Male 2	x	x	x	x	x	x	x	x	x	x	x	x
Female	x	x	x	x	x	x	x	x	x	x	x	x
Ovigerous Female						x	x					
Cambarus thomai												
Male 1				x	x	x	x	x	x	x		
Male 2			x	x	x	x	x	x	x	x		
Female			x	x	x	x	x	x	x	x		
Ovigerous Female			x	x								
Cambarus veteranus												
Male 1				x			x	x		x		
Male 2						x	x	x		x		
Female						x	x	x	x	x		
Ovigerous Female												

Table 3.2: West Virginia's Southwestern Coalfields *Cambarus bartonii cavatus* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	2	38.7	40.3	39.5	1.1
Male II					
Carapace length	79	5.2	39	15.4	9.3
Female					
Carapace length	237	4.9	47.8	12.6	8.4

Table 3.3: West Virginia's Southwestern Coalfields *Cambarus hatfieldi* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	8	28.8	39.5	33.7	3.4
Male II					
Carapace length	145	12.8	39.3	26.8	6.0
Female					
Carapace length	191	8.6	42.6	22.6	8.1

Table 3.4: West Virginia's Southwestern Coalfields *Cambarus theepiensis* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	53	30.5	50.6	40.5	4.7
Male II					
Carapace length	484	5.2	52.5	25.07	10.1
Female					
Carapace length	742	4.8	52.4	22.0	11.9

Table 3.5: West Virginia's Southwestern Coalfields *Cambarus veteranus* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	1	38.7	38.7	38.7	n/a
Male II					
Carapace length	1	34.9	34.9	34.9	n/a
Female					
Carapace length	4	20.0	38.9	32.5	8.6

Table 3.6: Seasonal data for West Virginia's Southwestern Coalfields *Orconectes* species

Species	J	F	M	A	M	Jn	J	A	S	O	N	D
Orconectes cristavarius												
Male 1				x	x		x	x	x	x		
Male 2					x	x	x	x	x	x		
Female				x		x	x	x	x	x		
Ovigerous Female				x		x						
Orconectes rusticus												
Male 1				x					x			
Male 2				x	x	x	x	x	x	x		
Female			x	x	x	x	x	x	x	x		
Ovigerous Female												
Orconectes sanbornii												
Male 1	x	x	x	x	x	x	x	x	x	x	x	
Male 2	x	x	x	x	x	x	x	x	x	x	x	x
Female	x	x	x	x	x	x	x	x	x	x	x	x
Ovigerous Female				x	x							
Orconectes virilis												
Male 1				x			x	x	x			
Male 2		x					x	x	x			
Female		x		x			x	x	x			
Ovigerous Female				x								

Table 3.7: West Virginia's Southwestern Coalfields *Orconectes sanbornii* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	133	17.1	44	28.0	4.5
Male II					
Carapace length	391	4.4	36.3	15.0	5.4
Female					
Carapace length	588	4.1	38.5	16.8	7.3

Table 3.8: West Virginia's Southwestern Coalfields *Orconectes cristavarius* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	9	24.9	35.3	30.7	4.2
Male II					
Carapace length	303	7	34	19.0	7.1
Female					
Carapace length	341	7.7	35.7	19.4	7.5

Table 3.9: West Virginia's Southwestern Coalfields *Orconectes rusticus* measurements

Sex	N	Minimum	Maximum	Mean	Standard Deviation
Male I					
Carapace length	3	32.9	40.7	36.0	4.1
Male II					
Carapace length	10	10.6	15.4	13.7	1.5
Female					
Carapace length	18	9.5	33.2	16.0	7.9

Table 3.10: Major watershed distribution and global/state conservation rankings of West Virginia's Southwestern Coalfields crayfish species. BS = Big Sandy River, FP = Fourpole Creek, LG = Lower Guyandotte, TF = Tug Fork, TP = Twelvepole, and UG = Upper Guyandotte. Single asterisk denotes primary burrowing species. Double asterisk denotes invasive species.

Crayfish Species	**BS**	**FP**	**TF**	**TP**	**LG**	**UG**	**Gl**	**St**
Cambarus (C.) b. cavatus Hay, 1902 (Appalachian Brook Crayfish)	x	x		x	x	x	G5	S4
Cambarus (C.) hatfieldi Loughman et al., *in press* (Tug Valley Crayfish)			x				U	S2
Cambarus (Jugicambarus) dubius Faxon, 1884 (Upland Burrowing Crayfish)*		x	x	x	x	x	G5	S4
Cambarus (Puncticambarus) theepiensis Loughman et. al, 2013 (Coalfields Crayfish)	x		x	x	x	x	G3	S3
Cambarus (P.) veteranus Faxon, 1914 (Big Sandy Crayfish)			x			x	G3	S1
Cambarus (Tubericambarus) thomai Jezarinac, 1993 (Little Brown Mudbug)*	x			x	x	x	G5	S4
Orconectes (Crockerinus) sanbornii (Faxon, 1884) (Sanborn's Crayfish)	x	x		x	x	x	G5	S4
Orconectes (Gremicambarus) virilis Hagen, 1870 (Virile Crayfish)**		x					G5	I
Orconectes (Procericambarus) cristavarius Taylor , 2000(Spiny Stream Crayfish)	x		x	x	x	x	G5	S4
Orconectes (P.) rusticus (Girard, 1852) (Rusty Crayfish)**		x		x			G5	I

Chapter III: Crayfishes of the Coalfields figures

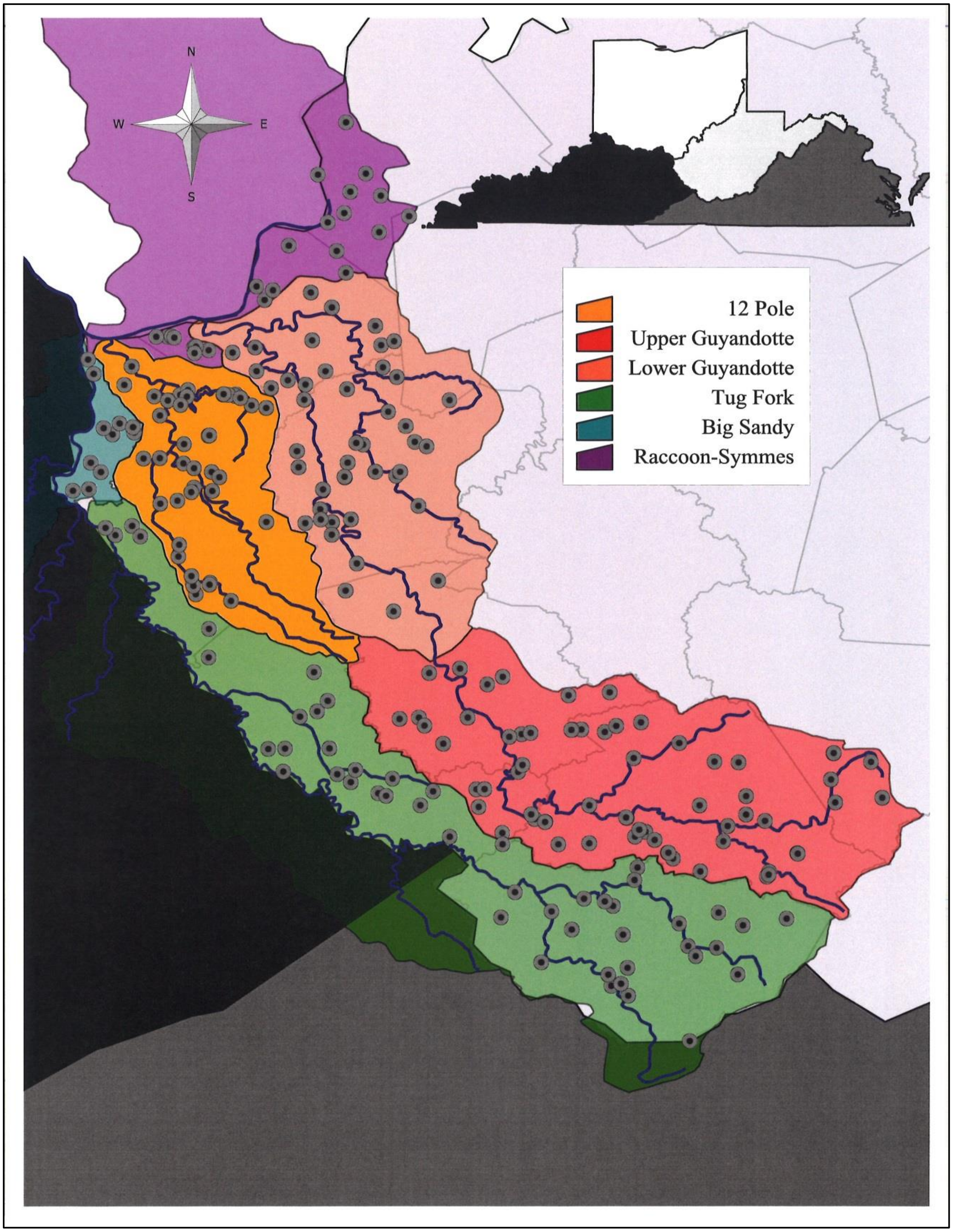

Figure 3.1: Map depicting drainage basin extent and sampling events for drainages located within West Virginia's Southwestern Coalfields.

Figure 3.2: Photo of Panther Creek, McDowell County (Tug Fork basin) a high gradient stream with intact riparian buffers and suitable habitat such as slabs, boulders, and large cobbles.

Figure 3.3: *Cambarus bartonii cavatus*. Taken from Loughman and Simon 2011.

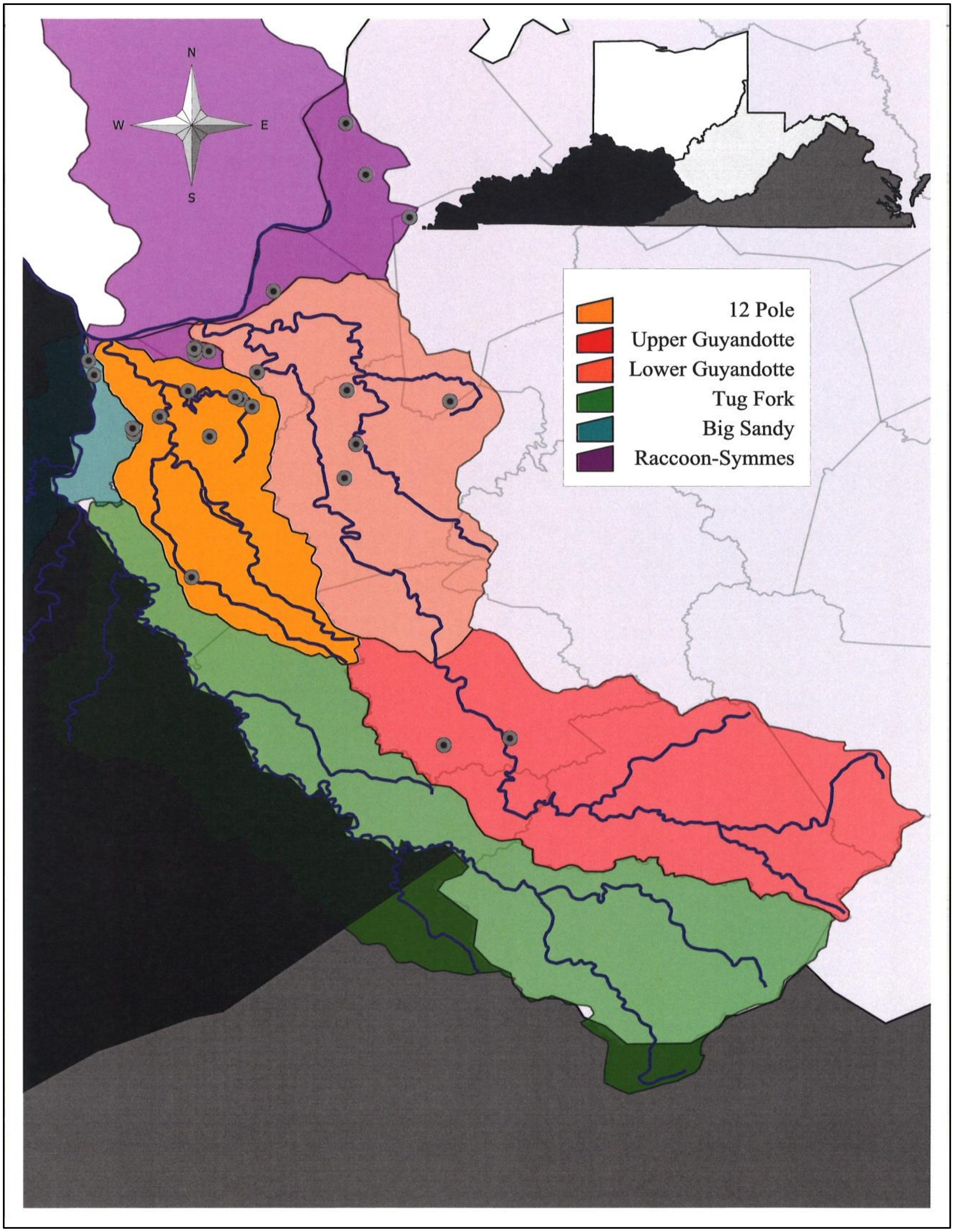

Figure 3.4: Distribution of *Cambarus bartonii cavatus* within West Virginia's Southwestern Coalfields.

Figure 3.5: *Cambarus hatfieldi* from Tug Fork mainstem, McDowell County (Tug Fork Basin).

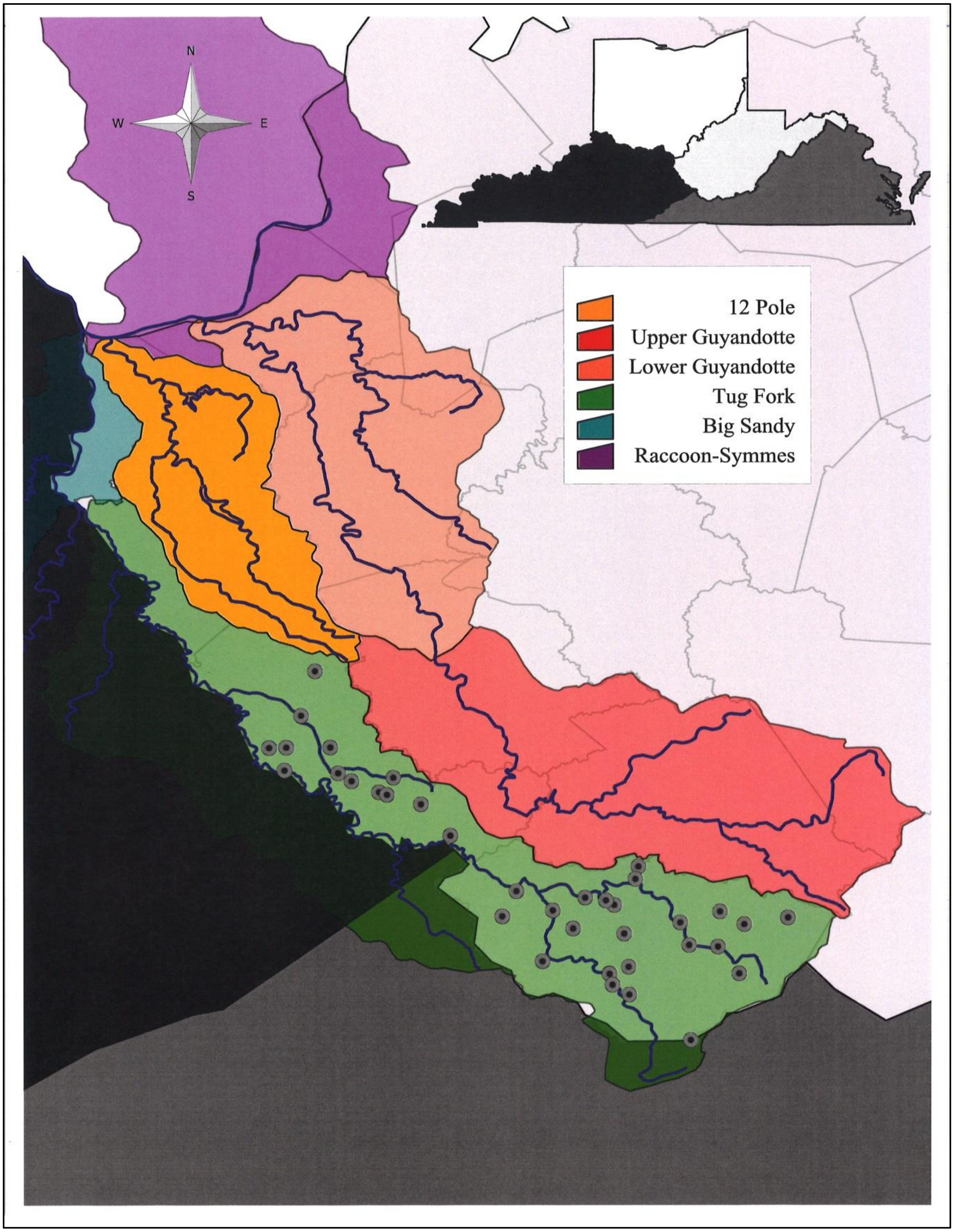

Figure 3.6: Distribution of *Cambarus hatfieldi* within West Virginia's Southwestern Coalfields.

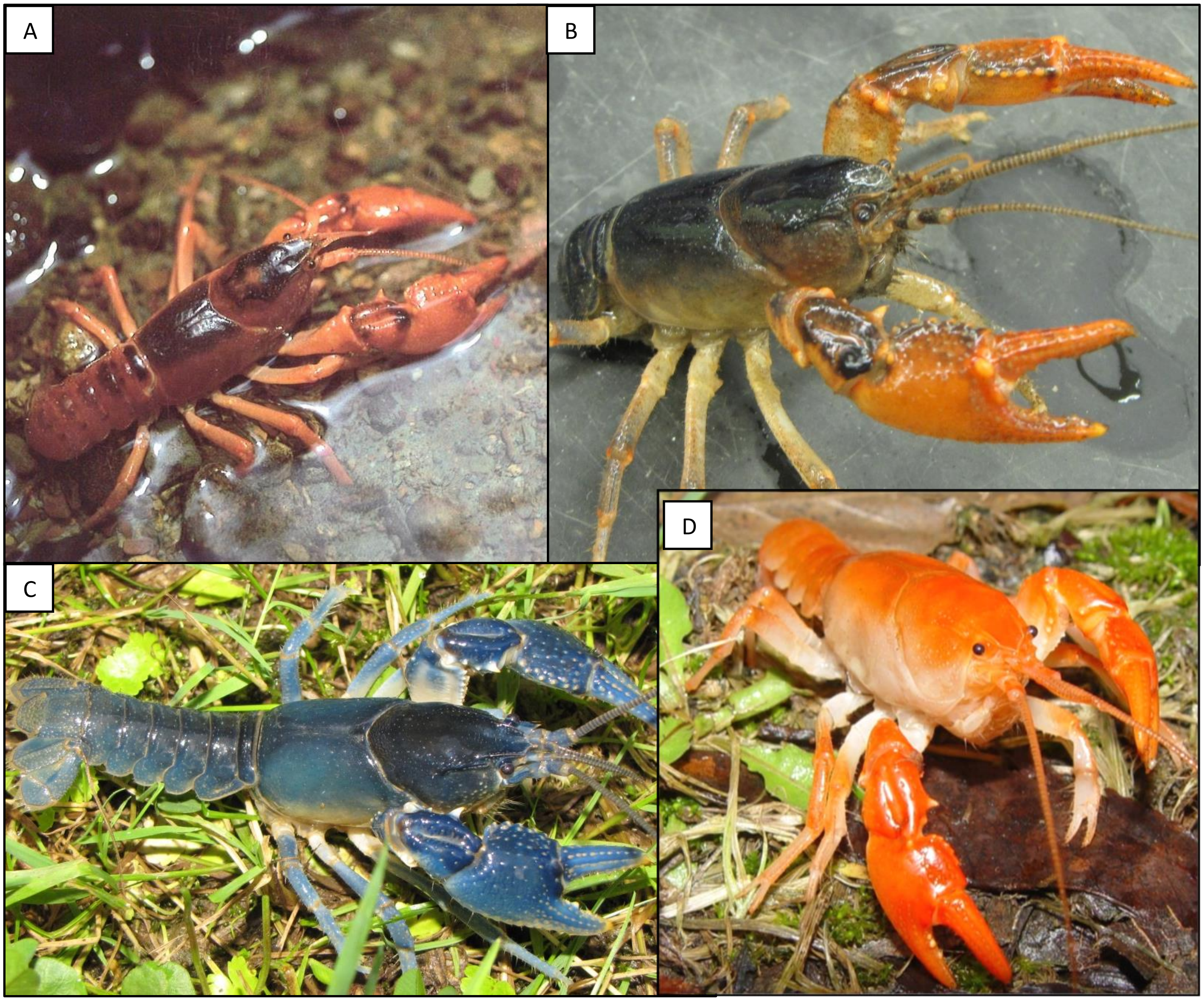

Figure 3.7: Morphs of *Cambarus dubius* found within WV. A. Halloween morph (taken from Jezarinac et al. 1995). B. Kanawha morph. C. Blue morph D. Orange morph. Both the halloween morph and the Kanawha morph are found within the study area while the blue morph can be collected just north of the study area. The Orange morph is found within northeastern West Virginia.

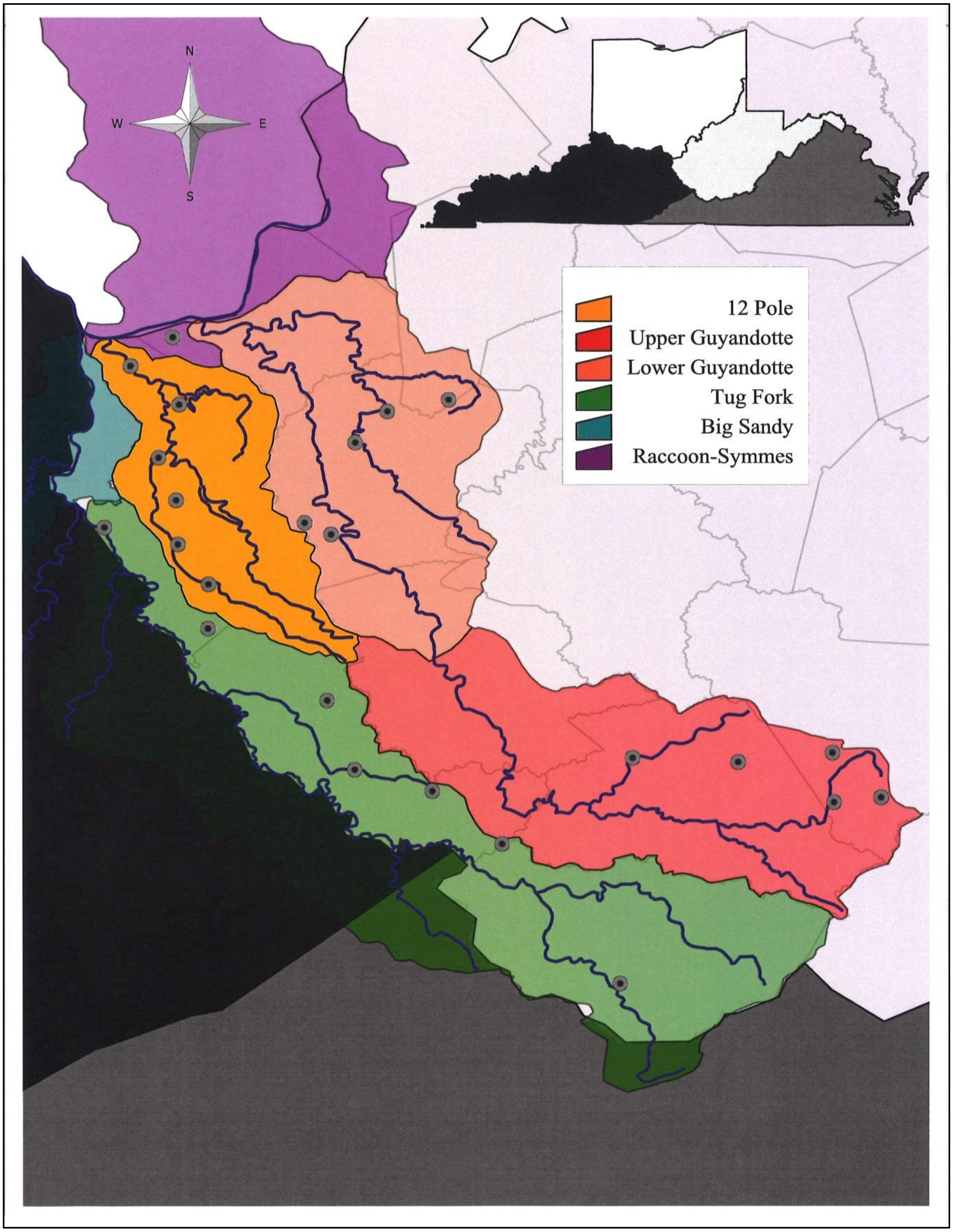

Figure 3.8: Distribution of *Cambarus dubius* within West Virginia's Southwestern Coalfields.

Figure 3.9: *Cambarus theepiensis* from Right Fork Miller's Creek, Wayne County (Twelvepole basin).

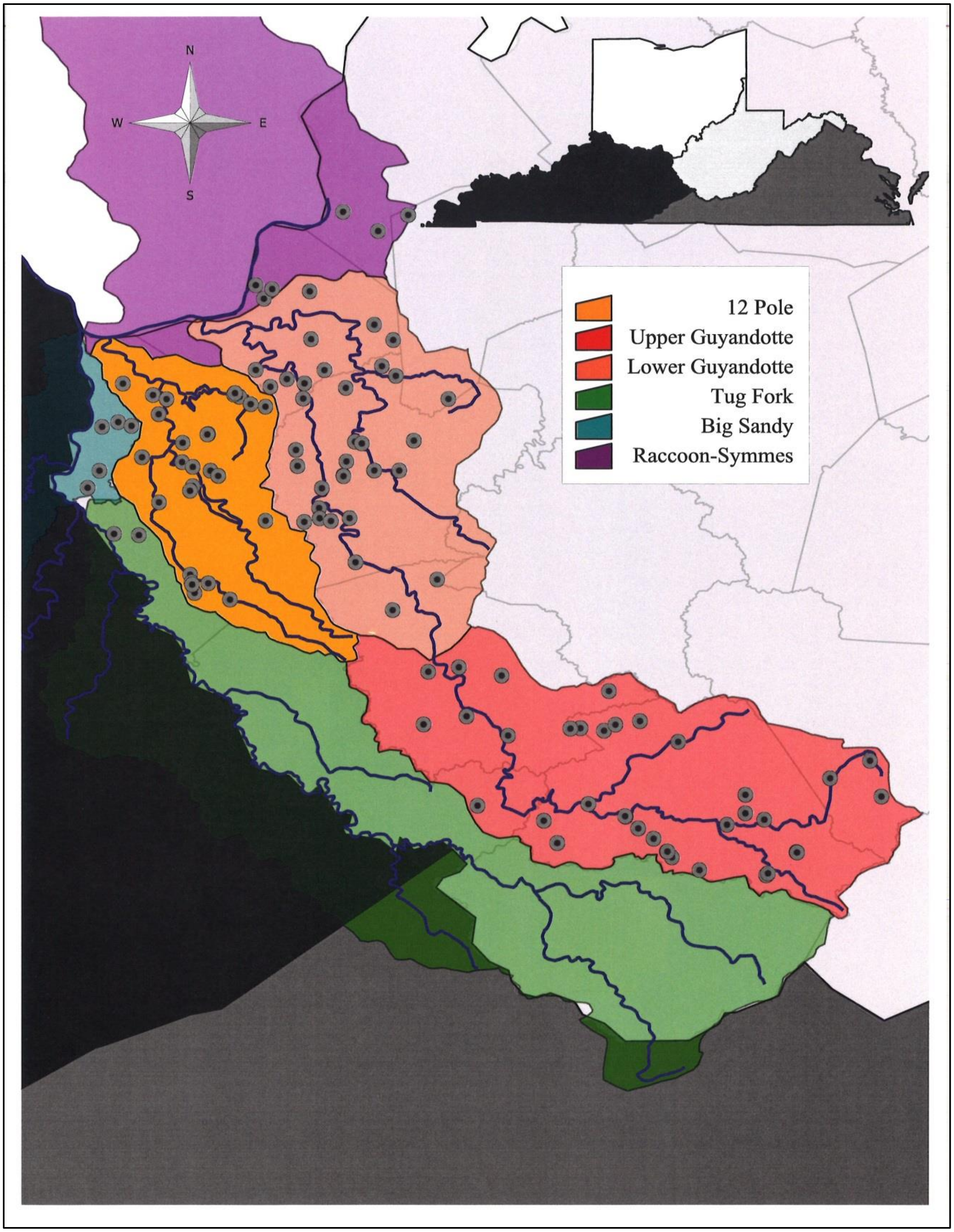

Figure 3.10: Distribution of *Cambarus theepiensis* within West Virginia's Southwestern Coalfields.

Figure 3.11: *Cambarus veteranus* from Tug Fork mainstem, McDowell County (Tug Fork basin).

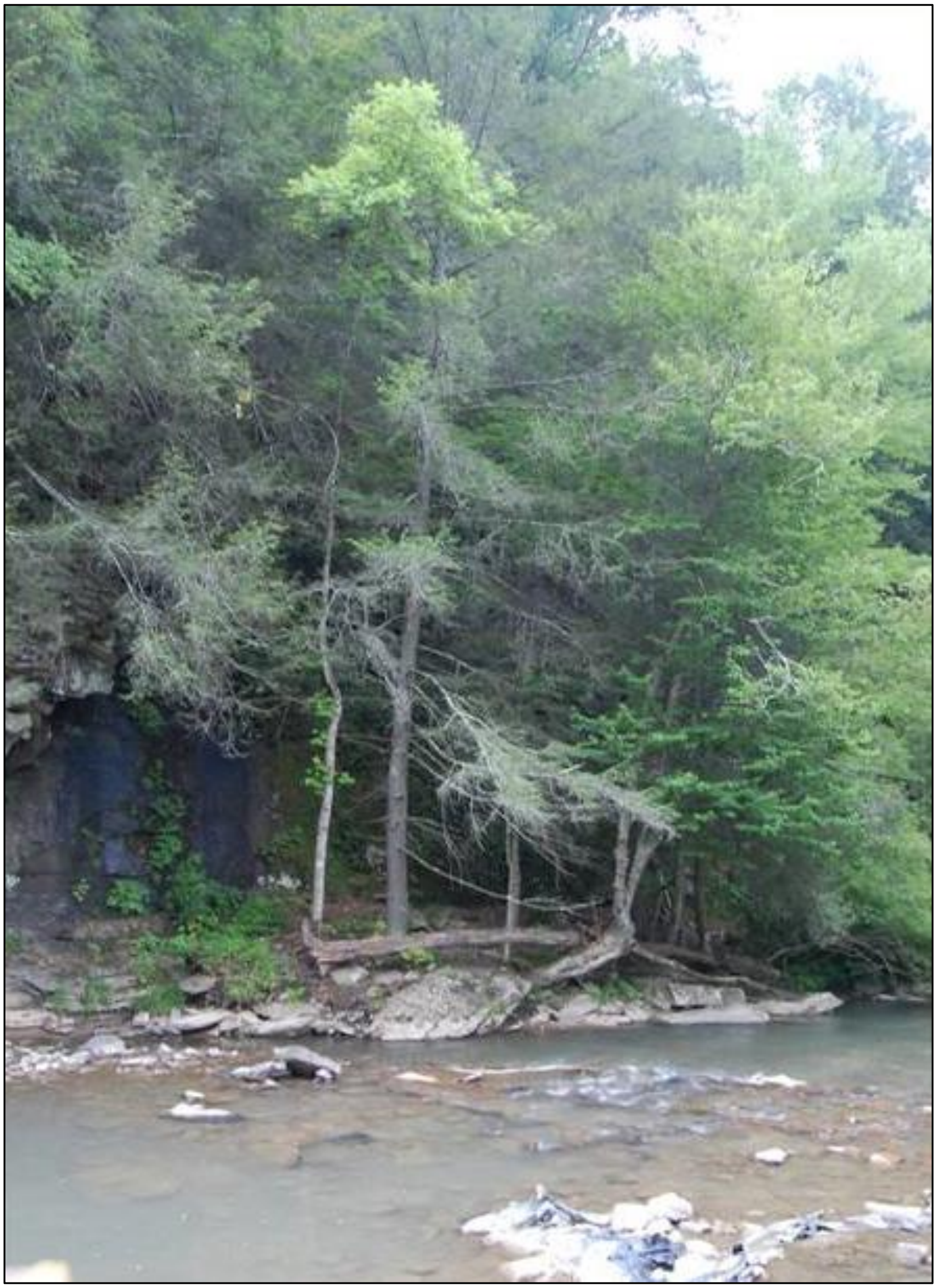

Figure 3.12: Photo of Pinnacle Creek, Wyoming County (Upper Guyandotte basin). The last known historic site within West Virginia where *Cambarus veteranus* is still present.

Figure 3.13: View of the Upper Guyandotte, Wyoming County (Upper Guyandotte basin) facing upstream in Mullens. Adequate habitat in the form of fast flowing water and large slab boulders are present, but declining stream quality ranging from trash, displaced riparian buffers, increased siltation, and adverse physiochemical readings threaten *Cambarus veteranus*.

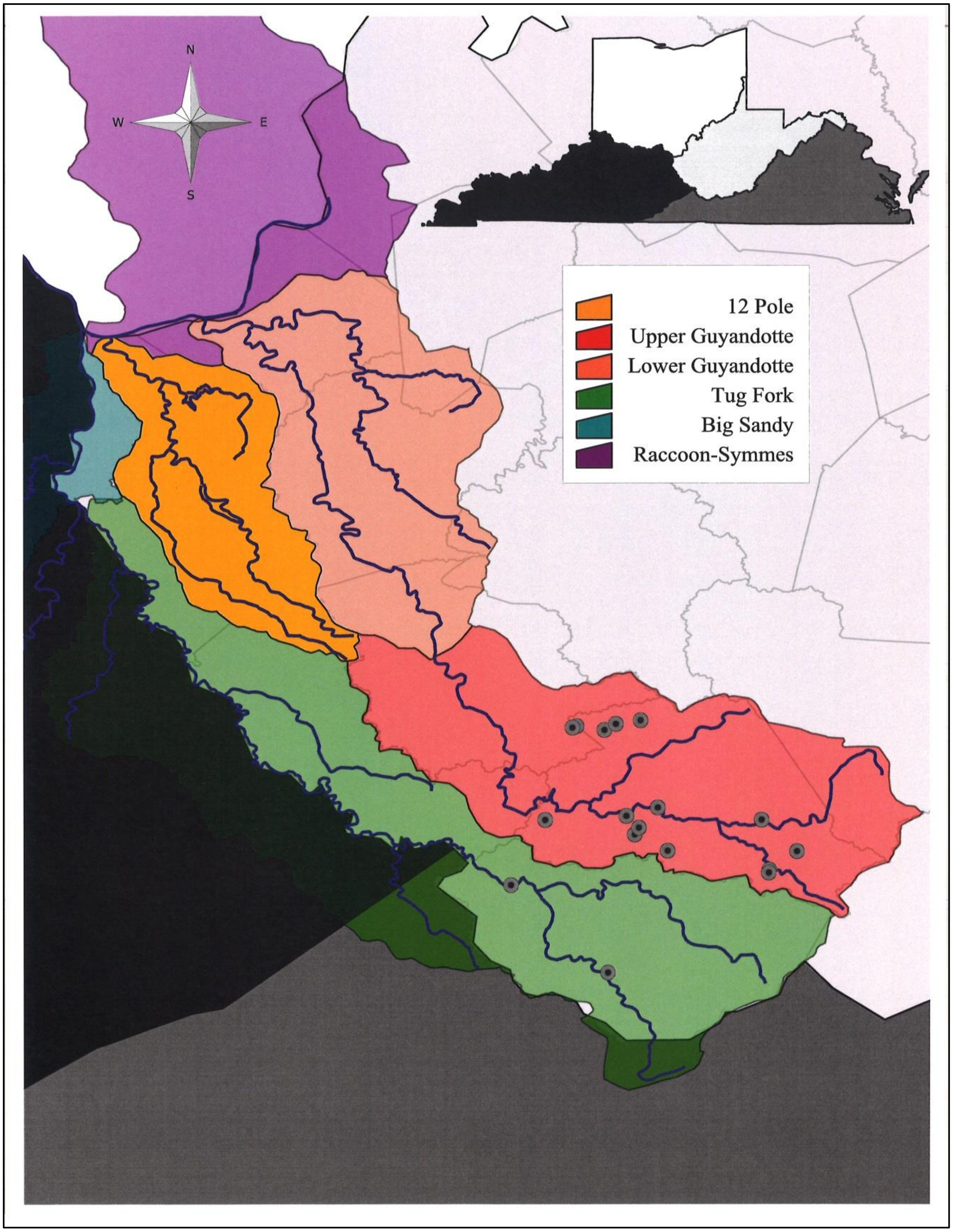

Figure 3.14: Distribution of *Cambarus veteranus* within West Virginia's Southwestern Coalfields.

Figure 3.15: *Cambarus thomai* from Beech Fork State Park, Wayne County (Twelvepole basin).

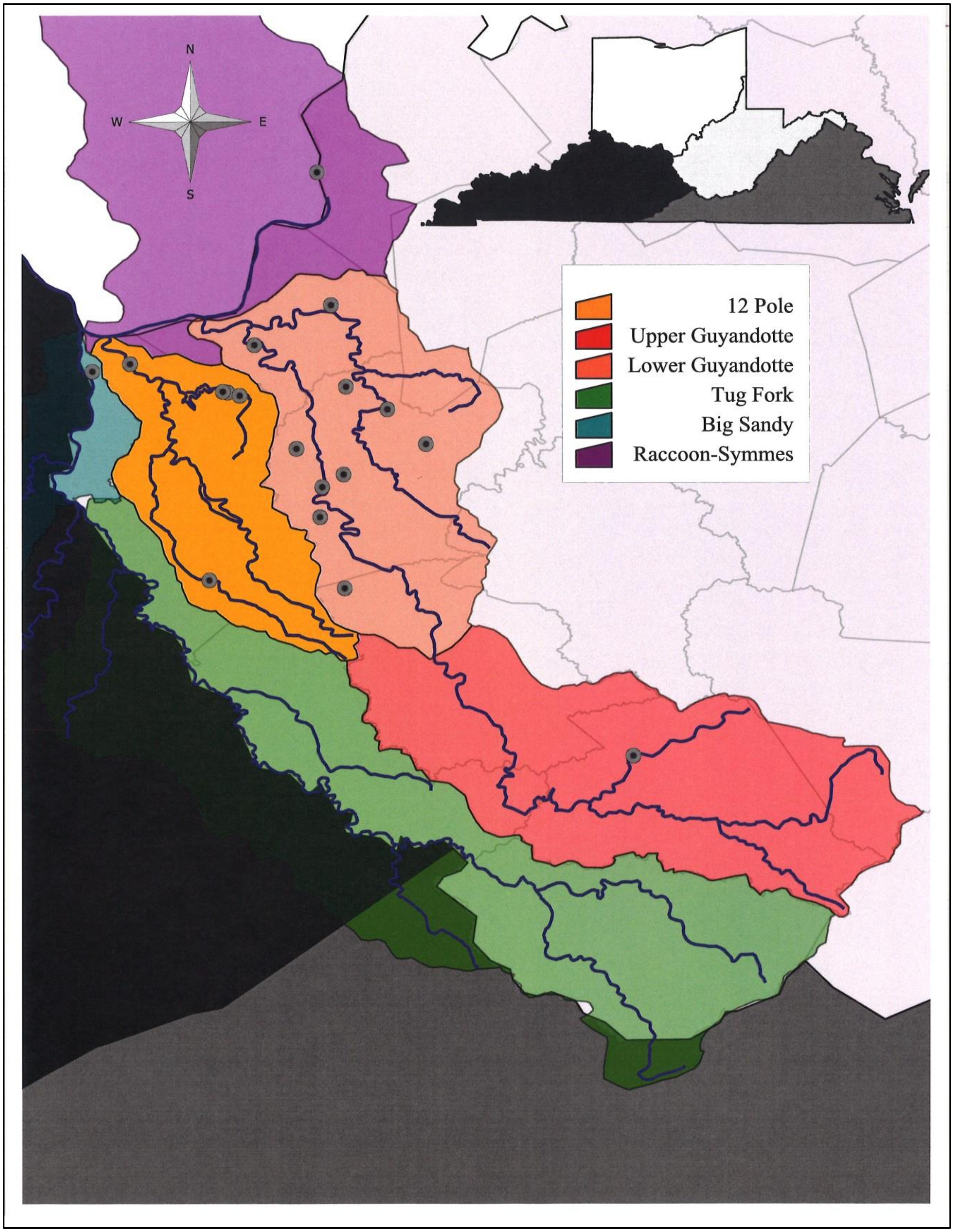

Figure 3.16: Distribution of *Cambarus thomai* within West Virginia's Southwestern Coalfields.

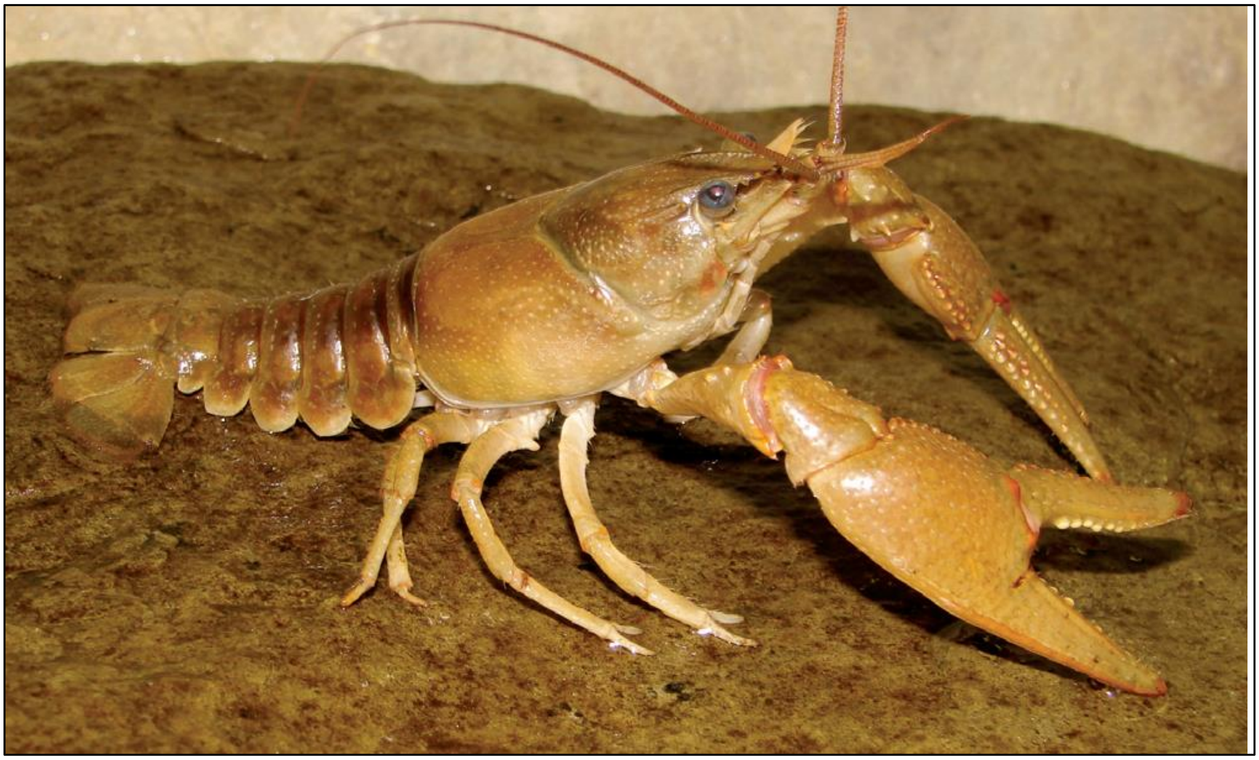

Figure 3.17: *Orconectes sanbornii.* Taken from Loughman and Simon 2011.

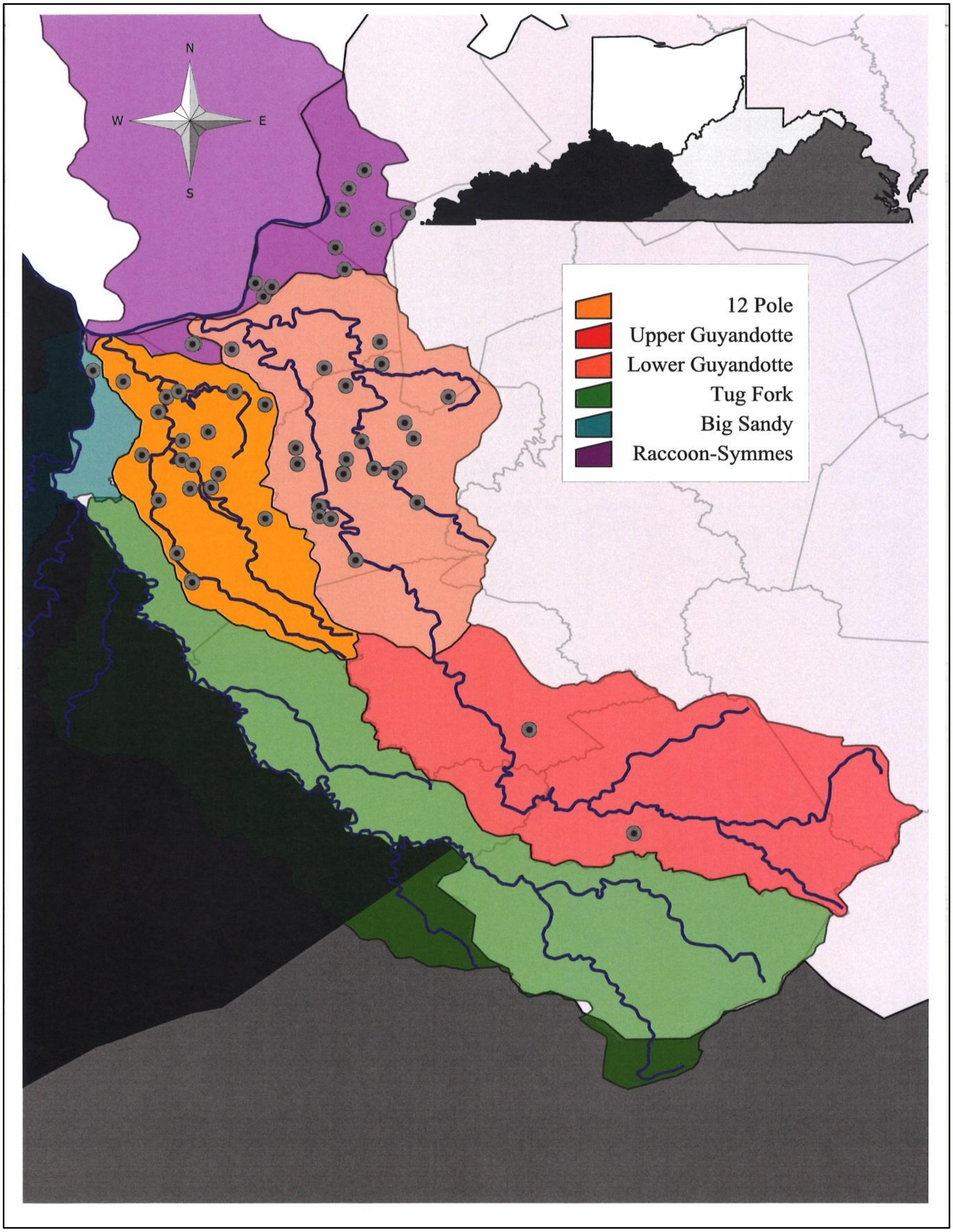

Figure 3.18: Distribution of *Orconectes sanbornii* within West Virginia's Southwestern Coalfields.

Figure 3.19: *Orconectes virilis.* Taken from Loughman and Simon 2011.

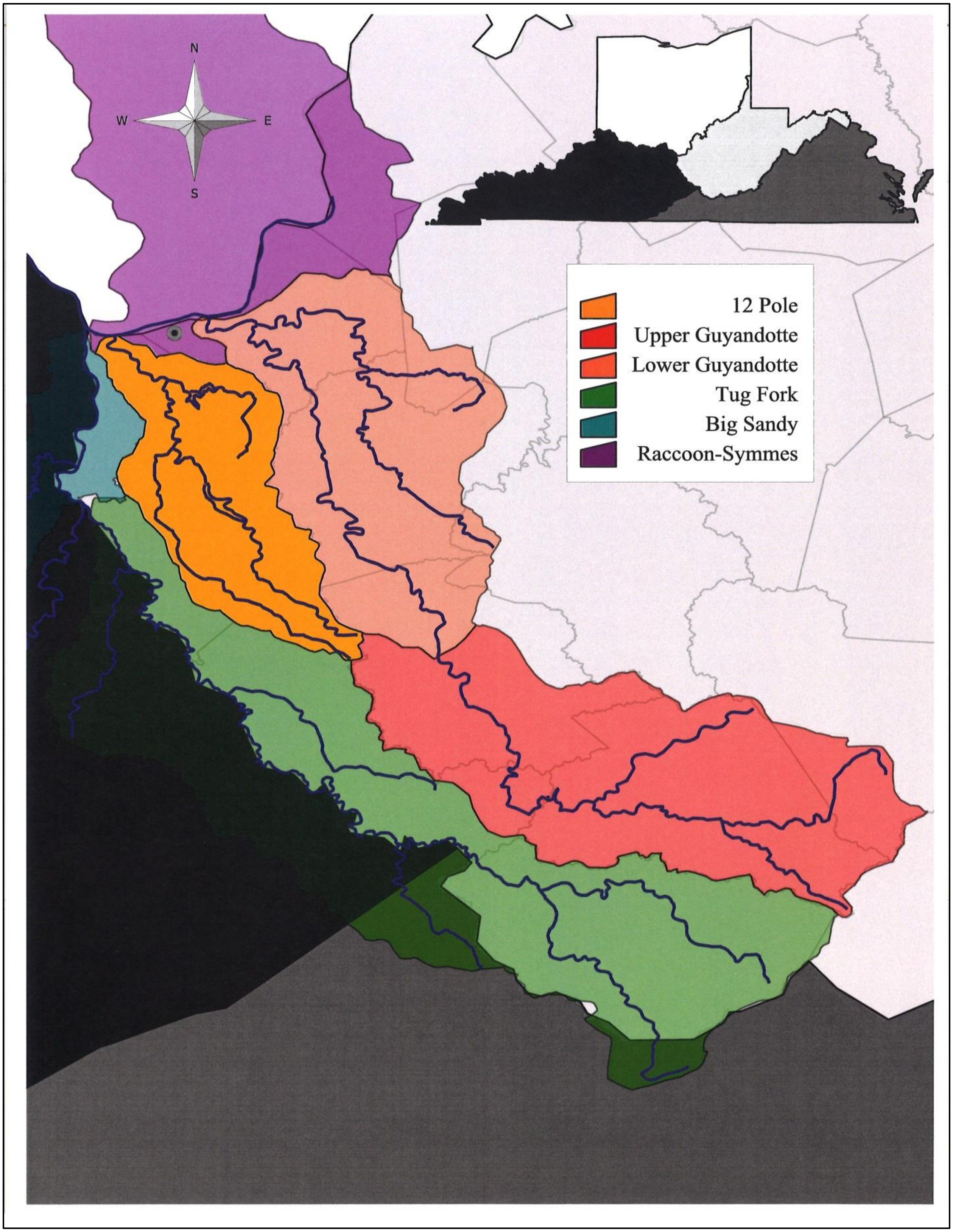

Figure 3.20: Distribution of *Orconectes virilis* within West Virginia's Southwestern Coalfields.

Figure 3.21: *Orconectes cristavarius* from Panther Creek, McDowell County (Tug Fork basin)

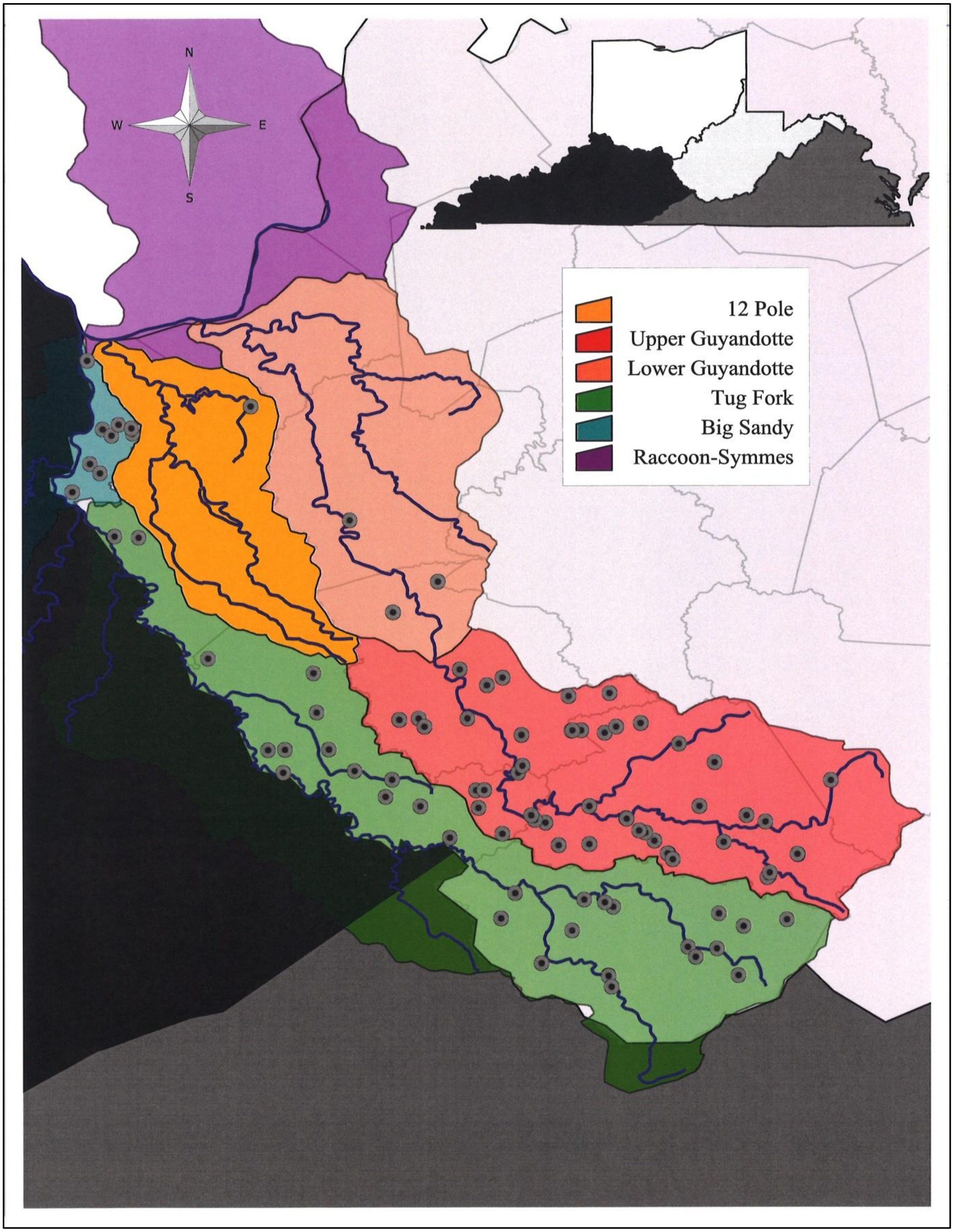

Figure 3.22: Distribution of *Orconectes cristavarius* within West Virginia's Southwestern Coalfields.

Figure 3.23: *Orconectes rusticus.* Taken from Loughman and Simon 2011.

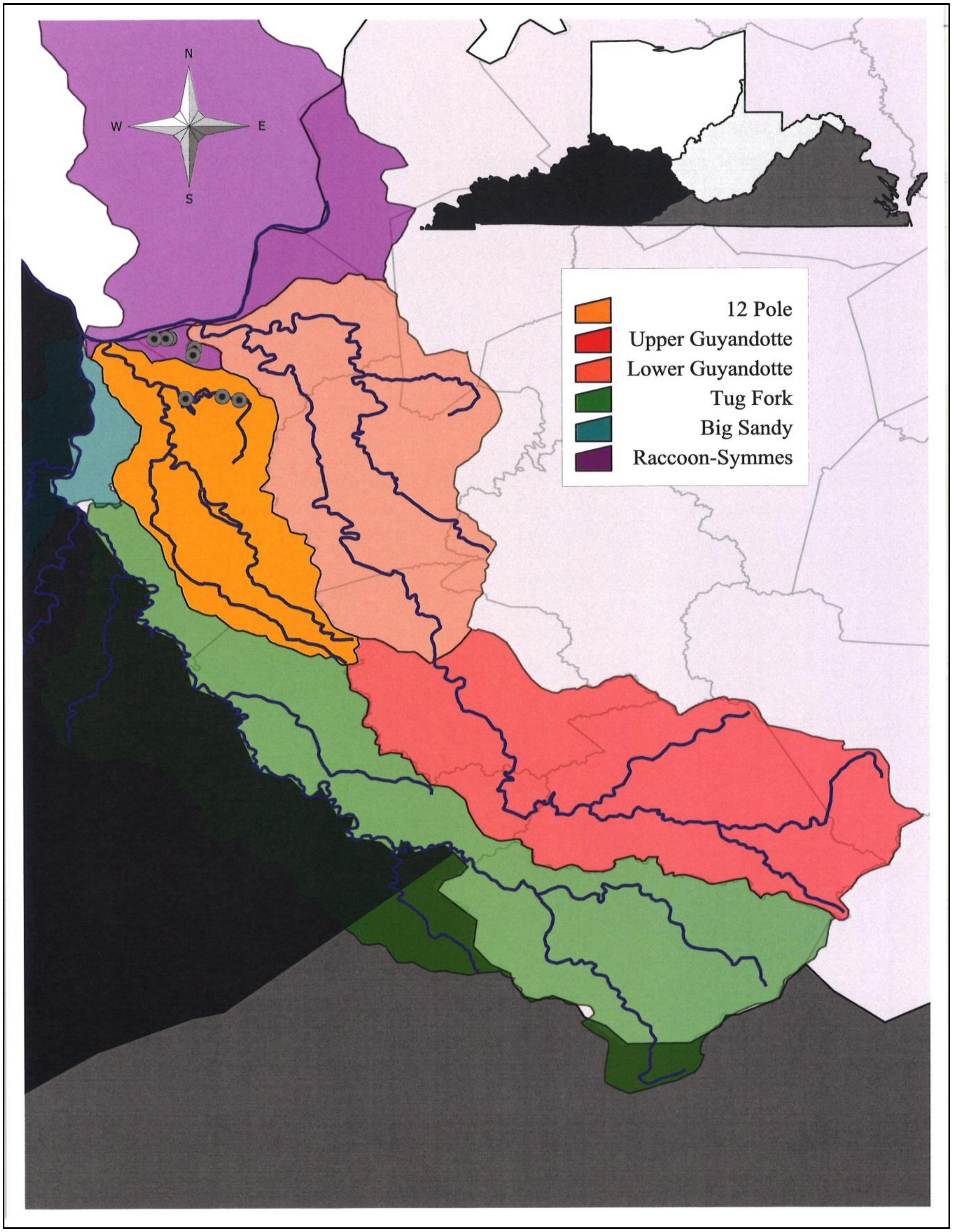

Figure 3.24: Distribution of *Orconectes rusticus* within West Virginia's Southwestern Coalfields.

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Office of Research Integrity

November 14, 2013

David A. Foltz II
566 Montclaire Lane
New Cumberland, WV 26047

Dear Mr. Foltz:

This letter is in response to the submitted thesis abstract entitled *"The Crayfishes of West Virginia's Southwestern Coalfields Region with an Emphasis on the Life History of Cambarus theepiensis."* After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) Chair has also deemed this not to be animal research requiring their approval. The information in this study is not considered human subject or animal research as set forth in the definitions contained in the federal regulations. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

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