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THE NATURAL LIFE HISTORY OF *CAMBARUS (PUNCTICAMBARUS) SMILAX*, (THE GREENBRIER CRAYFISH)

A thesis submitted to
the Graduate College of
Marshall University
In partial fulfillment of the
requirements for the degree of
Master of Science
in
Biological Sciences
by
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Approved by
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ABSTRACT

The natural life history of *Cambarus* (*Puncticambarus*) *smilax*, the Greenbrier Crayfish, was studied in West Fork of the Greenbrier River and in Thorny Creek, a tributary of the main stem of the Greenbrier River. The Greenbrier Crayfish gets its name from the Greenbrier River watershed where it is thought to occur exclusively. Among described members of the subgenus *Puncticambarus*, *C. smilax* is a sister taxon most similar to *Cambarus* (*Puncticambarus*) *robustus*. Monthly collections were made within the two study sites, from August 2010 to July 2011. Collecting techniques included dip-netting, seine-netting, and hand collecting. *Cambarus smilax* and all other species of crayfish found within the sites were recorded with sex, molting status, and reproductive status. Data collected from *C. smilax* specimens including weight, total carapace length (TCL), and chelae length. All gravid females collected were preserved in a 70% ethanol solution; fecundity and length of offspring were recorded in the lab. Seasonal breeding and molting showed a positive correlation and were synchronous with *C. robustus* populations studied by Hamr and Berrill (1985), Corey, S. (1990), and Guiasu and Dunham (2001). Habitat selection analysis indicated competition between *C. smilax* and *Cambarus* (*Hiatacambarus*) *chasmodactylus*, where *C. chasmodactylus* partially excludes *C. smilax* from the principal habitat found in runs. The limited range of *C. smilax* makes it a species of special concern and a candidate for protection.

CHAPTER 1

INTRODUCTION

With over 350 crayfish species North America represents the greatest proportion of crayfish fauna in the world. Australia is second with over 100 species of crayfish. In contrast, Europe has only 5 endemic species of crayfish. Africa has no native crayfish species, but some species such as *Procambarus clarkii* have been introduced for aquaculture (Holdich 1993). North American crayfish species are represented by two families, Astacidae and Cambaridae. Astacidae is represented by one genus in North America, *Pacifastacus,* which is located on the Pacific slope. This genus has only five current species, of which one is thought to be extinct and another critically endangered. The family Cambaridae is represented by 12 genera: *Barbicambarus, Bouchardina, Cambarellus, Cambaroides, Cambarus, Distocambarus, Fallicambarus, Faxonella, Hobbseus, Orconectes, Procambarus,* and *Troglocambarus*. The Cambaridae family is very diverse east of the Rocky Mountains. The highest diversity is concentrated in the southeastern United States (Loughman & Welsh 2010). The southeastern area of the Appalachian Mountain range is an epicenter for crayfish diversity, as well as salamanders, fish and other aquatic organisms. Several factors make this area prone to diversity, including the geologic age of the Appalachian Mountains (300 – 500 million years old), the meeting of different terrains and biomes, and the rain shadow effect which drops a lot of precipitation on this region (Karr & Chu 1998).

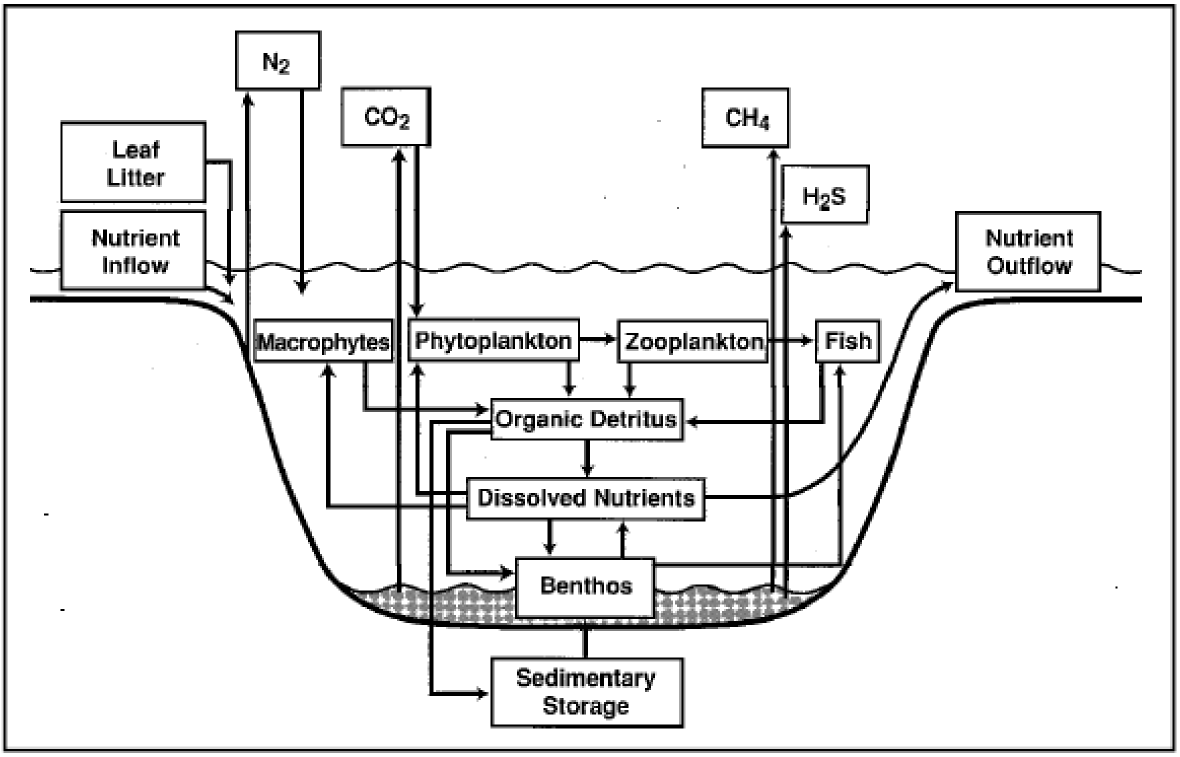
Crayfishes Effects On Their Environment

Crayfish are of great importance to their environment. The roles crayfish play in their environment have significant trophic and nontrophic effects (Momot 1995; Covich et al.

1999). Crayfish are an important sources of food for many aquatic organisms, including fish. Semi-aquatic species such as amphibians and snakes, and terrestrial animals such as birds and mammals also utilize crayfish as a food source. Crayfish are omnivores and detritivores. One study (Momot 1995) found that some crayfish species are opportunistic carnivores, preferring animal protein whenever possible, but will become facultative herbivores when these sources of animal protein are exhausted. The diverse trophic positions crayfish occupy make them functionally important within complex aquatic food webs.

The ways in which crayfish cycle energy is of critical importance to their aquatic ecosystems. Figure 1 displays how crayfish and other benthic invertebrates accelerate nutrient cycling. Crayfish play an important role in the conversion of allochthonous inputs (e.g., leaf litter) into fine particulate organic matter and dissolved nutrients. Crayfish are primary, secondary or tertiary burrowers. Their burrowing in sediment creates aeration and therefore accelerating nutrient cycling. Burrowing can be critical for releasing macronutrients such as phosphorus and nitrogen to be used by bacteria, alga and plants (Covich et al. 1999). Because crayfish are central in the aquatic food web, and accelerate nutrient cycling, disturbing native crayfish populations would affect other species within the ecosystem. Crayfish can play a role as a keystone species in their ecosystem.

Figure 1. Roles of crayfishes and other benthos in the aquatic food web, modified from Covich, et al. 1999



Human Impacts on Crayfish

An especially alarming trend in North America is the loss of native freshwater fauna. This trend can be emphasized when comparing the number of threatened aquatic species to terrestrial species in North America. With 14% to 18% of terrestrial species such as birds, mammals, reptiles and butterflies classified as vulnerable, imperiled, or extinct in the US, aquatic species are disproportionately endangered with 35% to 37% of amphibians and fishes, 65% of crayfish, and 67% of unionid mussels listed. As a group crayfishes represent 35% of all North American fauna listed as vulnerable, endangered or possibly extinct. Around 51% of the native species of crayfish in North America are listed as extinct, critically imperiled, imperiled, or vulnerable (Richter et al. 1997).

Using an exponential decay model Ricciardi and Rasmussen (1999) derived that recent and future extinction rates for North American freshwater fauna are five times higher than terrestrial fauna. When projecting mean future extinction rates for freshwater fauna they hypothesized that freshwater extinction rates will contend with extinction rates of tropical rainforest communities.

One of the foremost problems in preserving native crayfish populations is the introduction of invasive crayfish species. The translocation of the alien crayfish species through bait-bucket introduction, escape from aquaculture facilities, or other methods can have decimating effects on native crayfish species. *Orconectes rusticus* (The Rusty Crayfish) has been spread through a large area of North America, mostly as bait by fishermen (Holdich 1993). When introduced *O. rusticus* will often out-compete native species and remove complete macrophyte beds. This will affect benthic organisms like gastropods and juvenile fish that depend on macrophyte beds for refuge (Covich et al. 1999).

The native range of *O. rusticus* is the mid-lower Ohio River drainage in central Kentucky, western Ohio, and eastern and central Indiana and the western Ohio, and eastern and central Indiana and the western Lake Erie drainage in southeastern Michigan (Taylor & Schuster 2004). Presently *O. rusticus* has expanded its range widely over North America, where it is an invasive species. Where introduced *O. rusticus* may out-compete native species for habitat and food.

One scientific study carried out in northern Wisconsin lakes established trends for the replacement of a native crayfish population of *Orconectes virilis* (Virile Crayfish) by *O. rusticus* (Hill & Lodge 1999). Due to roles in competition and predation *O. rusticus* had advantages over *O. virilis* in competing and establishing populations. After analyzing the data

gathered from the gradual invasion by *O. rusticus* it was disturbingly concluded that there were no natural occurring domains were *O. virilis* would favor *O. rusticus*. Thus the extirpation of *O. virilis* was inevitable where *O. rusticus* was found. The outlawing of live crayfish as fishing bait was recommended and has been implemented in Wisconsin and other states (Hill & Lodge 1999).

Sedimentation from nonpoint sources such as agriculture, road construction, mining and urban development can lead to a decrease in interstitial space needed by crayfish and other aquatic fauna for habitat. According to the U.S. Environmental Protection Agency's (EPA) 1992 Clean Water Act Section 305 (b) reports, the leading cause of water quality impairment across the U.S. was siltation, accounting for 45% of the impaired assessed river miles. Siltation was followed by nutrient pollution (37%), pathogen indicators (27%), pesticides (26%), and organic enrichment and resultant low levels of dissolved oxygen (24%) (Richter et al. 1997). The EPA has reported that the number one cause for water quality degradation is agriculture. Agricultural activities include those such as crop production, animal pasture, grazing, and feeding operations. Other major causes in water quality degradation include hydro-modification, such as water diversions, channelization, and dam construction, and habitat alteration such as resource extraction, silviculture, and urban development (US EPA 1992).

Historically, crayfish and other aquatic organisms have been greatly imperiled by chemical pollutants like those from acid mine drainage (AMD), industrial point-sources, and fertilizer runoff. Beginning with the EPA's establishment of the Clean Water Act of 1972, significant efforts by federal and state environmental protection agencies to reduce and regulate point-source discharges have successfully reduced chemical pollutants. Even with

increased regulations, chemical pollution remains a serious problem for more than 20% of imperiled aquatic fauna (Richter et al. 1997). Best Management Practices (BMP) must be put into place to limit sedimentation, pollution, hydromodification, and other practices that impair streams and rivers.

Crayfishes of West Virginia

The southeastern area of North America is an epicenter for crayfish diversity. West Virginia is a critical area for crayfish research. West Virginia's terrain varies from mountainous ridge/valley terrain to piedmonts. The southern area of West Virginia is being greatly impacted by deep mining, mountain top removal, and acid mine drainage. The northeastern area of the state is being developed for natural gas extraction from Marcellus Shale reservoirs. These anthropogenic effects heavily impact West Virginia aquatic ecosystems.

It appears the first publication on West Virginia Crayfish was from Faxon in 1914 (Loughman et al 2009). Faxon reported on two taxa of West Virginia crayfish fauna referred to as *Cambarus bartonii* (Common Crayfish) and *Cambarus dubius* (Upland Burrowing Crayfish), both of these species are still recognized and present in West Virginia (Loughman et al 2009).

One of the first attempts to comprehensively list and describe crayfish species in West Virginia was in 1929 by Newcombe (Newcombe 1929). Newcombe identified 15 species from 30 counties within three months of collecting. His data was influenced by previous work from other biologist including Ortmann, Faxon, Hay, Hagen, Turner, and Williamson. Their studies were founded in neighboring states of Pennsylvania, Ohio, Maryland and

Kentucky, as well as collecting a number of times in West Virginia. When Newcombe published his findings in 1929 he listed just 17 species but was careful to mention that he had not explored the ranges or differences in some crayfish species (Newcombe 1929).

The most comprehensive crayfish survey carried out in West Virginia was Jezerinac and Stocker in the summers of 1988 and 1989. They used seine nets, dip nets, and bare hands to collect from a minimum of four sites from each of the 55 counties in West Virginia. Jezerinac concluded that West Virginia's watersheds have 21 existing crayfish species that have resident populations in West Virginia's watersheds (Jezerinac et al. 1995).

Since Jezerinac's initial survey, there have been some more changes to taxonomy. Thoma and Jezerinac (1999) elevated *Cambarus* (*Cambarus*) *bartonii carinirostris* (The Rock Crayfish) from a subspecies to a species. A population in West Virginia previously allocated as *Orconectes* (*Procericambarus*) *spinosus* has been described by Taylor (2000) as *Orconectes* (*Procericambarus*) *cristavarius* (Spiny Stream Crayfish). Loughman (2007) added *Procambarus* (*Ortmannicus*) *acutus* (White River Crayfish) to the list of states crayfish species (Loughman et al. 2009).

In 2011 Loughman described an additional crayfish species in West Virginia. The species was named *Cambarus* (*Punticambarus*) *smilax* (The Greenbrier Crayfish). With the addition of *C. smilax*, there are 23 listed crayfish species in West Virginia as of 2012 (Loughman et al. 2011). Of the 23 species in West Virginia species 6 are given an S1 (critically imperiled) status: *C. elkensis*, *C. longulus*, *C. nerterius*, *C. veteranus*, *F. fodiens* and *O. limosus*. *Orconectes limosus* is thought to be extirpated within the last decade, to some extent by the introduction of non-native *O. virilis*. (Swecker et al. 2010).

Table 1. Crayfishes by major river drainage and physiographic province in West Virginia (modified table from Loughman et al., 2011).

Species	Founder	Common Name	P	M	O	J	K	S	RV	AM	AP	Gl	St
Cambarus (Cambarus) b. bartonii	Fabricius	Common Crayfish	X			X			X	X	X	G5	S5
C. (C.) b. cavatus	Hay	Appalachian Brook Crayfish			X		X	X			X	G5	S5
C. (C.) carinirostris	Hay	Rock Crayfish		X	X		X			X	X	G5	S5
C. (C.) sciotensis	Rhoades	Teays River Crayfish					X	X		X	X	G5	S5
C. (Hiatacambarus) chasmodactylus	James	New River Crayfish					X			X	X	G5	S3
C. (H.) elkensis	Jezerinac and Stocker	Elk River Crayfish					X				X	G2	S1
C. (H.) longulus	Girard	Atlantic Slope Crayfish				X						G5	S1
C. (Jugicambarus) dubius	Faxon	Upland Burrowing Crayfish	X	X	X	X	X	X	X	X	X	G5	S3
C. (J.) monogalensis	Ortmann	Blue Crayfish	X	X	X		X		X	X	X	G5	S5
C. (Puncticambarus) nerterius	Hobbs	Greenbrier Cave Crayfish					X				X	G2	S1
C. (P.) robustus	Girard	Big Water Crayfish			X		X	X			X	G5	S5
C. (P.) smilax	**Loughman**	**The Greenbrier Crayfish**					X		X	X		G3*	NA**
C. (P.) veteranus	Faxon	Big Sandy Crayfish						X^H			X	G3	S1
C. (Tubericambarus) thomai	Jezerinac	Little Brown Mudbug			X		X	X			X	G5	S5
Fallicambarus (Creaserinus) fodiens	Cottle	Digger Crayfish					X				X	G5	S1
Orconectes (Crockerinus) obscurus	Hagen	Allegheny Crayfish	X	X	X		X		X	X	X	G5	S5
O. (C.) sanbornii	Faxon	Sanborn's Crayfish			X		X	X			X	G5	S5
O. (Faxonius) limosus	Rafinesque	Spinycheek Crayfish	X^H						X^H			G5	S1
O. (Germicambarus) virilis	Hagen	Virile Crayfish	X		X		X		X		X	G5	I
O. (Procericambarus) cristavarius	Taylor	Spiny Stream Crayfish				X	X	X			X	G5	S5
O. (P.) rusticus	Girard	Rusty Crayfish			X		X				X	G5	I
Procambarus (Ortmannicus) acutus	Girard	White River Crayfish					X				X	G5	U
P. (O.) zonangulus	Hobbs and Hobbs	Southern White River Crayfish	X						X			G5	I

Potomac River (P), Monongahela River (M), Ohio River (O), James River (J), Kanawha River (K), Ohio River basin southwestern West Virginia (S), Ridge and Valley (RV), Allegheny Mountains (AM), and Appalachian Plateau (AP).
XH indicates historic records.
Global (Gl) and state (St) conservation status ranks (WVNHP 2007) are as follows: G5 = secure, G3 = vulnerable, G2 = imperiled, S5 = secure, S3 = vulnerable, S1 = critically imperiled, I = introduced, and U = unrankable.
* Global status of G3 is recommended by Z. Loughman.
** A State status has not yet been established.

The Greenbrier Crayfish - *Cambarus (Puncticambarus) smilax*

It is believed that *Cambarus (Puncticambarus) smilax* evolved from an isolated population of *Cambarus* (*Puncticambarus*) *robustus* (Big Water Crayfish). *Cambarus robustus* was first described by Girard in 1852; it has the most extensive range of any *Puncticambarus* species. *Cambarus robustus* occurs throughout the Great Lakes region of Lake Superior east to Lake Erie, throughout New York, Michigan, and Ohio, and into regions of Pennsylvania, West Virginia, Kentucky and Indiana. In West Virginia, *C. robustus* is found continuously across the Allegheny Plateau Physiographic Province and within the Ohio River,

Little Kanawha River, Kanawha River, Guyandotte River, and Big Sandy River systems (Jezerinac et al. 1995). *Cambarus robustus* is present in the Kanawha River upstream to Kanawha Falls. Kanawha Falls is a well-known zoogeographic barrier to aquatic fauna in West Virginia (Loughman et al. 2011). At a maximum height of 7.3 m (24 feet), and running the entire width of the Kanawha River, Kanawha Falls is a physical barrier for aquatic fauna (Rahel 2007). *Cambarus robustus* is replaced directly upstream of Kanawha Falls by *Cambarus* (*Cambarus*) *sciotensis* (Teays River Crayfish) (Jezerinac et al. 1995). There is a population, previously known as *C. robustus*, 105 km upstream from Kanawha Falls in the Greenbrier River (Loughman et al. 2011). Newcombe (1929) and Jezerinac (1995) reported finding this population of *C. robustus* in the West Fork of the Greenbrier and the Greenbrier River's main-stem. These and other previous investigators commented on the range of *C. robustus* within West Virginia, but they did not comment on unique morphologic differences between the two populations.

When a population of interbreeding individuals becomes isolated, whether by physical or biological boundaries, over many generations observable differences in morphology and physiology can occur from the original population. This is referred to as allopatric speciation, and it is thought to be the main process by which new species are created. (Constantz 2004). Physical characteristics in a geographical area, such as mountainous terrain, would fracture populations more readily and provide more opportunities for speciation and diversity. The Southeastern United States has numerous mountainous terrains that divide watersheds, leading this area to have a large amount of aquatic diversity. Species that are slow moving or have a smaller dispersion range for offspring such have a greater inclination towards speciation. For example, speciation amongst birds is thwarted by their ability to fly and travel

long distances, but crayfish are mostly contained within a water body, in a stream or river only able to move upstream or downstream. Manmade structures such as dams, or natural physical barriers such as waterfalls, can limit or prevent crayfish migration.

In 2008, Loughman led a crayfish survey on the Greenbrier River and collected specimens that shared morphological similarities with *C. robustus*, but had unique morphological characteristics to the Greenbrier River population (Loughman et al. 2011). After morphological comparison between the Greenbrier River specimens and other *C. robustus* specimens within West Virginia, the Greenbrier River population was determined to have enough morphological and genealogical difference to be considered a separate species (Loughman et al. 2011). In 2011 Loughman et al. submitted a manuscript to the Biological Society of Washington where the population was validated as a new species. Loughman named the species *Cambarus smilax*, (The Greenbrier Crayfish) (Loughman et al. 2011).

Taxonomy

All crayfish species are in the Kingdom: Animalia, Phylum: Arthropoda, Sub-phylum: Crustacea, Class: Malacostraca, and Order: Decapoda. *Cambarus smilax* is in the Super-family: Astacoidea, Family: Cambaridae, and Genus Cambarus. The *Cambarus* genus contains around 100 species that are divided among 12 subgenera. *C. smilax* is in the subgenus *Puncticambarus* with around 15 other species in this subgenus, all located in North America, and most in the southeastern region.

Since it is believed that populations of *C. smilax* derived from isolated populations of *C. robustus*, *C. robustus* is considered the sister taxon to *C. smilax* (Loughman et al. 2011).

Other species in the *Puncticambarus* in West Virginia include *C.* (*P.*) *robustus*, *C.* (*P.*) *nerterius* (Greenbrier Cave Crayfish), and *C.* (*P.*) *veteranus* (Big Sandy Crayfish).

Cambarus smilax can be distinguished from *C. robustus* populations elsewhere in the state by chelae palm length comprising 73-76% of palm width as opposed to 63-70% in *C. robustus*, a narrower rostrum shape of 47-52% rostrum width/length ratio compared to *C. robustus* shorter less narrowing rostrum with a 54-63% width/length ratio, the ventral surface of the chelae of *C. smilax* with 0-2 subpalmar tubercles, compared to *C. robustus* with 3-6 subpalmar tubercles, Form I males' gonopod angled ≥ 90° to the shaft compared to < 90° for *C. robustus* (Loughman et al. 2011).

Etymology

Cambarus smilax (The Greenbrier Crayfish) is named after the Greenbrier River watershed where it is found and thought to be endemic. The Greenbrier River is named after a group of herbaceous, thorny vines (smilax spp.) that frequently grow along its banks (Loughman et al. 2011).

Range

Cambarus smilax is thought to be endemic to the Greenbrier River Watershed (Loughman et al. 2011). The Greenbrier River is the longest undammed river in West Virginia, running 233 km south from its headwaters in northern part of Pocahontas County to its mouth where it flows into the New River in Hinton, West Virginia. The Greenbrier River drains nearly 4,289 km^2 of area (Stauffer 2007).

Cambarus smilax is found primarily in tributaries of the main stem of the Greenbrier River, with stable populations found in the East Fork and West Fork of the Greenbrier River, Knapps Creek, Deer Creek, and Thorny Creek (Loughman et al. 2011). The holotype, the specimen used as a reference to formally describe the species, was collected from the West Fork of the Greenbrier. Specimens have been collected from Laurel Creek of the Greenbrier River in Monroe County, which is the most southern known range of the species. Species have also been found in the main stem of the Greenbrier River.

CHAPTER 2

OBJECTIVES AND HYPOTHESES

Objective

The objectives of this research:

1. Collect data on its life cycle including habitat, life stage, breeding, and fecundity
2. Record morphological characteristics: chelae length, carapace length, and weight
3. Observe and record information on interspecies relations between *C. smilax* and other crayfish species

Hypothesis

The following hypotheses were formed prior to data collection:

- *Cambarus smilax* populations' life cycles will parallel those of *C. robustus* found in other studies such as Hamr and Berrill (1985), Corey, S. (1990), and Guiasu and Dunham (2001).

- The presence of *C. chasmodactylus* at the West Fork Site will reduce abundance and alter habitat selection of *C. smilax*.
- *Cambarus smilax* weight to total carapace length (TCL) ratio has a greater correlation than the chelae length to TCL.
- The majority of *C. smilax* will be found in run type habitat where water oxygenation and suitable habitat are abundant.

CHAPTER 3

METHODS

Site Selection

Two collection sites were chosen from the headwaters of the Greenbrier River watershed in Pocahontas County, West Virginia (Figure 2).

The West Fork of the Greenbrier River was chosen as a collection site due to the holotype specimen being collected in that area, and due to a stable population in a larger stream. The collection site on the West Fork of the Greenbrier River is located at N 38.60119°, W 79.82171°, at an elevation of 2,885 feet (Figure 3). This site is at Breacher, 7.72 km (4.8 mi) from origin of Forest Service Road 44 in Durbin, Pocahontas Co., West Virginia. The holotype, as well as the allotype and morphotype where collected here by Loughman et al. in 2008. Representative photographs of the West Fork Greenbrier River site are provided in Appendix B.

Thorny Creek was chosen as a collection site due to a stable abundant population in a smaller stream. Loughman et al. 2009 and 2011 had collected *C. smilax* specimens at both of these sites, in part to describe the species. The collection site on Thorny Creek is located at N 38.265537°, W 79.982827°, at an elevation of 2,540 feet (Figure 4). This site is just north of Pocahontas County High School, 2.25 km (1.4 mi) from the intersection on County Road 11-2 and Interstate Route 28 (CR 11-2/Rt 28) on County Road 11-2 (CR 11-2). Representative photographs of the Thorny Creek site are provided in Appendix B.

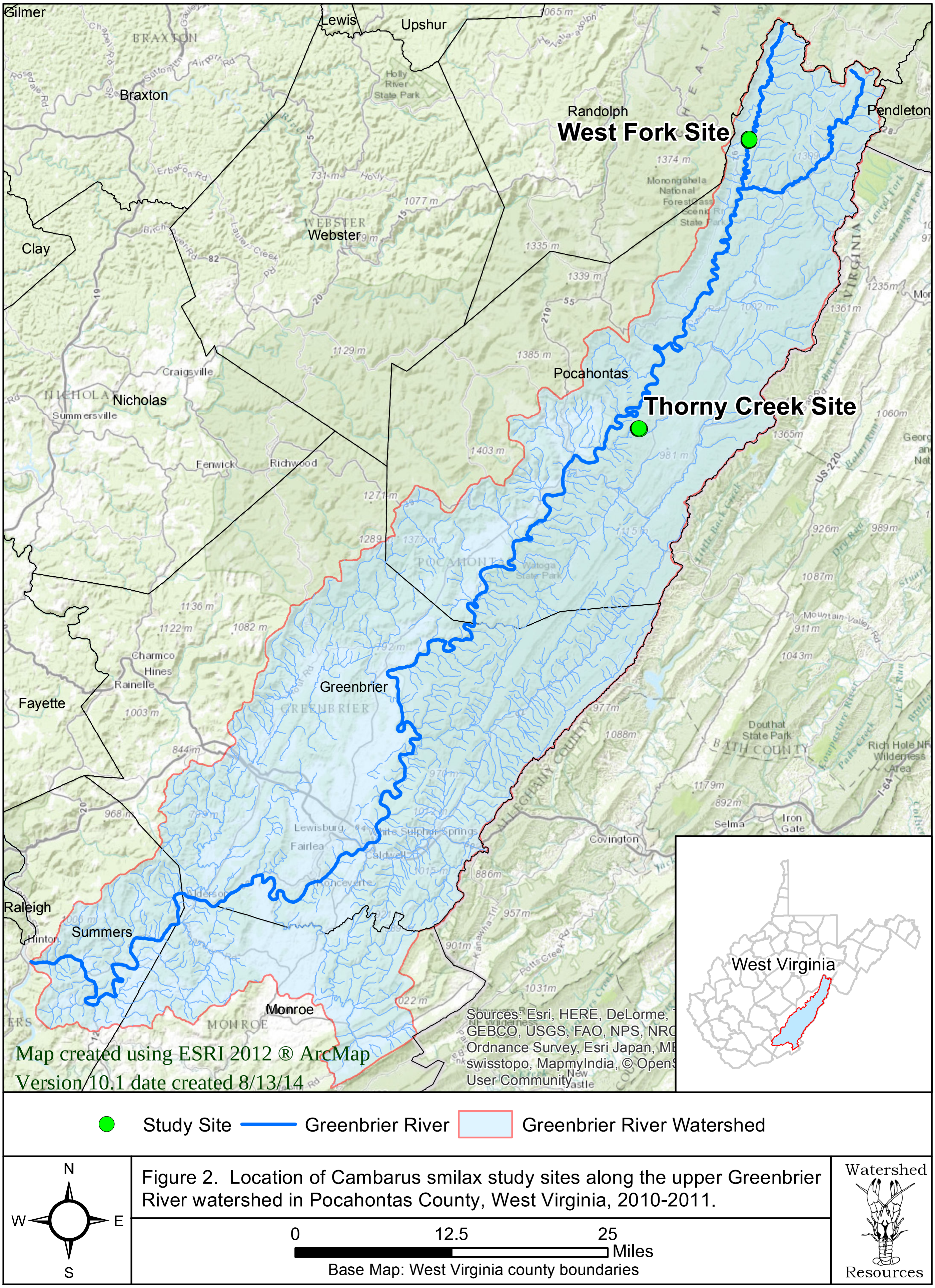


Figure 2. Location of Cambarus smilax study sites along the upper Greenbrier River watershed in Pocahontas County, West Virginia, 2010-2011.

Figure 3. Location of West Fork Greenbrier River *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

Figure 4. Location of Thorny Creek *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

Crayfish Sampling Methods

Collecting techniques included dip-netting, seine-netting, and hand collecting. All crayfish captured during sampling were identified and recorded with species and sex, reproductive status, and molting status were recorded. *Cambarus smilax* specimens included weight, total carapace length (TCL), and chelae length. All gravid females were collected and preserved in a 70% ethanol solution. The total number of offspring, egg diameter and length of offspring were recorded.

Crayfish sampling was limited to for 4 ½ person-hours per visit. Prior to sampling the proportion of pool, run and riffle habitats were assessed and used to ensure equal time for each habitat type. Monthly sampling of the sites was conducted within two consecutive days.

Different sampling techniques were more productive under certain conditions. Seine netting was most successful in areas were water velocity was higher, such as runs and riffles. Seine netting became necessary when higher water levels limited visibility and the ability to collect using dip nets or hand collecting. The dip netting using basic fish nets was most successful in deeper pools where visibility was limited but seine netting was not effective due to lack of water velocity. Hand collecting was most effective in pools and some runs where crayfish could be easily seen and grabbed with a quick hand grab.

Moving upstream, crayfish were collected and put into buckets labeled with the designation of pool, run, or riffle. After moving up through a pool, run, riffle complex data were collected from the crayfish in each bucket. All crayfish had data collected for species, life stage, molting status, chelae damage and any other notable characteristics. *Cambarus smilax* data was collected for total carapace length (TCL), chelae length, and weight. The

TCL and chelae length were measured using a SPI Caliper. Weight was measured using a clear plastic cup to hold the crayfish immobile, and a set of digital scales (accuracy ± 0.1g).

A complete list of equipment is provided in Appendix A

Site Habitat Evaluation

The stream conditions that support *C. smilax* and other crayfish species were recorded in order to study what conditions might be beneficial or unfavorable to their survival. Study sites were examined using physical, physiochemical, and GIS based spatial analysis.

Physical Stream Characteristics

Physical stream characteristics for each site were measured using the West Virginia Department of Environmental Protection's (WVDEP) Rapid Bioassessment Protocols (RBP). This protocol uses ten habitat parameters: 1) Epifaunal Substrate / Available Fish Cover, 2) Embeddedness, 3) Velocity / Depth Regimes, 4) Channel Alteration, 5) Sediment Deposition, 6) Riffle Frequency, 7) Channel Flow Status, 8) Bank Stability, 9) Bank Vegetation Protection, 10) Width of Undisturbed Vegetation Zone. These RBP parameters are scored 1 – 20 into categories ranked: Poor, Marginal, Sub-optimal, and Optimal respectively (WVDEP 2011).

A Marsh-McBirney Flo-mate 2000, flow-meter and rod was used to measure water flow velocity. Flow was measured in feet per second (fps) at the beginning, middle, and end of each pool, run, and riffle in the deepest area of the stream (thalweg) prior to collecting in these areas.

Physiochemical Stream Characteristics

Chemical characteristics such as pH and conductivity, and physical characteristics such as temperature and dissolved oxygen where measured with a Hach HQ40D: Dual Probe Multi-Parameter Meter water chemistry kit. Three readings for each characteristic measured were collected at the beginning, middle, and end of the sampling area. These reading were taken in thalweg after waiting approximately two minutes for the readings to stabilize.

Stream Characterization Using GIS Spatial Analyst

Stream data collected from copper-poling (i.e., point-intercept sampling) and a geographic information system (GIS) were used to model stream substrate composition, water velocity and water depth for the two sample sites. Data were collected in July 2010, prior to crayfish sampling. Point-intercept samples were systematically taken at approximately 3 m intervals throughout each site by moving upstream in a sinuous pattern. A copper pole (5' length by 7/8" diameter) was placed on the stream substrate and held the pole vertically in the stream to identify each point-intercept. Substrate was classified as fines, gravel, cobble, boulder or bedrock. A Marsh-McBirney Flo-mate 2000, flow-meter and rod was used to measure water velocity. Water depth was measured to the nearest 0.03 m. A handheld GPS receiver (Garmin 400t) was used to geo-reference each point-intercept and its corresponding data. ArcMap 10.1 (ESRI 2012) and inverse distance weighting (IDW) was used to create a surface model for each stream parameter (i.e., substrate composition, water velocity and water depth). Although these parameters vary over time, the surface models provided biologically relevant information within the temporal extent of this study and used the surface models in the *C. smilax* habitat analyses.

Data Processing and Analysis

Text was prepared in Microsoft Word ® (2007). Data was compiled and analyzed in MS Excel ® (2007).. Photographs were cropped and resized in IrfanView ® (2004).. Maps throughout this thesis were created using ArcMap 10.1 software by ESRI and are the intellectual property of ESRI and are used herein under license.

A box-and-whisker plot was made to display the size differences between the two major molting events. Histograms were used to compare percentages of gravid and in-glair females, percentages of molting individuals, the amount of Form I and Form II males, the difference between species found in each habitat type for both sites, and the percentage of individuals that had damaged chelae. The histograms in Appendix E display size cohorts for each month for the year.

Regressions were used to show the TCL to fecundity rate for gravid females, the correlation between TCL and chelae length, and the correlation between TCL and weight. Using linear regressions slopes were established ($y=mx+b$). Using the coefficient of determination, denoted R^2, this statistical formula indicates a best fit for the data points. Other line graphs were used to compare physical and chemical properties in the water, such as dissolved oxygen levels to water temperature. Pie charts in Appendix C display changes in the amount of each species caught per month for the year.

Using ArcMap 10.1 software, which is a GIS (ESRI 2012) program, Inverse Distance Weighting (IDW) was done on data points to create maps and analysis the values.

CHAPTER 4

RESULTS

There were four species observed: *Cambarus (Puncticambarus) smilax, Cambarus (Hiatacambarus) chasmodactylus* (The New River Crayfish), *Cambarus carinirostris* (The Rock Crayfish), and *Orconectes (Crockerinus) obscurus* (The Allegany Crayfish). Representative photographs of each species are located in Appendix B. Confidence that the species identifications were accurate is because of differences in chelae and rostrum morphology, and to some extent color. *Cambarus chasmodactylus* has very distinct chelae with a large gap and fine hairs called seta between the dactyls and fixed prodopods. *Cambarus chasmodactylus* also has an aquamarine coloration not present in the other species present. *Cambarus carinirostris* has a distinct 90° turn on the end of its rostral point. *Orcoectes obscurus* was the only species present with three rostral spines and it has a pinkish hue, with bright red highlights along its abdomen, carapace and chelae. *Cambarus smilax* was distinguished from other species by one rostral spine, no seta or gap in its chelae, a rostrum that turns in on a 45° angle, 4-5 tubercles along the palm of its chelae and a chestnut coloration.

Crayfish Community Observations

The crayfish community collected between the West Fork site and the Thorny Creek site showed some variations in species observed, abundance, and behavior as it related to habitat selection. Abundance of *C. smilax* was greater at the Thorny Creek Site (n = 561) compared to the West Fork site (n = 369). *Cambarus chasmodactylus* was not collected at the

Thorny Creek site. Conversely, it was the most abundant species at the West Fork site (*n* = 448). *Orconectes obscurus* abundance was double at the Thorny Creek site (*n = 212*) compared to numbers observed at the West Fork site (*n = 102*). The abundance of *C. carinirostris* was also elevated at the Thorny Creek site (*n* = 134) compared to a much lower abundance at the West Fork site (*n* = 21).

Although the overall abundance of crayfish specimens collected between the West Fork site and the Thorny Creek site were similar (*n = 940* and *n = 907* respectively) the only species found in greater abundance at the West Fork site was *C. chasmodactylus*, which was absent from the Thorny Creek Site. The abundance of each species collected by month is listed in Table 2 and 3.

Table 2. Crayfish community collected monthly from August 2010 to July 2011 for the West Fork site.

Crayfish Community, West Fork Site

	Aug. 13th 2010	Sep. 5th 2010	Oct. 10th 2010	Nov. 6th 2010	Dec. 31st 2010 *	Feb. 27th 2011	Mar. 19th 2011	Apr. 21st 2011	May 9th 2011	Jun. 19th 2011	Jul. 17th 2011	Total for Year
C. smilax	49	46	45	17	16	36	21	41	30	42	26	369
C. chasmodactylus	56	62	50	26	18	22	10	70	33	58	43	448
C. carinirostris	0	0	1	1	0	5	7	6	1	0	0	21
O. obscurus	12	25	14	3	0	3	9	13	3	6	14	102
Total	117	133	110	47	34	66	47	130	67	106	83	**940**

*Stream surface was frozen; collecting was only possible in one run approximately 25 meters long.

Table 3. Crayfish community collected monthly from August 2010 to July 2011 for the Thorny Creek site.

Crayfish Community, Thorny Creek Site

	Aug. 14th 2010	Sep. 4th 2010	Oct. 9th 2010	Nov. 6th 2010	Dec. 31st 2010*	Feb. 26th 2011	Mar. 18th 2011	Apr. 21st 2011	May 9th 2011	Jun. 18th 2011	Jul. 16th 2011	Total for Year
C. smilax	94	43	74	20	0	36	45	62	84	57	46	561
C. carinirostris	2	3	16	8	0	16	14	27	35	10	3	134
O. obscurus	30	66	16	3	0	5	11	3	12	36	30	212
Total	126	112	106	31	0	57	70	92	131	103	79	**907**

*Thorny Creek stream surface was frozen solid, collecting was not possible.

Four species of crayfish were collected at the West Fork site: *C. smilax*, *C. chasmodactylus*, *C. carinirostris*, and *O. obscurus*. The most abundance species collected was *C. chasmodactylus* (The New River Crayfish). *Cambarus chasmodactylus* abundance was greater in all months except February and March, with *C. smilax* number slightly greater. The greatest number of *C. chasmodactylus* collected from a single month was in June (n = 58). The most *C. smilax* collected was in August (n = 49), September (n = 46), and October (n = 45). The most *O. obscurus* collected was in September (n = 25), October (n = 14), and July n = 14. The other species collected was *C. carinirostris* (The Rock Crayfish). *Cambarus carinirostris* had limited collections at the West Fork site. Collecting in August, September, December, June and July yielded zero specimens. The most *C. carinirostris* collected was in March (n = 7), April (n = 6), and February (n = 5).

The crayfish community at the West Fork Site had the most specimens collected in September (n = 133), April (n = 130), and August (n = 117). The months that had the lowest

number of specimens collected was in November (*n* = 47), March (*n* = 47), and December (*n* = 34). The total 940 crayfish from four species was collected at the West Fork site: *C. chasmodactylus*: (*n* = 448), *C. smilax*: (*n* = 369), *O. obscurus*: (*n* = 102), and *C. carinirostris*: (*n* = 21).

There were three species of crayfish collected at the Thorny Creek site: *C. smilax*, *C. carinirostris*, and *O. obscurus*. *Cambarus chasmodactylus* was not collected at this site. *Cambarus smilax* was the most abundant species collected. The most *C. smilax* collected was in August (*n* = 94), May (*n* = 84), and October (*n* = 74). The only month that *C. smilax* was not the most abundant species collected was September with a larger number of *O. obscurus* collected (*n* = 66 to *n* = 43). The most *O. obscurus* collected was in September (*n* = 66), June (*n* = 36), July (*n* = 30), and August (*n* = 30). *C. carinirostris* had the lowest abundance collected at the Thorny Creek site, but with higher abundance than the West Fork site. The most *C. carinirostris* collected was in May (*n* = 35), April (*n* = 27), October (*n* = 16), and February (*n* = 16).

The overall crayfish community at the Thorny Creek site had the most specimens collected in May (*n* = 131), August (*n* = 126), and September (*n* = 112). December yielded no specimens collected at the Thorny Creek site due to the freezing of the water surface. The other months that had the lowest number of specimens collected was in November (*n* = 31), February (*n* = 57), and March (*n* = 70). A total of 907 crayfish of three species were collected from the Thorny Creek site: *C. smilax* (*n* = 561), *O. obscurus* (*n* = 212), and *C. carinirostris* (*n* = 134).

A series of pie charts depicting changes in the abundances and overall percentages of species from month-to-month are provided in Appendix C. Trends can be observed, such as

the increase in capture abundance of *C. carinirostris* in the colder months, and the increased abundance of *O. obscurus* collected in the warmer months.

Population Observations

The composition of sex and lifestage of *C. smilax* populations can be seen in Tables 4 and 5. Every *C. smilax* specimen collected had data collected for sex, mating status, TCL, chelae length, weight, and any notes such as chelae damage. The smallest gravid female *C. smilax* collected had a TCL of 26.7mm. There were several form I males that had a TCL of 24mm. To distinguish between specimens that could possibly be sexually active and inactive, a minimum TCL of 24mm was established. Form I males were separated from form II males by examining the gonopods. The gonopods are the first pair of male pleopods that are highly specialized for copulation (Guiasu & Dunham 2001). The lateral view of the gonopod on form I males the terminal element is movable, translucent, and downturned at a greater angle. Whereas form II male's first pleopod is fixed and calcified. Secondary characteristics such as the prominent ischial hook on form I males were also examined to differentiate between form I and form II males (Loughman et al. 2011). Females that were gravid were obvious with eggs or young attached to their pleopods. Females that were "In Glair" displayed white patches of polysaccharides around the base of each swimmerette or pleopod (Muck et al. 2002) this acts as cement that will attach the eggs to the pleopods. Juvenile *C. smilax* specimens were under 18mm TCL and with ambiguous sexual characteristics. Neonates were young-of-the-year (YOY) that had become dislodged from gravid *C. smilax* in recent days.

Table 4. West Fork Site breakdown of the composition of *C. smilax* population collected.

***C. smilax* Population Composition, West Fork Site**

	Form I Males	Form II Males	Immature Males	Gravid Female	In Glair Females	Mature Females	Immature Females	Juveniles	Neonates	**Total**	Male to Female Ratio
Aug. 13 2010	7	8	6	0	0	22	6	0	0	**49**	0.75 **:** 1
Sep. 5 2010	13	7	1	0	0	15	2	8	0	**46**	1.24 **:** 1
Oct. 10 2010	10	8	3	0	0	14	6	4	0	**45**	1.05 **:** 1
Nov. 6 2010	2	4	0	0	0	8	1	2	0	**17**	0.67 **:** 1
Dec. 31 2010*	0	5	3	0	0	5	0	3	0	**16**	1.6 **:** 1
Feb. 27 2011	9	6	1	0	0	16	2	2	0	**36**	0.89 **:** 1
Mar. 19 2011	6	1	0	0	0	9	2	2	1	**21**	0.64 **:** 1
Apr. 21 2011	5	6	2	0	0	11	4	12	1	**41**	0.87 **:** 1
May 9 2011	8	9	0	0	3	4	5	1	0	**30**	1.42 **:** 1
Jun. 19 2011	3	17	1	1	1	7	4	8	0	**42**	1.62 **:** 1
Jul. 17 2011	3	13	0	1	2	5	1	1	0	**26**	1.78 **:** 1
Total for Year	66	84	17	2	6	116	33	43	2	**369**	1.06 **:** 1

Table 5. Thorny Creek Site breakdown of the composition of *C. smilax* population collected.

***C. smilax* Population Composition, Thorny Creek Site**

	Form I Males	Form II Males	Immature Males	Gravid Female	In Glair Females	Mature Females	Immature Females	Juveniles	Neonates	Total	Male to Female Ratio
Aug. 14 2010	8	18	10	3	0	23	16	16	0	94	0.86 **:** 1
Sep. 4 2010	5	3	4	1	0	8	11	10	1	43	0.6 **:** 1
Oct. 9 2010	13	9	5	0	0	22	7	18	0	74	0.93 **:** 1
Nov. 6 2010	3	5	1	0	0	6	2	3	0	20	1.13 **:** 1
Dec. 31 2010*	---	---	---	---	---	---	---	---	---	0	* **:** *
Feb. 26 2011	9	6	2	0	0	11	2	6	0	36	1.31 **:** 1
Mar. 18 2011	10	7	1	2	0	11	3	11	0	45	1.13 **:** 1
Apr. 21 2011	13	6	2	0	0	21	2	18	0	62	0.91 **:** 1
May 9 2011	16	8	3	0	2	18	5	32	0	84	1.08 **:** 1
Jun. 18 2011	5	9	5	0	2	19	15	2	0	57	0.53 **:** 1
Jul. 16 2011	5	9	8	6	5	4	4	5	0	46	1.16 **:** 1
Total for Year	87	80	41	12	9	143	67	121	1	**561**	0.9 **:** 1

There were more *C. smilax* collected at the Thorny Creek site (*n* = 561) than the West fork site (*n* = 369) throughout the year. One of the more notable differences between the sites was the ratio of Form I to Form II males between the two sites. At the Thorny Creek site the ratio was 1.00 : 0.92, Form I to Form II males. The West Fork site had a ratio of 1.00 : 1.27, Form I to Form II males. The reduced proportion of Form I males at the West Fork site could

indicate an increased mortality for *C. smilax* at this site. The male to female ratio was almost 1.00 :1.00 at both sites. At the West Fork site the ratio was 1.06 : 1.00 and at the Thorny Creek site the ratio was 0.90 : 1.00. There were several more gravid females collected at the Thorny Creek site (*n* = 12) compared to the West Fork site (*n* = 2). This could also indicate increased mortality at the West Fork site. There were significantly more juvenile and neonate specimens collected from the Thorny Creek site (*n* = 122) compared to the West Fork site (*n* = 45).

Gravid Females

There were 14 gravid female *C. smilax* collected throughout the year. There were only two gravid females collected at the West Fork site, one in June and one in July. The Thorny Creek site yielded many more gravid females with a total of 12. Six were collected in July, three in August, one in September, and two in March. *Cambarus smilax* females that displayed glair were also recorded. There were a total of 17 individuals that were recorded as displaying glair. There were six at the West Fork site, and there were nine at the Thorny Creek site. At both sites individuals with glair were in May, June and July.

The months of May, June, July, August and September were consecutive months that displayed females that were gravid or in glair at one or both sites. The only instance where there were gravid or in glair females outside of these month occurred, was March when two gravid females were collected at the Thorny Creek site. These results are represented in Figures 5 and 6 below. Hamr and Berrill (1985) found similar results with seasonal offspring development, with all of the gravid *C. robustus* females found in the months of July, August and September. Corey (1990) found the majority of gravid females occurred in the month of

July, followed by August and September. The comparison between this study and these seems to correlate, and begin to display a comparison between *C. smilax* life history and *C. robustus*.

Figure 5. *Cambarus smilax* females that were gravid or in glair at the West Fork Site.

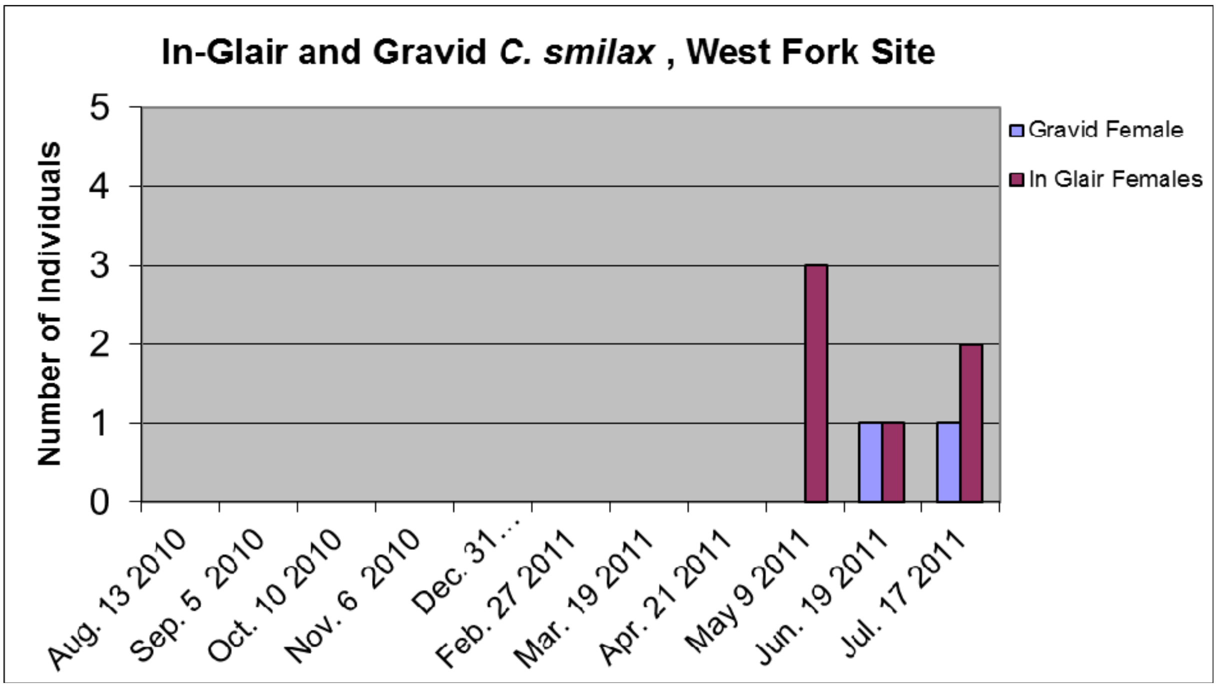
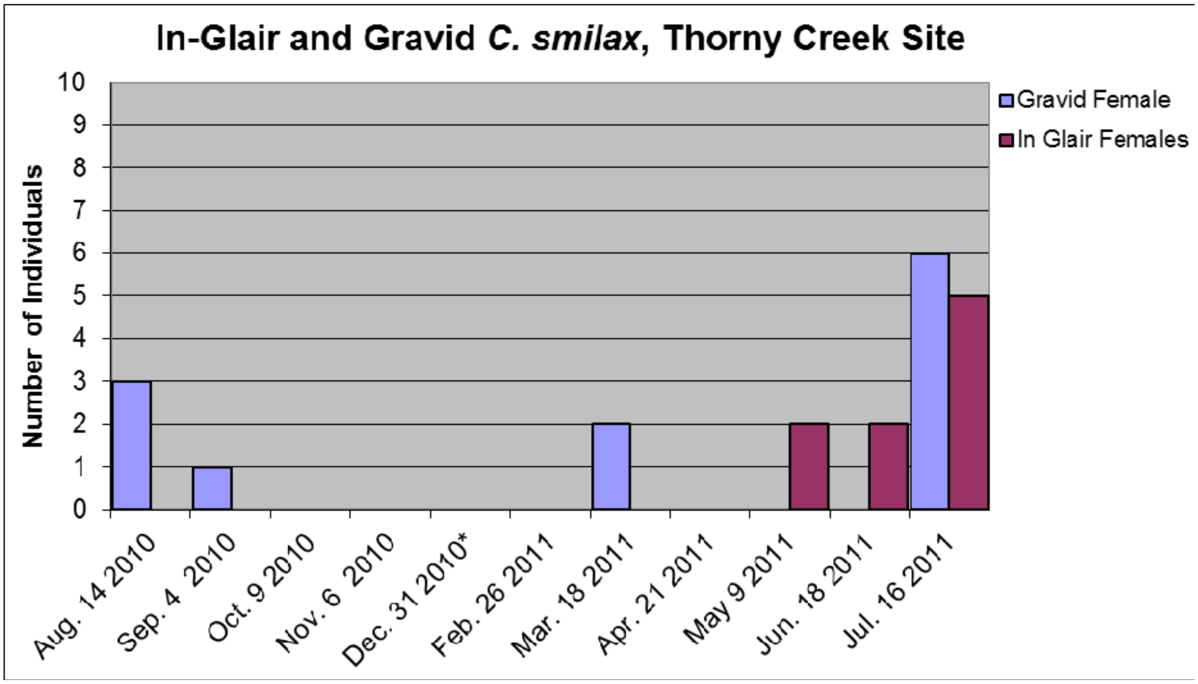


Figure 6. *Cambarus smilax* females that were gravid or in glair at the Thorny Creek Site.



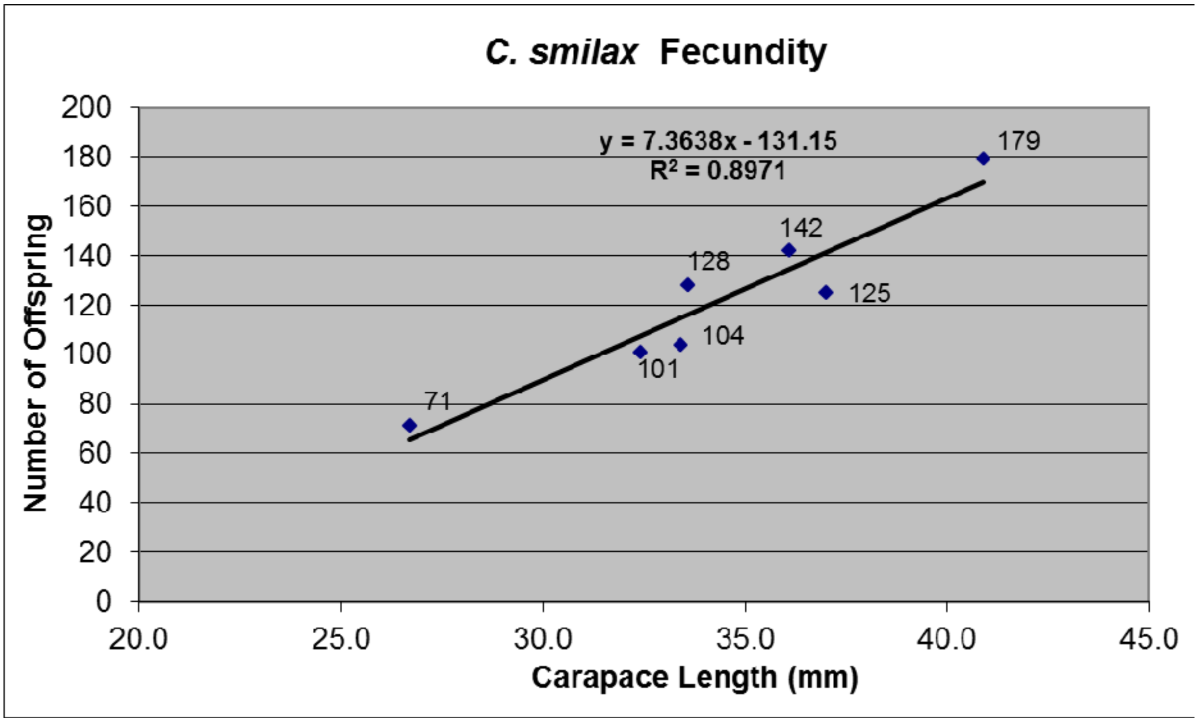
Fecundity

Cambarus smilax specimens that were gravid were put into containers containing 70% ethanol solution. In the lab the fecundity was recorded with each specimen. Some gravid specimens displayed eggs, referred to as berries (see photo Appendix B). Other gravid specimens had early instar young, already developed from eggs (see photo Appendix B) All egg extrusion was found in May, June, and July. Early instar larva were collected in July, August, and September taking about 30 days to hatch. Then another 30 days to reach 3rd instar and become dislodged from the female in September and October, or as described by Corey (1990) 'overwintering young' stay attached to the female throughout the winter and are released in March, such as the two gravid females collected at the Thorny Creek site.

The total number of offspring was compared to the TCL of the gravid female to establish a regression line. As can be seen with most crayfish species, the larger the female

the more offspring produced. There were only seven of the 14 total gravid females collected, used to create this regression (Figure 7). This is due to young becoming dislodged (see photo Appendix B).

Figure 7. Gravid *C. smilax* linear regression between TCL and number of offspring.



The smallest ovigerous female had a TCL of 26.7 mm, and it had the fewest offspring (n = 71). The largest ovigerous female had a TCL of 40.9 mm, and it had the largest number of offspring (n = 179). The mean carapace length for ovigerous females was 34.3 mm, with a range of 26.7 to 40.9 mm. The mean egg number was 121.4, with a range of 71 to 179. Looking at ovigerous *C. robustus* in Southern Ontario, Corey (1990) found a mean carapace length of 28.6 mm, with a range of 24.3 to 35.4 mm. And the mean egg number was 61.1,

with a range of 30 to 108. These numbers display a smaller overall ovigerous female carrying fewer offspring. Also in Southern Ontario, Hamr and Berrill (1985) give accounts for ovigerous *C. robustus* much larger in TCL and brood size; the mean TCL was 44 mm, with a range of 35 – 57 mm. Some ovigerous females had over 200 offspring, but the data points were more widely dispersed displaying a coefficient of determination of $R^2 = 0.25$. The egg diameter measured from each *C. smilax* in berry had an average egg diameter found to be 2.78 mm. This is very close to the 2.7 mm average found by Hamr and Berrill (1985) and the 2.6 mm average found by Corey (1990).

Molting

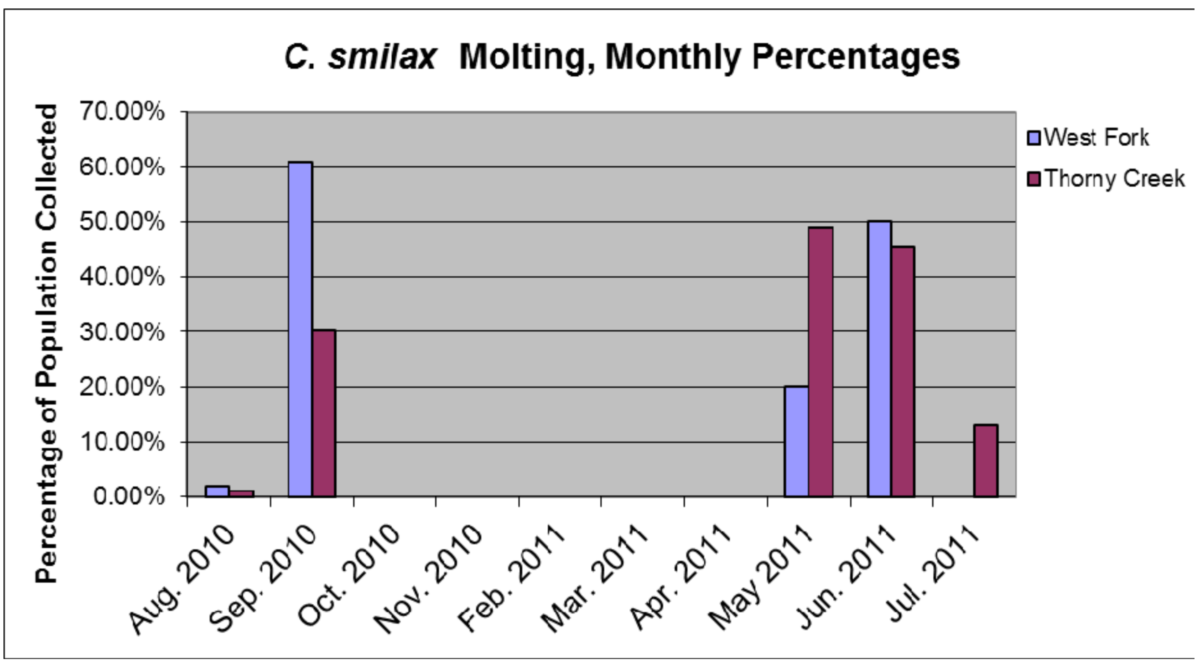
Molting is an essential part of the life cycle of *C. smilax* and all crustaceans for growth and development. Molting involves the shedding of the hard exoskeleton, leaving the crayfish soft-bodied and vulnerable for two to several days (Guiasu & Dunham 2001). Molting also allows crayfish in the Cambaridae family to alternate between sexually active (form I males) and inactive (form II males) forms (Guiasu & Dunham 2001). Newly molted crayfish often seek refuge under embedded rocks and crevices until their new exoskeleton becomes hardened.

Cambarus smilax was first observed molting in August 2010 with only a single individual from each study site. The following month of September yielded many freshly molted individuals at both the West Fork site (n = 28) and the Thorny Creek site (n = 13). Then it was eight months before molting was again observed. In May 2011 there molting specimens at both the West Fork site (n = 6), and at the Thorny Creek site (n = 41). The

following month, June, also yielded more molting specimens at the West Fork site (n = 21), and at the Thorny Creek site (n = 26). The following month, July, yielded a few molting individuals at the Thorny Creek site (n = 6) but no molting specimens from the West Fork site.

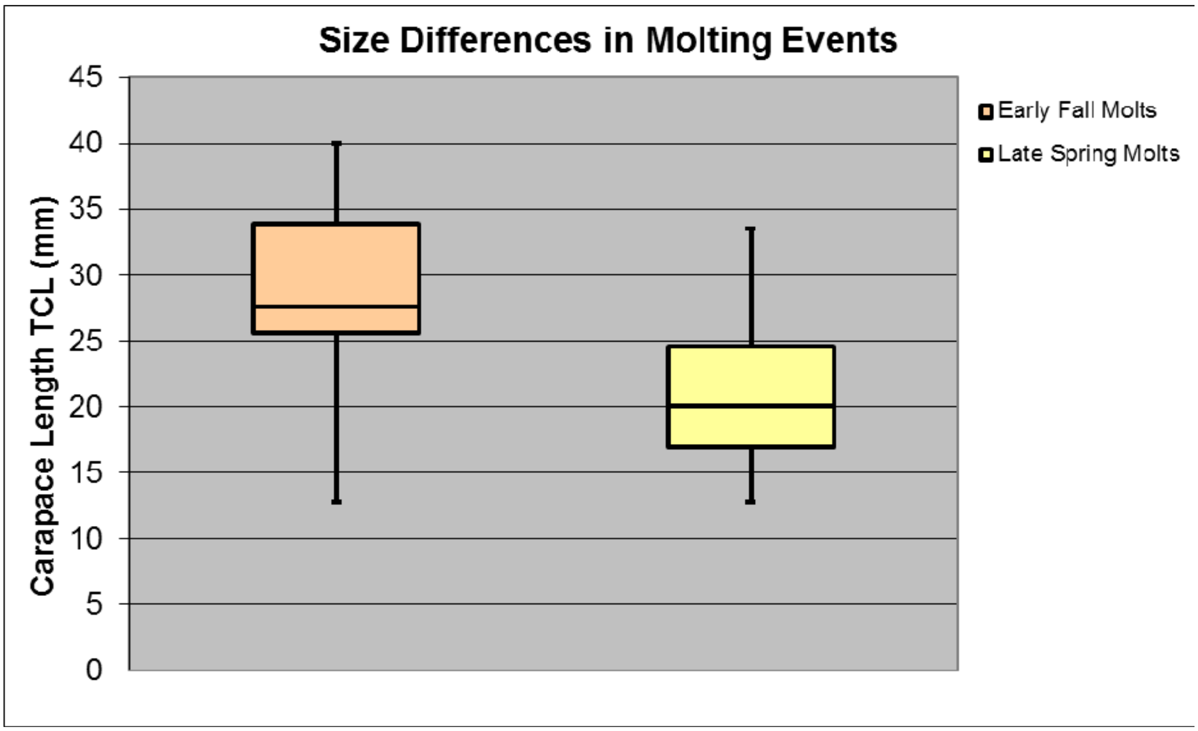
From these observations it appears that there are two separate molting events. One molting event centered in September and included some of August (Early Fall). The other molting event centered about mid-June and included May, June, and July (Late Spring). The monthly molting percentages of *C. smilax* collected at both sites are represented in Figure 8.

Figure 8. Monthly molting percentages of *C. smilax* at both the West Fork and Thorny Creek sites.



The size differences between the Early Fall and Late Spring molting *C. smilax* were notable. The larger size class of the early Fall molts is emphasized by a median size of 27.6 mm over the late Spring median size of 20.0 mm. The box-and-whisker plot in (Figure 9) displays the differences in sizes between the molting events. These differences indicate that different age classes of *C. smilax* are molting during these different times; older specimens in the early fall, and younger specimens in the late spring.

Figure 9. Box-and-whisker plot showing differences in size classes between molting events.



Hamr and Berrill (1985), Corey (1990), and Guiasu and Dunham (2001) all found similar patterns in molting *C. robustus*. The majority of molting took place mainly in two events, one around May, June and July which involved mostly immature specimens, and the

other around September when the majority of molting specimens were mature. Hamr and Berrill (1985) study most closely correlated with the molting patterns found here for *C. smilax*, where all mature crayfish molted in September, and most immature crayfish molted in May, June and July. Guiasu and Dunham (2001) found the majority of mature males and females molted in September, October or November. Further, there was no example of a form I male molting to a form II taking place outside of October or November found in any of their studies.

Male Form I to Form II

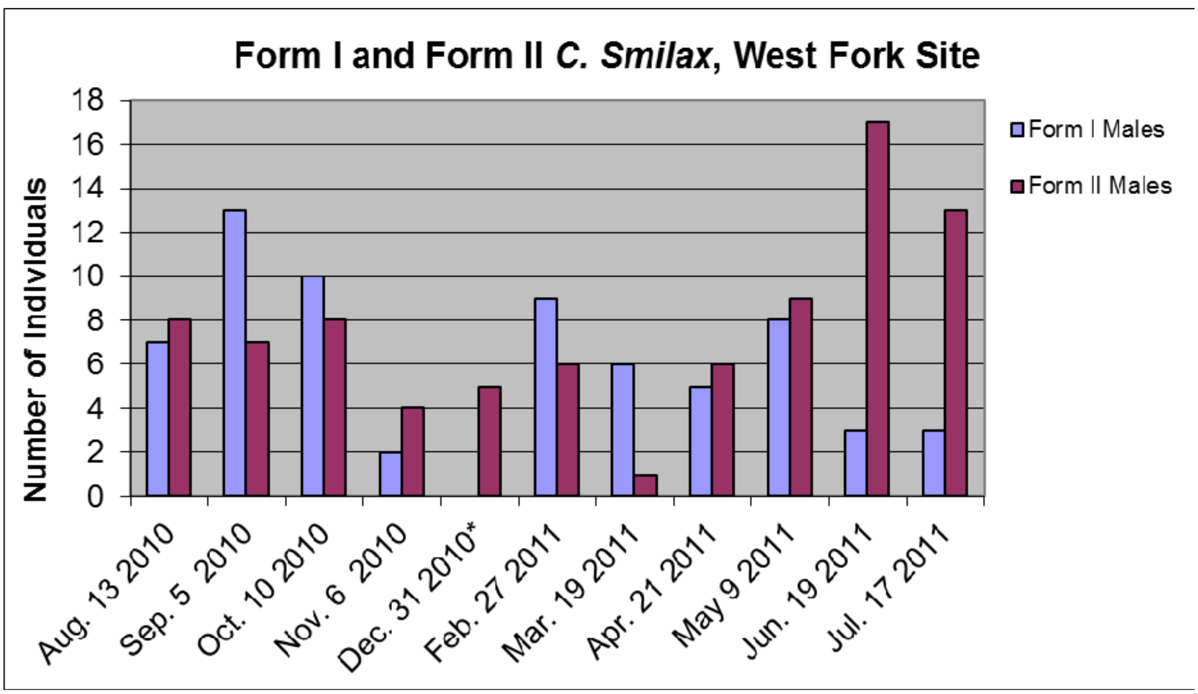
The proportion of *C. smilax* form I to form II males is important because it gives insight into when males are becoming sexual mature and sexually active. Form I males are found throughout the year. However there were more form I males collected in months of September – May prior to the hypothesized main breeding season in May, June, and July. A representative photograph of a form I male with active gonopods and enlarged ischial hooks is displayed in Appendix B. This specimen was collected on 18 March when water temperatures were cooler (7.3° C).

There were 150 males with a TCL > 24mm at the West Fork site (*n* = 66 form I and *n* = 84 form II). The ratio for form I to form II at the West Fork site was 0.79: 1. There were a total of 167 males with a TCL > 24mm at the Thorny Creek site (*n* = 87 form and *n* = 80 form II). The ratio for the form I to form II at the Thorny Creek site was 1.09: 1. There was a greater ratio of form I to form II males at the Thorny Creek site. There could be many reasons for the disproportionate ratios between the two sites. The larger stream size of the West Fork

could influence *C. smilax* longevity. The absence of *C. chasmodactylus* may allow some greater longevity for *C. smilax* males to molt into sexually mature form I.

The months with the greatest amount of form I males at the West Fork site were September (*n* = 13) and October (*n* = 10). The months with the greatest number of form II males at the West Fork site were June (n = 17), July (n = 13), and May (n = 9). These results can be seen in Figure 10.

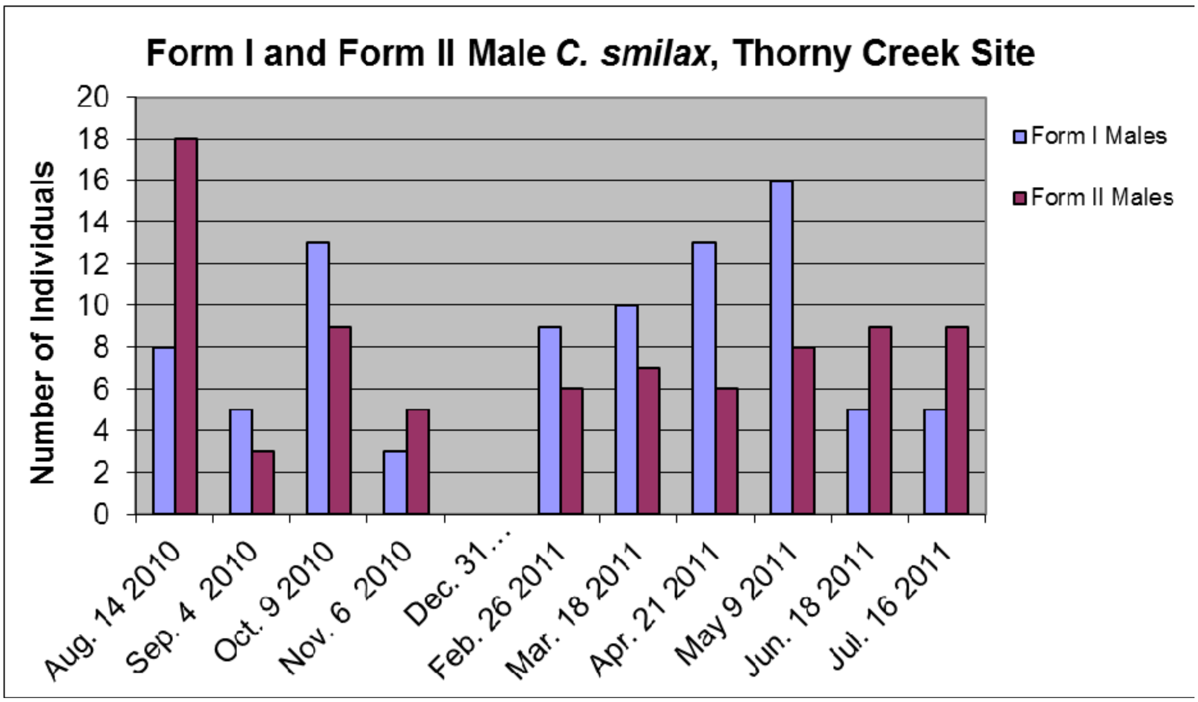
Figure 10. The number of *C. smilax* males in form I and form II for each month at the West Fork site.



The months with the greatest amount of form I males at the Thorny Creek site were May (*n* = 16) and October (*n* = 13). October was one of the two top months for the greatest amount of form I males collected at both sites, this would be just after the molting event in

September. The months with the greatest number of form II males for the Thorny Creek site was August (*n* = 18) and October (*n* = 9). Significantly the months that followed were June (*n* = 9), July (*n* = 9), and May (*n* = 8); which corresponds with the West Fork site. These results can be seen in Figure 11.

Figure 11. The number of *C. smilax* males in form I and form II for each month at the Thorny Creek site.



There seems to be some slight trends established at both sites. When applying what is known about the two molting events, it seems that the late spring molt (May, June, and July) leads to a greater proportion of form II. This is most likely due to males reaching a size that could necessitate maturity (TCL > 24 mm) but not yet have molted into form I for the first time. Accordingly it can be observed that the there is a switch over to more form I males in September at both sites. This also corresponds with a molting event, that being the fall

(September) molting event. This tendency is probably influenced by many males molting into sexual maturity for the first time in their second complete year of life. As a result of mortality of mature males, this trend could possibly be a yearly cycle that can be observed each year. It is also possible that this trend changes from year-to-year.

There are a majority of form I males in the months of September, October, February, March, April and May. Excluding the cold winter months (November, December, and January) it is hypothesized that these are the months when most of the mating for *C. smilax* takes place. Corey (1990) concluded the mating season for his population of *C. robustus* reached its peak in May and June. He based this time-line in part due to a greater ratio of form I to form II males in the month of May. The months of April and May had the greatest ratio of form I to form II at the Thorny Creek site (April 2.17 :1.00 and May 2.00 : 1.00). It would stand to reason that the warmer weather in Northern West Virginia compared to Southern Ontario would lead to an earlier mating season. Hamr and Berrill (1985) also put the mating season for their *C. robustus* population in May, June and July. It should be noted that the only copulation observed in this study was on October 9th, and took place inside a holding bucket. Since mating was observed in October, (this could account for late instar being found on two gravid females in March) the mating season for *C. smilax* should include the months of September and October, as well as April, May, June and July, with the peak taking place in May and June.

Interspecific Habitat Partitioning

Interspecific habitat partitioning is a term to describe the decipherable areas of the stream such as pools, runs, and riffles utilized between crayfish species. Previous studies have

shown that when two or more crayfish species are present at a site they may exhibit habitat preferences and dominant species shift subordinate species habitat (DiStefano 2003; Rabeni 1985). At the beginning of the study it was observed that there habitat types where certain species were more likely to occur. When collecting crayfish they were put into a bucket labeled with the habitat they were found in: Riffle, Run, or Pool. Although this is a qualitative value, it gives some comparison between habitat selections at both sites.

The most obvious habitat partitioning was the lack of *C. smilax* in runs and riffles at the West Fork site, replaced by *C. chasmodactylus*. However, runs and riffles were utilized by *C. smilax* at the Thorny Creek site. As the closest relative, *C. robustus*, is found in 3^{rd} to 5^{th} order streams and prefers microhabitats of leaf packs and boulders (Loughman et al. 2011). *Cambarus chasmodactylus* is found in 4^{th} and 5^{th} order streams under boulders mid-stream in larger streams and rivers (Loughman et al. 2009). It appears that the larger *C. chasmodactylus* is more adapt to living in higher water velocities and therefore dominant to *C. smilax* at West Fork site. The effect is that there are more *C. chasmodactylus* found in runs and riffles, and *C. smilax* is pushed into lotic water such as pools and bank margins. The results of habitat partitioning are represented in Figures 12 and 13.

Figure 12. Qualitative habitat type of all crayfish species collected at the West Fork site.

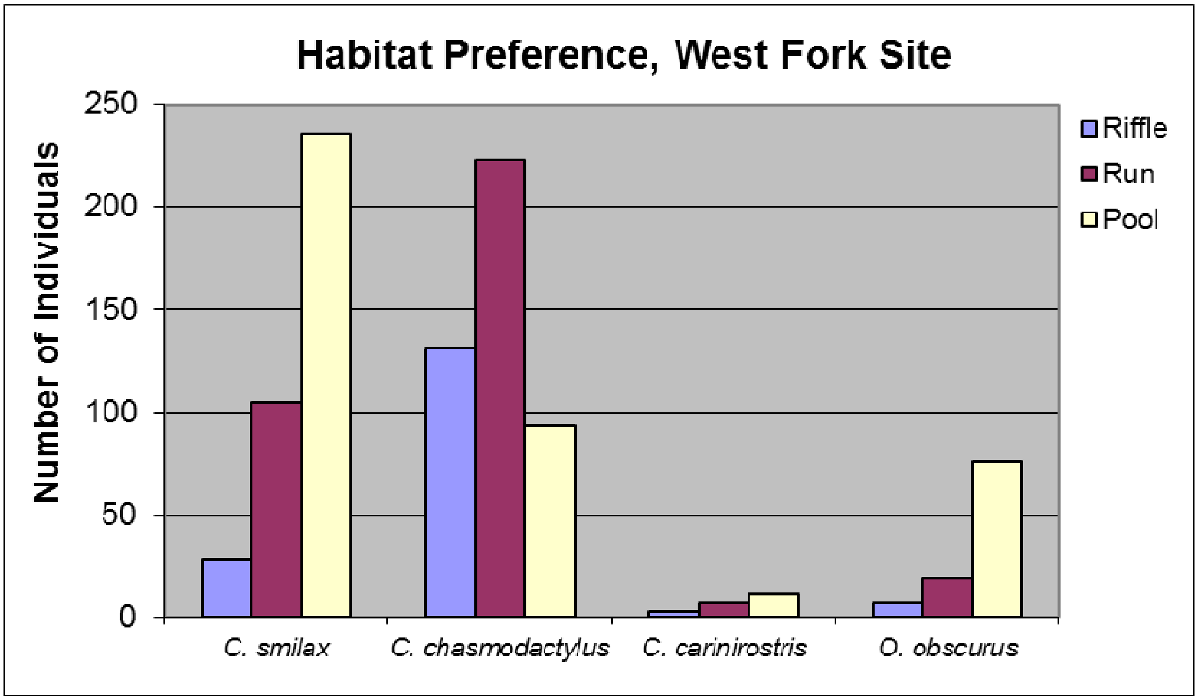
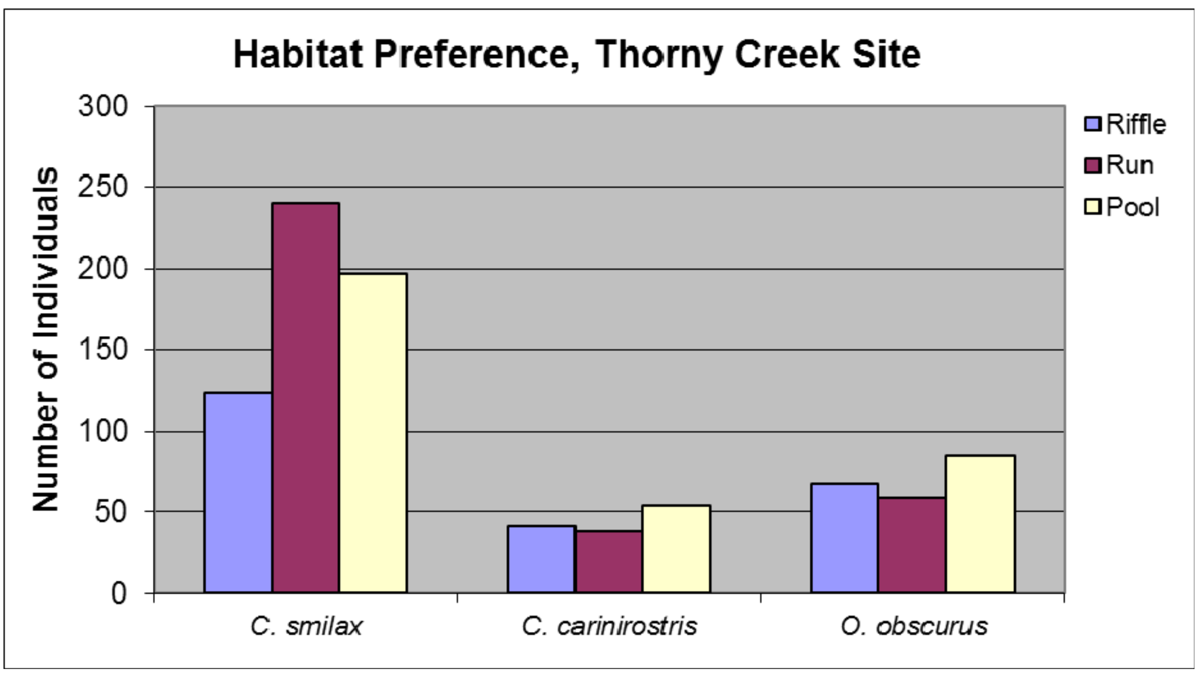


Figure 13. Qualitative habitat type of all crayfish species collected at the Thorny Creek site.



At the West Fork site *C. smilax* was collected from lotic pool habitat and the littoral zone more than twice as often as run and riffle combined. *Cambarus chasmodactylus* was

collected in the greater velocities found in runs and riffles. At the Thorny Creek site *C. smilax* was collected from runs, riffles, and pools with increased similarity. It could indicate that with the lack of competition from an absence of *C. chasmodactylus*, *C. smilax* expands its range throughout the stream. It is also possible that *C. smilax* simply can't occupy areas with higher velocity like those found at the West Fork site.

Another phenomenon observed was *C. carinirostris* selecting habitat areas around the banks and under isolated boulders on the bank. *Cambarus carinirostris* inhabits mainly 1st and 2nd order streams but can be founding larger streams were it inhabits side pools, eddies, and stream margins (Loughman et al. 2011). *Cambarus carinirostris* was found at both sites, but more were collected at the smaller Thorny Creek site. If there were crayfish present under an isolated boulder on the bank, it would almost always be *C. carinirostris*. It seems as though *C. carinirostris* is subordinate to *C. smilax* and *C. chasmodactylus* and it is being pushed to marginal habitat.

Crayfish Morphometric Analysis

Size Cohorts

As each generation of *C. smilax* grows from month-to-month there can be recognizable size cohorts observed. This data was put into a histogram in 1 mm increments. Observable size classes can be seen. Each size class is hypothesized to be mostly from the same generation.

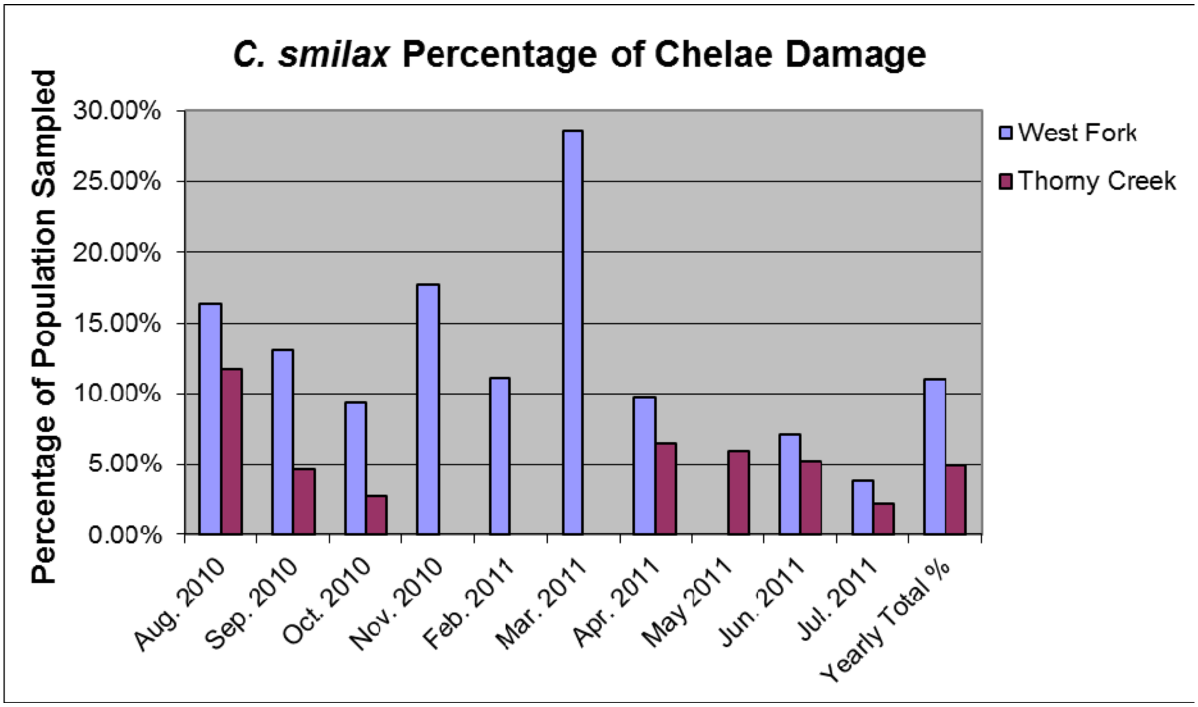
Some months have more distinct cohort groups, where there is almost a bell curve for each group. The more recognizable size groups are from the Thorny Creek site, due to there

being more individual collected at this site. The data from both sites was kept separate due to noticeable differences between the two sites. These size cohorts can be seen in Appendix E.

Chelae Damage

Examining each crayfish specimen collected, notes were taken if there was damage to one or both chelae. This would include chelae that were damaged, missing, or regenerating after loss. Because the number of crayfish collected each month varied with such factors as water temperature and water velocity, percentages give a more accurate account of chelae damage per month than the total number. Looking at the *C. smilax* population at each site, a percentage was calculated for the overall number collected that had damaged chelae (number individuals with damaged chelae / total number collected). These percentages for both sites can be seen in Figure 14.

Figure 14. The percentage of *C. smilax* species that had damaged, missing, or regenerating chelae at both the West Fork and Thorny Creek sites.



The Thorny Creek site has a considerably lower percentage of *C. smilax* individuals displaying chelae damage. The only month that displayed a higher percentage at the Thorny Creek site was May. The months and amounts with the highest values was August (n = 11) for the Thorny Creek site, and August (n = 8) for the West Fork site. Even though the amount of individuals was higher at the Thorny Creek site, the total amount of *C. smilax* collected was also significantly higher (Thorny Creek [n = 94] to West Fork [n = 49]) thus a percentage of 10.64% for the Thorny Creek site compared to the 16.33% for the West Fork site is a better representation of the amount of chelae damage happening to the *C. smilax* population at each site.

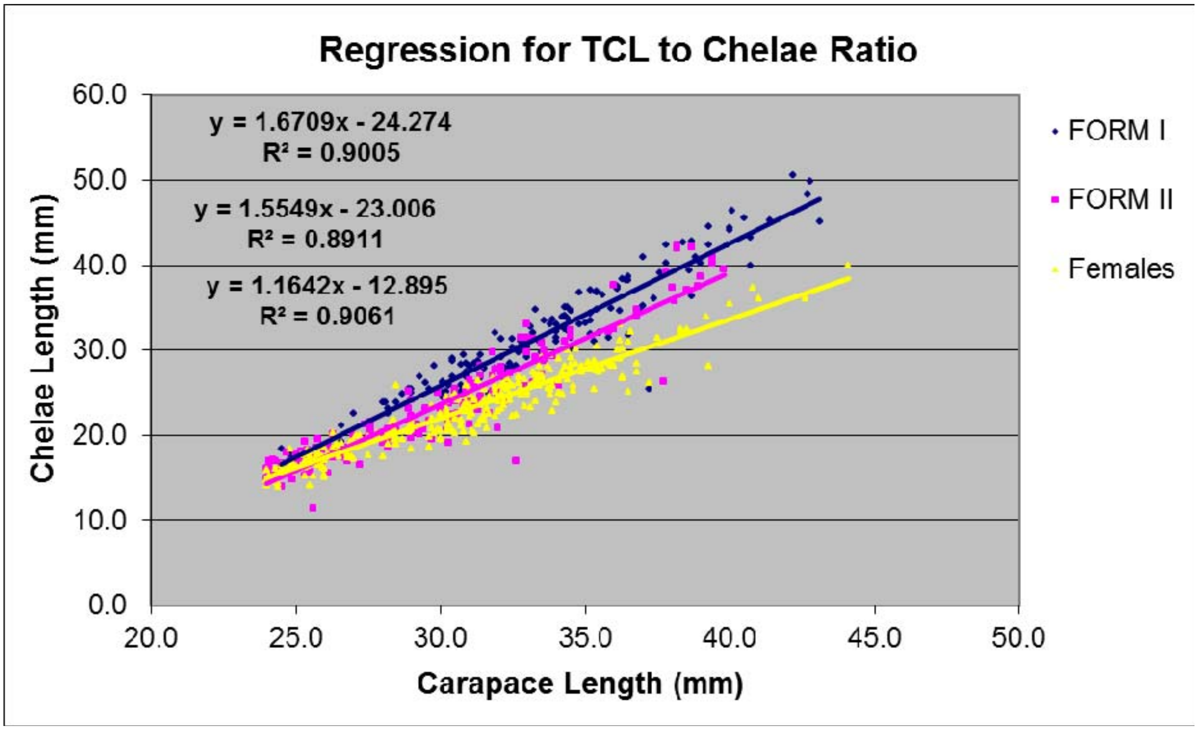
The total percentage for chelae damage for the entire year was 4.99% at the Thorny Creek site. The West Fork site displayed more than twice that percentage for damaged chelae in the collected population for the year with 11.05%. These calculations suggest that the

significantly greater amount of chelae damage occurring to the *C. smilax* population at the West Fork site is indicative of some type of competition, such as interspecies competition from *C. chasmodactylus*, or possibly more intraspecies competition within the *C. smilax* population.

Chelae to Total Carapace Length Relationship

It has been shown that there are larger chelae found on form I males compared to form II males in *C. robustus* and other crayfish species (Jezerinac et al. 1995). Larger chela assists in copulating with females, competing with males, and may possibly be used in sexual selection by females. To display the chelae differences between *C. smilax* form I males, form II males and females a regression between TCL length and chelae length was established (Figure 15).

Figure 15. Regression displaying the chelae length to TCL relationship between form I males, form II males, and females.

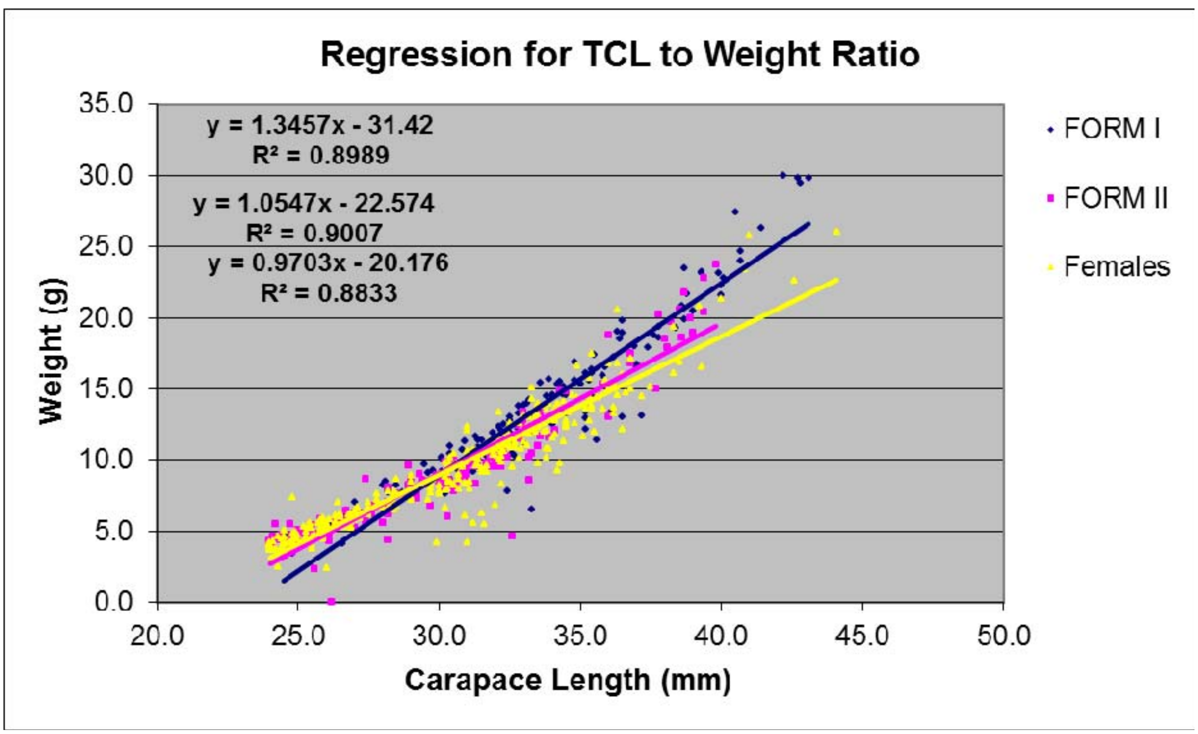


It can be seen in Figure 15 that form I males have a larger chelae to TCL correlation compared to form II males. And females have smaller chelae to TCL correlation than form I or form II males. The slope, denoted *m* value, is the rise (chela) divided by the run (CLP). Therefore the slope will become steeper the larger the ratio of chela size is to carapace size. The greatest ratio of chela size compared to TCL size is for form I males with *m* = 1.67, followed by form II males with *m* = 1.55, and females with the smallest slope *m* = 1.16. Using the coefficient of determination, denoted R^2, this statistical formula indicate a best fit for the data points of $R^2 = 0.90$ for form I males, $R^2 = 0.89$ for form II males, and $R^2 = 0.91$ for females. With a strong coefficient of determination, this data does show that *C. smilax* form I males do have larger chela compared to form II males.

Weight to Total Carapace Length Relationship

It is hypothesized that form I males will have greater weight allocations compared to form II males. After thorough literature review, no previous studies displayed weight as a characteristic differing between form I and form II males. The extra weight may be used as energy reserve in order to molt into form I and then mate. Further, the added size can be useful in competing with other males and copulating with females. To display the weight differences between form I males, form II males and females a regression between weight and chelae length was established (Figure 16).

Figure 16. Regression displaying the weight to TCL relationship between form I males, form II males, and females.



It can be seen in Figure 16 that form I males have a greater weight to TCL correlation compared to form II males. Females have smaller weight to TCL correlation than form I or

form II males. The slope, denoted *m* value, is the rise (weight) divided by the run (TCP). The slope will become steeper as the ratio of weight increases to TCL. The largest ratio is for form I males with *m* = 1.33, followed by form II males with *m* = 1.05, and females with the smallest slope *m* = 0.97. Using the coefficient of determination, denoted R2, this statistical formula indicate a best fit for the data points of R^2 = 0.90 for form I males, R^2 = 0.90 for form II males, and R^2 = 0.88 for females. Weight to TCL also displayed a strong coefficient of determination; approximately the same as chela to TCL. This data does show that *C. smilax* form I males do have larger weight compared to form II males.

Slope change for weight was greater between form I and form II males than the slope change for chela length. The slope change for chela to TCL was *m* = 1.67 for form I and *m* = 1.55 for form II, for a difference = 0.12. The slope change for weight to TCL was *m* = 1.33 for form I and *m* = 1.05 for form II, for a difference = 0.28. Looking at chela and weight difference between form I and form II males it is evident that morphologic changes are taking place in both chela length and weight.

Physical Stream Characteristics

Physical stream characteristics were assessed at each site using the West Virginia Department of Environmental Protection's (WVDEP) Rapid Bioassessment Protocols (RBP). This protocol uses ten habitat parameters: [1] Epifaunal Substrate / Available Fish Cover, [2] Embeddedness, [3] Velocity / Depth Regimes, [4] Channel Alteration, [5] Sediment Deposition, [6] Riffle Frequency, [7] Channel Flow Status, [8] Bank Stability, [9] Bank Vegetation Protection, [10] Width of Undisturbed Vegetation Zone. These RBP parameters are scored 1 – 20 into categories ranked: Poor, Marginal, Sub-optimal, and Optimal respectively (WVDEP 2011).

After several visits and walking the sites thoroughly a qualitative score was given to each parameter. These results can be seen in Table 6.

Table 6. WVDEP Rapid Bioassessment Protocol displaying qualitative scores for physical characteristics that impact stream quality for both sites.

West Fork Site			
	Habitat Parameter	Score	Category
1	Epifaunal Substrate/ Available Fish Cover	18	Optimal
2	Embeddedness	19	Optimal
3	Velocity/ Depth Regimes	19	Optimal
4	Channel Alteration	18	Optimal
5	Sediment Deposition	18	Optimal
6	Riffle Frequency	19	Optimal
7	Channel Flow Status	18	Optimal
8	Bank Stability	18	Optimal
9	Bank Vegetative Protection	15	Sub-Optimal
10	Width of Undisturbed Veg. Zone	14	Sub-Optimal
	Total	176	Optimal
	Esitmated Milage of Upstream Watershed Evaluated		

Thorny Creek Site			
	Habitat Parameter	Score	Category
1	Epifaunal Substrate/ Available Fish Cover	17	Optimal
2	Embeddedness	19	Optimal
3	Velocity/ Depth Regimes	18	Optimal
4	Channel Alteration	19	Optimal
5	Sediment Deposition	19	Optimal
6	Riffle Frequency	18	Optimal
7	Channel Flow Status	17	Optimal
8	Bank Stability	18	Optimal
9	Bank Vegetative Protection	17	Optimal
10	Width of Undisturbed Veg. Zone	18	Optimal
	Total	180	Optimal
	Esitmated Milage of Upstream Watershed Evaluated		

Both sites scored in the optimal category displaying either Optimal or Sub-Optimal scores for each parameter. The only Sub-Optimal scores were at the West Fork site for ‘Bank Vegetative Protection’ and Width of Undisturbed Vegetation Zone’. These scores were lowered by the direct impact of the rail-to-trail hiking and biking trail. This trail does impact the riparian zone which affects both of these parameters. The Thorny Creek site’s greatest

impact came from a slightly widened channel that affects the amount of the channel that has water in it throughout the year.

Stream Characterization Using GIS Spatial Analyst

ESRI's GIS ArcMap 10.1 software was used to characterize water depth, stream flow in the form of water velocity, and substrate type throughout each study site. Approximately 120 depth, water velocity, and substrate data points were taken at each site for interpolation using inverse distance weighting (IDW) spatial analysis GIS tools. Data for stream characterization analysis was collected during normal flow conditions in June 2010.

Water Depth

Stream depth was measured in tenths of a foot. A maps depicting water depth for the study sites is located in Appendix D.

The West Fork site had a maximum depth of 1.8' and an average depth of 0.7'. The West Fork site was separated into three habitat types Riffle (31.6%), Run (29.1%), and Pool (40.2%). In riffle type habitat the minimum depth was 0.2', the maximum depth was 0.7', and the average was 0.4'. In run type habitat the minimum depth was 0.4', the maximum depth was 1.4', and the average was 0.9'. In pool type habitat the minimum depth was 0.4', the maximum depth was 1.8', and the average was 1.0'.

The Thorny Creek site had a maximum depth of 2.0' and an average depth of 0.7'. The Thorny Creek site was separated into three habitat types Riffle (34.7%), Run (42.7%), and Pool (21.8%).In riffle type habitat the minimum depth was 0.2', the maximum depth was 1.0', and the average was 0.5'. In run type habitat the minimum depth was 0.5', the maximum

depth was 1.7’, and the average was 0.9’. In pool type habitat the minimum depth was 0.3’, the maximum depth was 2.0’, and the average was 0.8’.

These conditions are normal for June but lower than average for the year. It should be noted that although these readings display greater depths at Thorny Creek than the West Fork, this is due to low water conditions. The wider channel at the West Fork site displaces water over a greater area. Most of the year depths will be greater at the West Fork.

Stream Flow

Stream velocity was measured in feet per second using the Marsh-McBirney Flow-Meter. A maps depicting stream flow velocity for the study sites is located in Appendix D.

The West Fork site had a maximum velocity of 1.66 fps and an average velocity of 0.45 fps. In riffle type habitat the minimum velocity was 0.25 fps, the maximum velocity was 1.66 fps, and the average was 0.85 fps. In run type habitat the minimum velocity was 0.19 fps, the maximum velocity was 0.80 fps, and the average was 0.47 fps. In pool type habitat the minimum velocity was 0.00 fps, the maximum velocity was 0.42 fps, and the average was 0.12 fps.

The Thorny Creek site had a maximum velocity of 2.78 fps and an average velocity of 0.96 fps. In riffle type habitat the minimum velocity was 0.46 fps, the maximum velocity was 2.78 fps, and the average was 1.38 fps. In run type habitat the minimum velocity was 0.24 fps, the maximum velocity was 2.05 fps, and the average was 1.04 fps. In pool type habitat the minimum velocity was 0.00 fps, the maximum velocity was 0.75 fps, and the average was 0.15 fps.

This is about normal conditions for June but lower than average for the year. It should be noted that although these readings display greater velocities at Thorny Creek than the West Fork, this is due to low water conditions. The wider channel at the West Fork displaces water over greater area so flow is thwarted. Most of the year greater velocities are found at the West Fork.

Substrate Condition

Stream substrate was measured using a Wolman Pebble Count technique; where substrate was randomly picked up through the stream and measured across its intermediate axis. Qualitatively substrate is rated as fines/sand, gravel, cobble, boulder or bedrock. Due to a large quantity of slab boulders at both sites, boulder was the most abundant substrate type found at both sites. A maps depicting substrate type for the study sites is located in Appendix D.

At the West Fork site the dominant substrate was cobble (48.7%), followed by boulder (44.4%). There were small areas where sand/fines (5.2%) were observed. Very little gravel (0.9%) and bedrock (0.9%) persisted.

At the Thorny Creek site the dominant substrate was boulder (56.5%), followed by cobble (39.5%). There were a few small areas of gravel (3.2%). No discernible areas of fines/sand or bedrock were measured.

Physiochemical Stream Characteristics

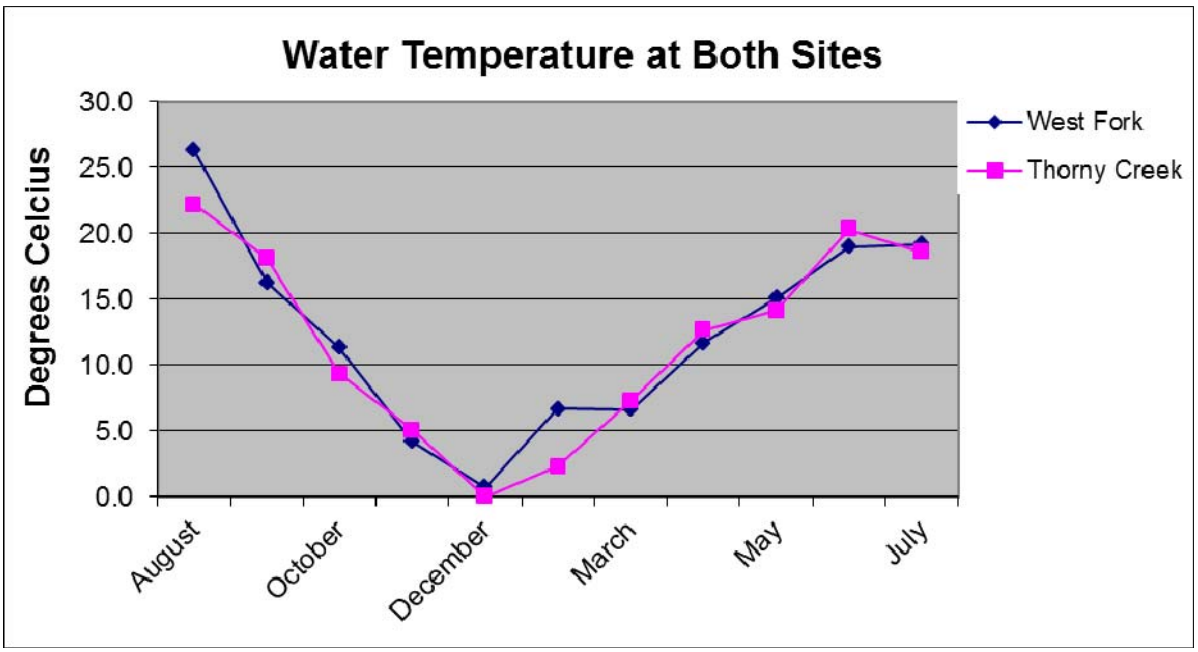
Water chemistry parameters were measured in the field using a Hach Model: HQ40D multi-probe. Water temperature, pH, Dissolved Oxygen (DO), and conductivity were

measured at each site monthly. At least three readings for each parameter were recorded for each site. The readings were averaged and graphed to compare data collection events.

Temperature

Water temperature at each site was similar (Figure 17). The highest water temperature observed was in August at 26.3 ºC for the West Fork site and 22.2 ºC for the Thorny Creek site. The lowest water temperature observed was in December at 0.7 ºC for the West Fork site and 0.0 ºC at the Thorny Creek site where the surface was completely froze over.

Figure 17. Water temperature (Cº) recorded monthly at each site.



Dissolved Oxygen

There is a strong negative correlation between water temperature and DO. As the water cools it is able to hold more DO. Water temperature and DO are so correlated that the

data sets in Figure 18 and 19 appear inverted; as water temperature warms or cools, DO levels drop or rise proportionally.

Figure 18. Displaying the correlation between water temperature (Cº) and DO (mg/L), at the West Fork site.

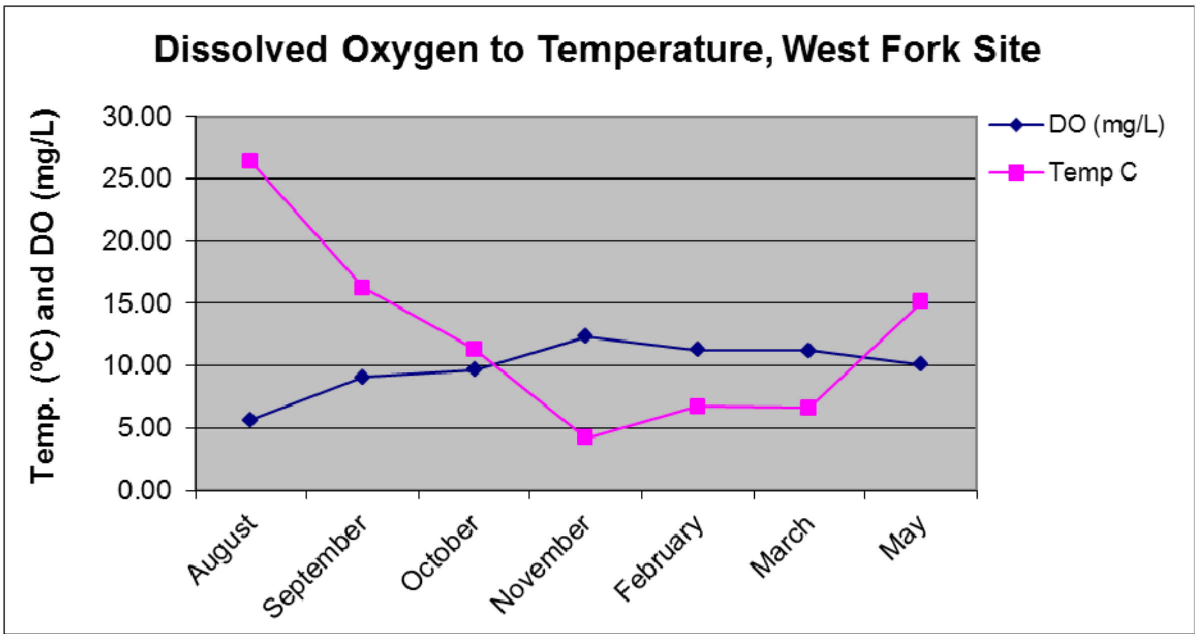
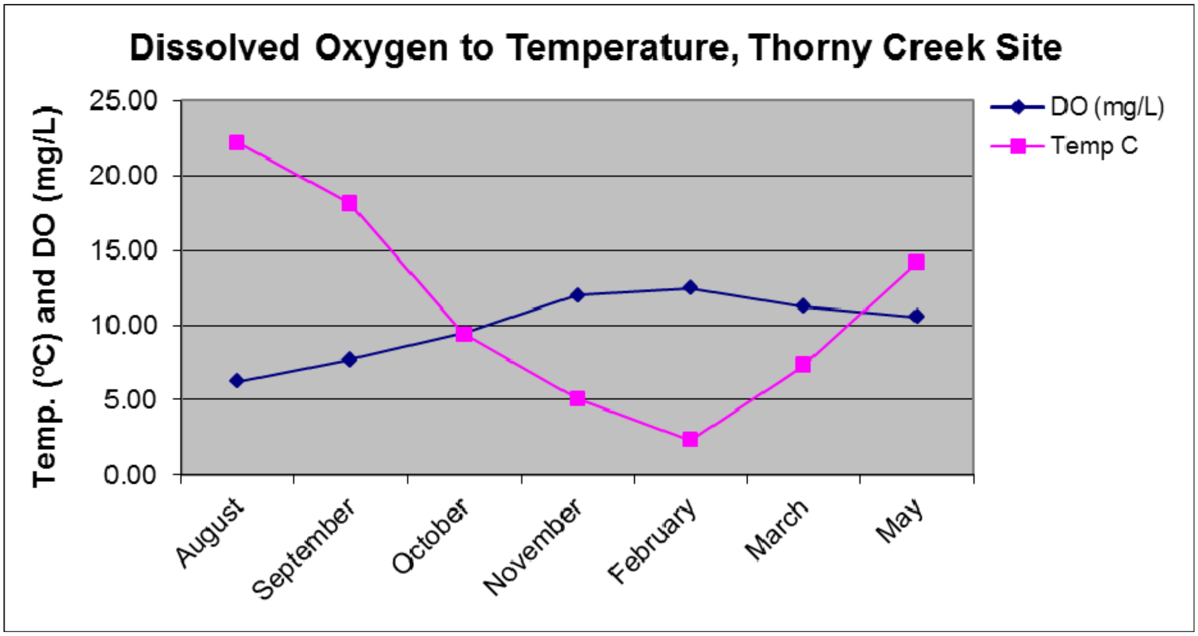


Figure 19. Displaying the correlation between water temperature (Cº) and DO (mg/L), at the Thorny Creek site.



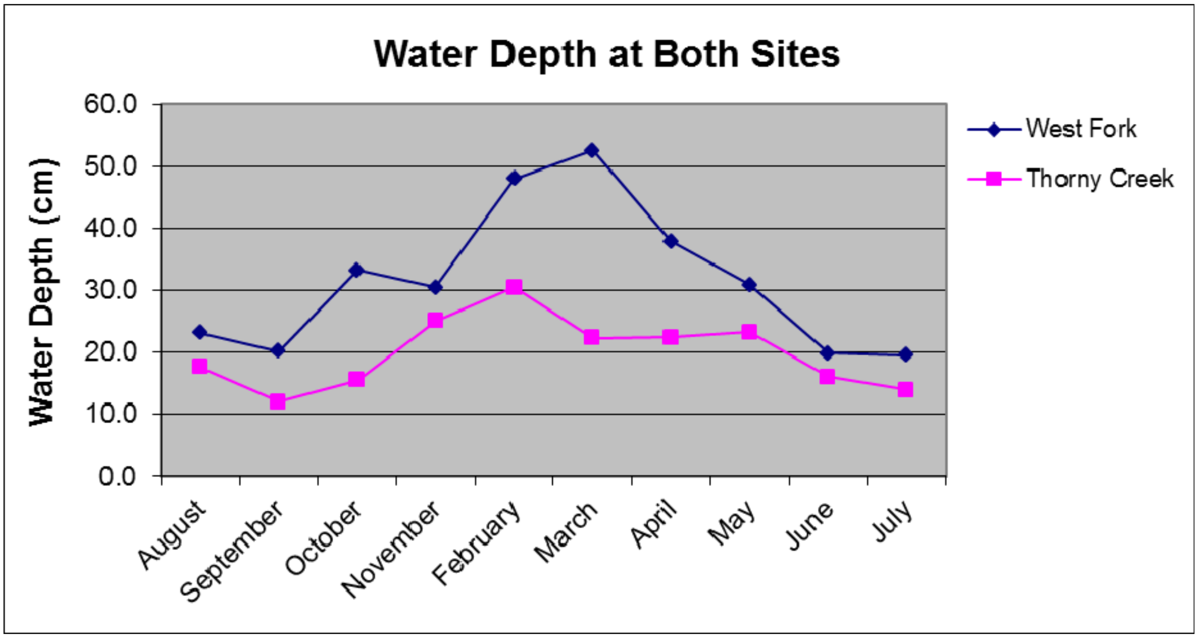
The coldest month at the West fork site (besides December when DO was not measured) was November with 4.2 Cº, and it had the largest amount of DO with 12.34 mg/L. The coldest month at the Thorny Creek site was February with 2.3 Cº, and it had the largest amount of DO with 12.47 mg/L. The warmest month at both sites was August with 26.3 Cº at the West Fork site and 22.2 Cº at the Thorny Creek site; the amount of DO was the smallest for the year with 5.67 mg/L at the West Fork site, and 6.23 mg/L at the Thorny Creek site.

Water Depth

Water depth measurements were taken throughout the collecting site using the flow-rod. There were nine measurements taken in each habitat of riffles, runs, and pools for 27 total measurements. These measurements were converted into centimeters (cm) and averaged together. Comparing water depth between the two sites the larger West Fork site had greater

depth throughout the year, but there is some correlation of seasonal water levels rise and fall between the two sites (Figure 20).

Figure 20. Water depth for each site for each month.



The greatest depth recorded for the West Fork site was in March with 52.6 cm, and the Thorny Creek site was in February with 30.5 cm. The least depth recorded for the West Fork site in July with 19.5 cm, and the Thorny Creek site was in September with 12.0 cm.

Conductivity

Conductivity levels recorded at both sites were extremely low ranging from 9.4 us – 57.2 us. There is strong negative correlation between water depth and conductivity. As the water levels decrease conductivity increases (Figures 21 and 22). The more water in the stream, the more elements such as salts and metals that increase conductivity are diluted.

Figure 21. Displaying the correlation between water level (cm) and conductivity (us), at the West Fork site.

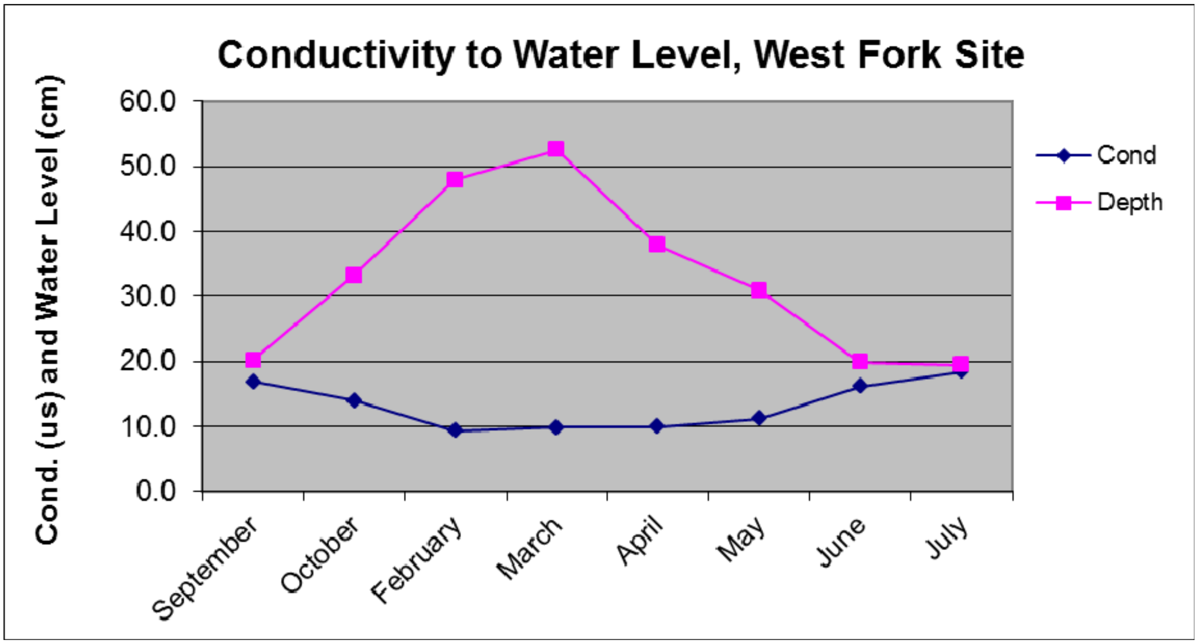
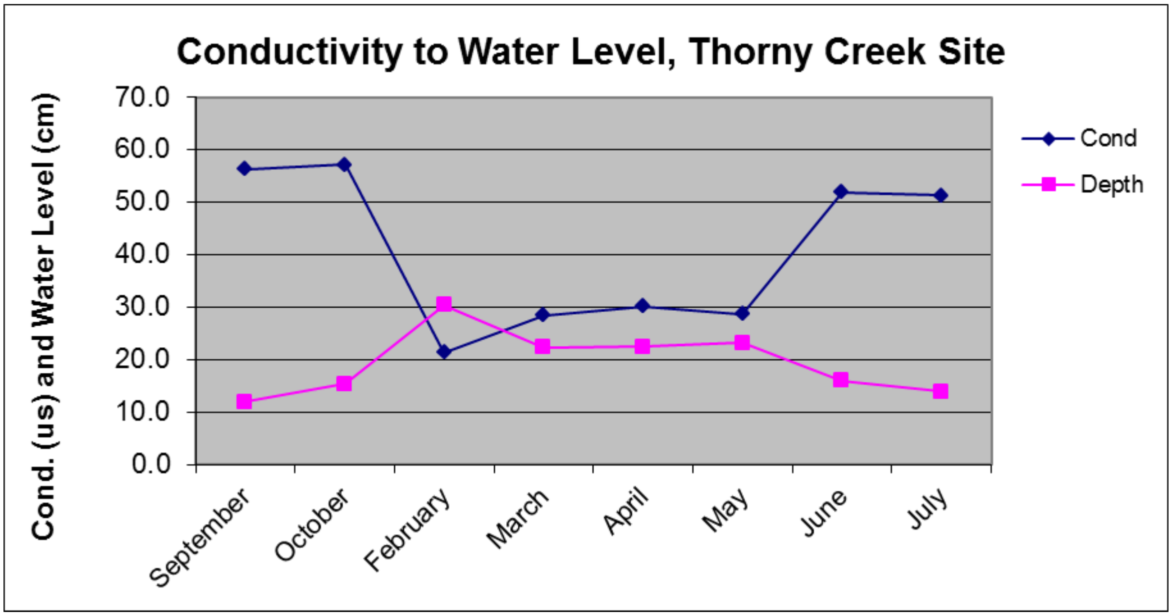


Figure 22. Displaying the correlation between water level (cm) and conductivity (us), at the Thorny Creek site.



The highest depth recorded at the West Fork site was in March with 52.6 cm, and it had the second lowest levels of conductivity with 9.9 us (March had slightly lower conductivity with 9.4 us). The highest depth recorded at the Thorny Creek site was February with 30.5 cm, and it had the lowest level of conductivity with 21.4 us. The lowest depth recorded at the West Fork site was in July with 19.5 cm, and it had the largest levels of conductivity with 18.5 us. The lowest depth recorded at the Thorny Creek site was in September with 12.0 cm, and it had the second largest levels of conductivity with 56.3 us (October had slightly higher conductivity levels with 57.2 us).

pH

Measurements for pH were recorded in Standard Units (S.U.) at each site. There did not seem to be a correlation between pH and any other parameter measured or in reference to seasonality. The West Fork site recorded pH that ranged from 6.67 to 7.82 with an average of

7.24. The Thorny Creek site recorded pH that ranged from 6.78 to 8.04 with an average of 7.43.

Crayfish Collection Rate Variability

As seen in Figures 23 and 24, water temperature affects the ability to collect crayfish. With cooler water temperatures, crayfish became less active and burrowed farther into substrate. Although collection times were the same, less crayfish were collected for the colder month, especially in November through February. Other factors such as water velocity and water clarity effect catch rate, but temperature appears to have the greatest influence.

Figure 23. Crayfish abundance collected with water temperature (°C) per month for the West Fork Site.

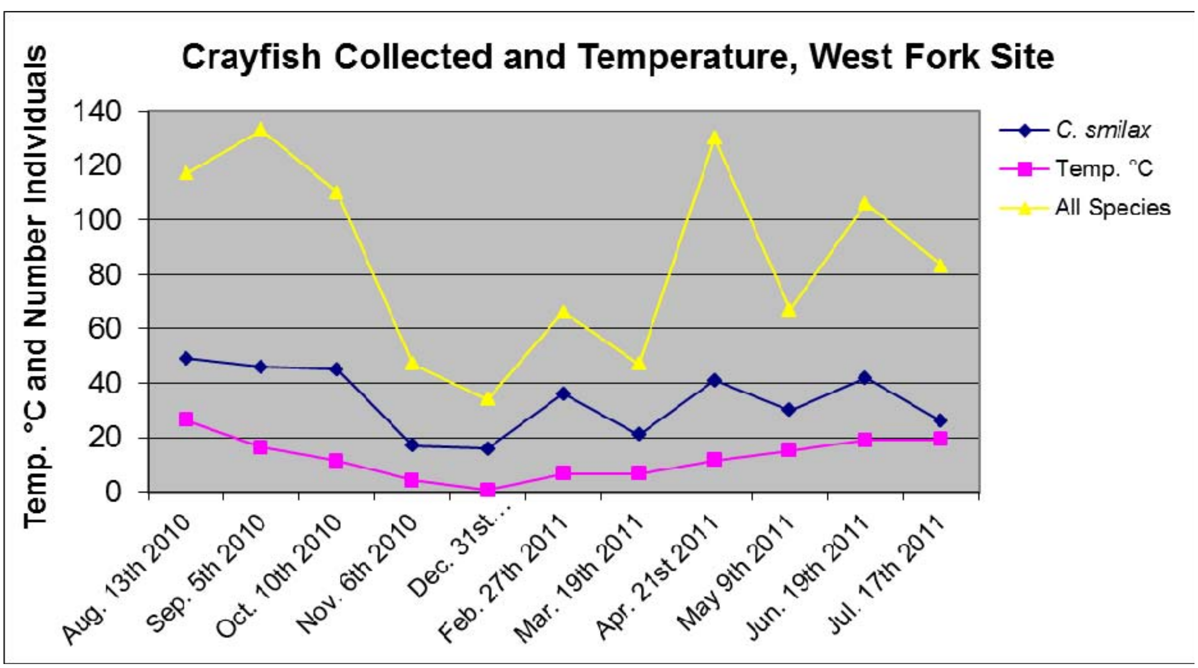
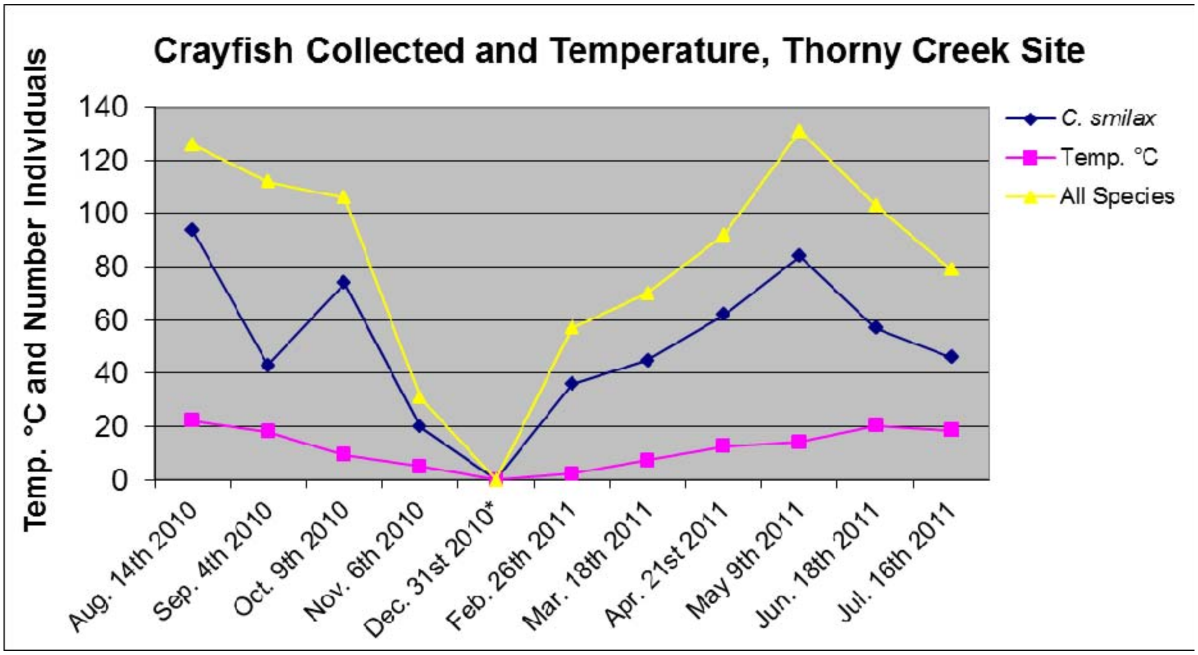


Figure 24. Crayfish abundance collected with water temperature (°C) per month for the Thorny Creek Site.



*water surface frozen solid, collecting was not possible.

CHAPTER 5

DISSCUSION

Crayfish Community Observations

There were similarities and differences between the species and abundances collected between the West Fork and Thorny Creek sites. There were four total species collected: *C. smilax*, *C. chasmodactylus*, *C. carinirostris*, and *O. obscurus*. The most obvious difference observed was the complete absence of *C. chasmodactylus* at the Thorny Creek site, while it

was the most abundant species at the West Fork site. The West Fork site, located on the West Fork of the Greenbrier River, is a much larger stream, with greater depths and velocities. *Cambarus chasmodactylus* has been documented as a larger stream and river crayfish (Helms & Creed 2005). The larger abundance and dominant role of *C. chasmodactylus* in the crayfish community at the West Fork site is most likely due to its adaptations for its affinity to larger streams and rivers. *Cambarus chasmodactylus* is also the largest growing species of crayfish in West Virginia, with a broad cephalothorax and very large chelae it can directly out compete other species, including *C. smilax*, for habitat and food (Helms & Creed 2005). Similarly, the adaptations that lead *C. smilax* to be a moderate stream species and *C. carinirostris* to smaller stream species can help explain the crayfish community differences between the larger West Fork site to the smaller Thorny Creek site where *C. smilax* and *C. carinirostris* are more prevalent.

The species most abundant was *C. chasmodactylus*. As a species more adapt to larger streams and rivers, it was the most collected and observed species of crayfish at the West Fork site. This species was found throughout pool, run, and riffle habitat. As seen in Figure 12, *C. chasmodactylus* was most dominant in run habitat. Runs provide a large portion of the stream, and runs are likely the best habitat for finding food and brooding offspring. *Cambarus chasmodactylus* has pushed other crayfish species out of the run habitats, and pushed them closer to the banks. A good example of this is found in the lack of abundance of *C. carinirostris* at the site. When *C. carinirostris* was collected it was within a few feet of the bank, or from isolated bank pools. As found by Loughman and Simon in their 2011 study of Ohio River floodplain crayfishes, *C. carinirostris* seems to limited to marginal habitats in larger ordered stream, through competition from other crayfish species such as *C. smilax* and

C. chasmodactylus found at the West Fork site (Loughman et al. 2011). The effect of *C. chasmodactylus* population was evident in the smaller abundance of *C. smilax* collected at the West Fork site (n = 369), to the Thorny Creek site (n = 561) which an absence of *C. chasmodactylus*.

The species most abundant was *C. smilax*. Since *C. smilax* is most closely related to *C. robustus*, a species most adapt to 2nd to 4th ordered stream, it makes sense that *C. smilax* was the dominant crayfish species at the Thorny Creek site (Thorny Creek is a 3rd order stream). There were no *C. chasmodactylus* found at this site, most likely because the stream is too small for its adaptations. *C. carinirostris* was found in higher abundance at the Thorny Creek site than the West Fork site. Due to the preference of the species *C. carinirostris* to prefer 1st and 2nd ordered streams, it is better established in Thorny Creek a 3rd order stream, than the West Fork a 4th order stream. Similarly to the West Fork site, *C. carinirostris* was found at the Thorny Creek site in marginal habitat along the banks and under isolated pocket water. *Orconectes obscurus* was found in higher abundance at the Thorny Creek site, but not likely competing with the two *Cambarus* species found here. *Orconectes obscurus* is not expected to greatly influence *Cambarus* species in these areas; however an invasive *Orconectes* species introduction could have damming effects on all native crayfish species.

Population Observations

The population of *C. smilax* found between the two sites varies in abundance. The greater abundance at the Thorny Creek site is most likely due to the smaller stream size, and the absence of *C. chasmodactylus*. The disproportional Form I to Form II males, gravid females, and abundance of juveniles and neonates between the two sites indicates that

presence of *C. chasmodactylus* at the West Fork site influences many aspects of the *C. smilax* population. These factors emphasize the importance of not introducing non-native crayfish. The *C. smilax* populations at both sites appear stable at this time with no known disturbances or non-native crayfish observed throughout the collecting year. The numbers of specimens of *C. smilax* varied from month to month, due to water temperatures, but did not indicate depletion throughout the year. The female to male ratio was nearly 1: 1, indicating that both male and females survive to maturity in equal proportions.

Gravidity and Fecundity

The months of May, June, July, August and September were consecutive months that displayed females that were gravid or in glair at one or both sites. There was also two gravid females were collected in March at the Thorny Creek site. Hamr and Berrill (1985) and Corey (1990) found similar results with the majority of *C. robustus* gravid females found in the months of July, August and September. The earlier gravid *C. smilax* found in this study may be due to warmer water temperature in West Virginia compared to southern Ontario.

Corey (1990) had a small percentage of gravid females in April with late instar development; this instance relates to the two gravid *C. smilax* found at Thorny Creek in March with 3rd instar young. Corey goes on to describe gravid females carrying eggs or early instar young through the winter months. This process which Corey terms ‘overwintering young’ seems like a likely scenario for the two gravid females with late instar young found at the Thorny Creek site in March. However, he explains that the offspring molt prior to winter, and thus cannot be referred to as stage III instars. More research needs to be done to conclude

whether or not ‘overwintering young’ is a viable explanation for finding a female *C. smilax* with late stage instar in March.

The fecundity levels and egg diameter of *C. smilax* was found to be comparable to populations of *C. robustus* in other studies (Hamr and Berrill (1985) and Corey (1990). The proximity to mating seasons, fecundity levels, and the comparable egg sizes found between this study, Corey (1990), and Hamr and Berrill (1985) suggests the life history of *C. smilax* parallels that of *C. robustus* as it relates to these breeding characteristics.

Molting

The seasonal molting of *C. smilax* found in this study parallels other studies of *C. robustus* (Hamr and Berrill (1985), and Guiasu and Dunham (2001). It appears that the late spring molting is for growth of immature individuals, and mature individuals are limited to molting in the early fall. In the first year of life, *C. smilax* will utilize the spring and fall to molt for growth. The following spring they can molt again to accommodate growth. Then after their second full summer ends most will molt into sexual maturity forms in the fall for the first time. They are then available to copulate in the fall or during the main mating season the following spring. *Cambarus smilax* that don’t molt into sexual majority that fall, will wait an entire year to molt into sexually active forms the next fall at the end of their third full year (Hamr and Berrill (1985), and Guiasu and Dunham (2001). As many large form II (TCL > 35mm) males were found, some individuals will molt from form I back to form II in their third or fourth year of life. With an estimated life span of around four years, it is possible, for a very few individuals to molt back into form I and possibly mate again before demise.

Male Form I to Form II

The proportion of form I to form II males changes every month. The tentative trend displays greater proportion of Form I males in September through May. It is hypothesized that most *C. smilax* males reach a sexually active state for the first time in the second full year of life around September after molting. They are then sexually active through the late fall through the spring. These form I males may then molt back to form II in the following September. If they did survive the third full year of life, they could molt back to form I for a second time the following September, then survive the winter into their fourth year of life to breed again. As Hamr and Berril (1985) found, winter mortality makes it unlikely that many of these crayfish will survive to mate again.

This cycle of mortality and growth would lead to the other part of the trend where more form II males were collected in the months of June, July, and August when a proportion of males are growing into a size where sexual maturity is possible, but will only molt and come into sexual maturity in the upcoming fall.

Interspecific Habitat Partitioning

Throughout this study there was an obvious inclination for *C. chasmodactylus* to inhabit areas of greater velocities in runs and riffles. *C. smilax* at the West Fork site was pushed into pools and margins around the banks. Runs, having more flow, may have the capacity to deliver more food to individuals and therefore be more desirable. When collecting gravid individuals of *C. smilax* and *C. chasmodactylus* most were found under partially embedded boulders with moderate velocity. Most of these boulders had an observable upstream burrow and a downstream burrow to allow some water to pass under the boulder and

oxygenate the female and her young. Since this type of habitat is desirable for brooding, *C. chasmodactylus* has an advantage over *C. smilax* by outcompeting for this habitat.

Even though *C. chasmodactylus* is outcompeting *C. smilax* for desirable habitat, both species coexist. The partitioning between areas in the stream may allow species to limit competition and allow these two species and *C. carinirostris* to live sympatrically.

Crayfish Morphometric Analysis

Size Cohorts

The size cohorts display that there are mostly two to three recognizable groups in each month. It would reason that since *C. smilax* lives for three to four years, that only three cohort groups are seen in any one time. It appears that the size cohort seem to grow 1 – 3 mm per month in the warmer months.

Chelae Damage

The West Fork site has twice the observable *C. smilax* chelae damage than the Thorny Creek site. Since *C. chasmodactylus* is the variable between the two sites, it appears that interspecies competition between *C. chasmodactylus* and *C. smilax* is the most obvious reason to account for the greater chelae damage to *C. smilax* populations at the West Fork site. One hypothesis might be that competition for food and habitat leads to more interactions between the two species. Since *C. smilax* is smaller on average, it is more likely to be damaged from such interactions.

Chelae to Total Carapace Length

Cambarus smilax males gain a larger chelae size when molting into Form I. The proportion of chelae to TCL is different between Form I and Form II males. This is a secondary sexual adaptation assisting in copulating with females, competing with males, and may possibly be used in sexual selection by females.

Weight to Total Carapace Length

Cambarus smilax males also seem to gain some weight as they molt into Form I. This may also assist in copulating with females and competing with males. The weight will also act as an energy reserve that may be needed as they seek and compete with mates. It is therefore necessary that males build up an energy reserve before molting into Form I.

Physical Stream Characteristics

The physical stream characteristics measured using the EPA RBP are listed: Epifaunal Substrate / Available Fish Cover, Embeddedness, Velocity / Depth Regimes, Channel Alteration, Sediment Deposition, Riffle Frequency, Channel Flow Status, Bank Stability, Bank Vegetation Protection, Width of Undisturbed Vegetation Zone. These characteristics are all important to the streams physical health, which will directly affect the biological health. The fact that all these characteristics are in good health now is a great sign for the present and future health of *C. smilax* and all aquatic fauna.

Although all of the parameters are important to healthy stream conditions, perhaps the most important to *C. smilax* populations is Embeddedness. Crayfish rely on interstitial space between rocks and substrate for Refugia. Refugia are an area in which a population of organisms can survive through a period of unfavorable conditions. If embeddedness was higher and interstitial space was limited, *C. smilax* would lack area to protect itself from

predators, flooding, or drought. Since embeddedness is very low at both sites the scores are very high. The main reason substrate becomes embedded is due to siltation which is often from logging, mining, and development.

When looking at *Cambarus veteranus* (The Big Sandy Crayfish) populations Loughman and Welsh (2011) state only two current populations exist in West Virginia and they are not stable. The main reasons stated was mining which leads to siltation and stream degradation. Comparing RBP scores found presently for the West Fork and Thorny Creek sites, 176 and 180 respectively, to those found for locations that historically held populations of *C. veteranus* but are now extinct at Little Huff Creek, Brier Creek, Little Indian Creek, and Little Indian Creek Jones et al. (2010) found that none of these sites scored above a 120 (low end of Sub-Optimal) for the EPA RBP. Undoubtedly these scores have diminished over the years with diminishing stream quality.

The scores found presently at the West Fork and Thorny Creek sites should be used as a baseline to monitor future physical stream health. Any sudden or continually gradual decline from this baseline would lead to concern for the *C. smilax* populations found therein.

Stream Characterization Using GIS Spatial Analyst

Water Depth

Stream depth measured in June of 2010 was lower than average for the year, but there was still enough water to support and protect the *C. smilax* populations found therein. The following months of July and August, and June of 2011 displayed equally low water levels; hypothetically a severe drought could make water levels dangerously low for crayfish and other aquatic species at both sites.

Stream Flow

The differentiating water velocities provide different habitats amongst the different crayfish species found therein. Pool type habitat is important to maintaining *C. smilax* populations, runs are a necessity to find proper aeration during gestation, and riffles facilitate water oxygenation.

Substrate Condition

The abundance of large slab boulders at both sites provides excellent refugia and habitat for *C. smilax*, as well as the other native species. Besides crayfish aquatic insects, fish and amphibians utilize these interstitial spaces created by the large slab boulders.

Physiochemical Stream Characteristics

Physical and chemical water characteristics such as temperature, DO, pH, conductivity and water levels affect crayfish and all aquatic life greatly. As seen in the results these characteristics change throughout the year and can vary greatly.

The pH levels did vary from 6.67 to 8.04 but are well within the survivability range of most crayfish and are actually desirable. The same can be said for the extremely low conductivity levels. The conductivity was highest at the Thorny Creek site at 57.2 in October, but this is still extremely low for any stream in West Virginia. The low conductivity can be an effect of the lack of mining and development.

Temperature ranged from 0.0 ºC to 26.3 ºC. These temperatures are well within the survivability of most warm water crayfish species. However, the warmer water temperatures in the summer into August lowered DO levels to a great degree. Indeed in the warmest

months of the year (June, July, and August) DO levels became low enough to impair aquatic life including *C. smilax*. Hobbs and Hall (1974) had suggested that cambarids were not found in hypoxic conditions less than 6 mg/L. If such low DO level persisted for extended periods of time, brought on by rising global temperatures and droughts, large numbers of *C. smilax* and other crayfish may not be able to survive.

Crayfish Collection Rate Variability

Water temperature was the most influencing factor to crayfish collection; the colder the water temperature, the less crayfish were collected. Crayfish were less active and burrowed more deeply into the substrate in the colder months of November through February. The exception might possibly by *C. carinirostris* where its abundances increase during the colder months at both sites and carried into March and April. A possible hypothesis might be that this species is taking advantage of the reduction of competition from other species, or it is simply more adapt to cooler water temperatures.

There were other influencing factor to collecting such as water level, sunlight, and foliage in the stream. When the water level was above a certain level and velocity was high, collecting could be more difficult. However, if the water level was too low and velocity was low, seine netting was not effective. In the fall months of October and November the leaf litter in the stream made collecting crayfish more difficult.

CHAPTER 6

CONCLUSIONS

The natural life history of *C. smilax* is described as the behavioral and physical changes individuals in the population go through in reaching maturity, reproducing, and then expiring. This life history can begin with first egg extrusion, follows these individuals through yearly cycles and reproduction, and is completed when it is expected the majority of individual in the population expire.

Egg Extrusion

Females with well-developed glair glands were seen in May at both sites when water temperatures were around 14ºC. Egg extrusion on gravid females was first observed in June at the West Fork site (n = 1), and was observed in July at the Thorny Creek site (n = 6) with temperatures around 20ºC. The average egg diameter was 2.78mm and the average fecundity for gravid females was 121.

Embryonic and Larval Growth

Hatching from eggs to attached 1st instar young was first seen in July at the West Fork site (n = 1), then in August at the Thorny Creek site (n = 3). This indicates that embryonic development required around 30 days at water temperatures around 20ºC to reach 1st instar. The 1st instar young had an average TCL of 4.35 mm. Embryonic development continued to 2nd and 3rd instar in August and September (n = 2). The 2nd and 3rd instar young had an average TCL of 5.41 mm, displaying about a 1.15 mm growth in 30 days.

Post-larval Growth

Only one free living neonate was collected in September with a TCL of 8.9 mm at the Thorny Creek site. It should be noted that neonates are extremely hard to collect, and there was undoubtedly many more present in the fall. Two more neonates were collected at the West Fork site in March and April with a TCL of 9.4 mm and 9.7 mm respectively. In May, June and July these immature crayfish molted to accommodate growth. By the end of July growth is expected to be between 12 – 16 mm. At the end of their first full summer in September they molted again to accommodate growth between 16 - 20 mm. No molting was observed from October until the following May when immature individuals molted again to accommodate growth. Most of these individuals may molt into sexual maturity after their second full summer in September. Size would be the most obvious determinant in determining whether individuals molted into sexual maturity.

Form I and Form II Males

Males first reached form I at 24 – 32mm (TCL). Size varies greatly because some molted into form I in September at two years old. The others will have to wait another full year to molt the following September at three years old and be much larger. Regardless if form I males were two or three years old, they would not molt back into form II until September; for sexually active form I males to molt into form II in May, June, and July during the mating season would be disadvantageous. Large form I males had a TCL of 33 – 43mm. A few of these large form I males may be three or four years old, and have molted back to form II prior to molting back into form I. Supporting this is form II males with size ranging from 30 – 40mm. These form II males would have to survive to the coming fall to molt back into form

I and then copulate that fall, or more likely the following spring. Winter mortality makes it unlikely that more than a few will molt back into form I and successfully mate.

Females

Females first reached sexual maturity between 24 – 34mm. Like males, this size varies greatly between the females that molt into sexual maturity in September of their second year, and those that will wait another year to molt the following September. The smallest ovigerous female was 26.7mm, but the average was 34.3mm. It is believed that females need to reach a larger size to mate and develop young, compared to males that simply need to mate. Therefore more females will wait until their third year to molt into sexual maturity for the first time. Larger mature females were 35 – 44mm. Like males, sexually mature females only molted in September.

Breeding

Females with distinct glair on their abdomens were seen in May, June and July. The climax of the breeding took place just prior to this in the months of April, May and June. However, the only instance of observed copulation was on October 9th 2010. This instance is thought to be indicative of individuals coming into sexual maturity in September and breeding extremely early. As the water temperature cools breeding would be less likely until temperature warmed back up in April.

Gravidity

Most gravid females were found in the consecutive months of June, July, August and September. Special considerations are necessary to account for the two gravid females with

late instar found in March at the Thorny Creek site. There are two possible hypotheses are being considered. Because the only copulation observed during this study took place in October, it is possible that a small portion of breeding takes place in the fall instead of the spring. The females breed in the fall would develop eggs slowly in colder months of fall and winter. The eggs would then hatch into early instar in mid-to-late winter, leading to the late instars observed in March.

The other possible scenario is described by Corey (1990) as ‘overwintering young’. Corey (1990) described that most gravid *C. robustus* breed in the spring develop late instar into the fall but do not become dislodged. Rather, these females overwinter with the late instar young until growth increases with water temperature in the early spring. It should be mentioned that Corey’s study took place in southern Ontario were water temperatures drop more quickly in the fall and take longer to warm in the spring.

The missing data to determine which scenario is taking place is to collect gravid females in the late fall through the winter. If these females displayed eggs through these months it would lend to late fall breeding hypothesis. If these females displayed late instar young it would lend to the ‘overwintering young’ hypothesis.

Life Expectancy

Cambarus smilax is expected to survive to three years, with some individuals surviving to their fourth year of life. After four years very few individuals would survive the following winter.

Interactions with Other Species

The most significant interactions that populations of *C. smilax* have with other species are those with other crayfish species. As found at the West Fork site, *C. chasmodactylus* populations compete with *C. smilax* populations for habitat and food. Although *C. carinirostris* populations are subordinate to *C. smilax* populations at both sites, they are still competing for resources. The three Cambarids found at the West Fork site, and two at the Thorny Creek site display similar breeding patterns throughout the year. This synchronicity in breeding leads to competition for brooding habitat such as slab boulders embedded at the right amount of velocity to allow aeration and protection. The presence of populations of *O. obscurus* does not have a significant effect on *C. smilax* populations. *Orconectes obscurus* as well as other species in the genera *Orconectes* are not as territorial as species in the genera *Cambarus*. Also, *O. obscurus* has mating and brooding seasons that are not synchronized with *C. smilax* (Loughman et al. 2011).

Predation from fish is certain. Only a few individuals od species such as smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*) that would prey on *C. smilax* throughout its life were observed at study sites. Species such as the bigmouth chub (*Nocomis platyrhynchus*) and the mottled sculpin (*Cottus bairdii*) were abundant but would only feed on neonates and smaller individuals. There were accidental collections of six hellbenders (*Cryptobranchus alleganiensis*) and two mudpuppys (*Necturus maculosus*) (see representative photographs in Appendix B) at the West Fork site. Crayfish make up the majority of the diet of *C. alleganiensis* Nickerson et al. (2002). At both sites there were many large slab boulders too heavy to lift, that would hold an abundance both hellbenders and mudpuppys, making these species a significant predator to *C. smilax* and other crayfish. The study site at the West Fork site is stocked with rainbow trout (*Oncorhynchus mykiss*).

Although occasionally a rainbow trout may make a meal out of a *C. smilax*, they aren't a significant predator. One occasion observed just the opposite as several *C. smilax* were feeding on a dead rainbow trout (see representative photograph in Appendix B). Other animals such as raccoons and birds also feed on *C. smilax* as evident by crushed shells found on the bank.

Threats to *C. smilax*

The current populations of *C. smilax* appear to be stable. However, the limited range of *C. smilax* to the northern to central part of the Greenbrier River watershed leaves it vulnerable. Possible threats could include the introduction of invasive species, and habitat destruction. Invasive crayfish species such as *O. rusticus* and *O. virilis* could disturb the entire crayfish community and possibly the entire ecosystem. Although the possibility of bait-bucket introduction is lower at these sites then heavily smallmouth bass fished southern sections of the Greenbrier River, just one non-native gravid female release could start an invasive population. Consider also that it does not have to be a totally alien species. The introduction of near local established crayfish species such as *C. sciotensis,* could create circumstances where competition from the intruding species would cause *C. smilax* to lose part of its range.

Cambarus robustus moved into the Greenbrier River watershed could crossbreed with *C. smilax* and reduce its genetic variation. Just as the zoogeographic barrier of Kanawha Falls isolated populations of what has become *C. smilax* over thousands of years, a person moving *C. robustus* upstream of Kanawha Fall into the Greenbrier River could decrease its genetic variation in a few decades. The best tool to prevent crayfish from being moved from water

body to water body is to educate the public, and perhaps regulate the use of crayfish as fishing bait.

Most of the established populations are limited to moderate to high gradient streams in Pocahontas and Greenbrier County between 1,200’ and 3,200’ elevation (Loughman et al. 2011). There was one population found in Monroe County representing the farthest southern extant the species as be located (Loughman et al. 2011). Forested land is mostly undisturbed, and therefore the most desirable for preserving local ecosystems. The agriculture in the area is mostly hay and corn fields and cattle. Agricultural land does pose some threats from fertilizer runoff, and sedimentation. The destruction of habitat to many benthic organisms from sedimentation is a huge problem. Sedimentation of streams can be decreased by increasing riparian zones around all streams, especially those through agriculture fields, maintaining stable stream crossings, and limiting overall land disturbances. Educating and regulating are the best ways to protects streams and water bodies from non-point source pollutants from agriculture.

Thoughts for Future Research

The hypothesis that *C. smilax* life history would parallel its sister taxon *C. robustus* appear to be true. This seems to be evident in similar molting patterns, mating patterns, fecundity levels, growth rates, and habitat selection. Evolutionary differences in morphometerics such as more slender carapace and chelae may indicate an inclination towards smaller streams. This trend might be studied by a further review of both species habitat selections for stream size and stream order.

The affect that a native population of *C. chasmodactylus* has on *C. smilax* seems most evident in habitat selection. When both species are found together *C. smilax* seems to be subordinate and moves to areas with less velocity such as pools and along banks. A more thorough study of velocity where species are found should show this effect on habitat selection.

Although current populations of *C. smilax* appear stable, future evaluations need to continually assess these levels. The limited range of *C. smilax* leaves it vulnerable to expanded land use such as mining and logging.

Cambarus smilax is a welcome addition to the Mountain State’s fauna. Being found solely in West Virginia it should be listed as a species of special concern, and protection should be considered.

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Appendix A

Equipment List

Equipment List		
Item	**Description**	**Quantity**
Seine Net	8' x 5' 1/8" mesh	1
Dip Nets	18" x 8" 1/8" mesh	2
Buckets	Labeled: Pool, Run, Riffle	3
Neoprene Gloves	5mm	2 pairs
Containers	For Preserving Specimens	several
70% Ethanol Solution	For Preserving Specimens	1 gallon
Calipers	SPI model 0.1mm precision	1 set
Digital Scales	Electronic Pocket Scales ± 0.1g accuracy	1 set
Flow-Meter	Marsh-McBirney Flow-Meter	1
Flow-Rod	6' Metal Flow-Rod	1
Water Chemistry Probe	The HQ40D: Dual Probe Multi-Parameter Meter	1
GPS	Garmin 400t Handheld Global Positioning System	1
Camera	Canon SX110 Power Shot Digital Camera	1
Data Forms, Pencils, Sharpies	Data Collecting Material	several

Appendix B

Representative Photographs

Photo 1. *Cambarus (Puncticambarus) smilax*.
Photo by Paul W. Hughes.

Photo 2. *Cambarus (Puncticambarus) robustus*.
Photo by Zachary J. Loughman.

Photo 3. *Cambarus (Hiatacambarus) chasmodactylus*.
Photo by Paul W. Hughes.

Photo 4. *Cambarus (Cambarus) carinirostris*.
Photo by Casey D. Swecker.

Photo 5. *Orconectes (Crockerinus) obscurus*.
Photo by Paul W. Hughes.

Photo 6. Gravid *C. smilax* displaying eggs or in berry.
Photo by Paul W. Hughes.

Photo 7. Gravid *C. smilax* displaying early instar young.
Photo by Paul W. Hughes.

Photo 8. Gravid *C. smilax* with young becoming detached.
Photo by Paul W. Hughes.

Photo 9. Form I Male *C. smilax* with active gonopods and enlarged ischial hooks.
Photo by Paul W. Hughes.

Photo 10. Hellbender (*Cryptobranchus alleganiensis*) collected from the West Fork Site.
Photo by Paul W. Hughes.

Photo 11. Mudpuppy (*Necturus maculosus*)
collected from the West Fork Site.
Photo by Paul W. Hughes.

Photo 12. *C. smilax* feeding on dead rainbow trout
(*Oncorhynchus mykiss*).
Photo by Paul W. Hughes.

Photo 13. West Fork of the Greenbrier River
during low flow conditions.
Photo by Paul W. Hughes.

Photo 14. West Fork of the Greenbrier River
during base flow conditions.
Photo by Paul W. Hughes.

Photo 15. West Fork of the Greenbrier River
during high flow conditions.
Photo by Paul W. Hughes.

Photo 16. West Fork of the Greenbrier River
almost completely froze over.
Photo by Paul W. Hughes.

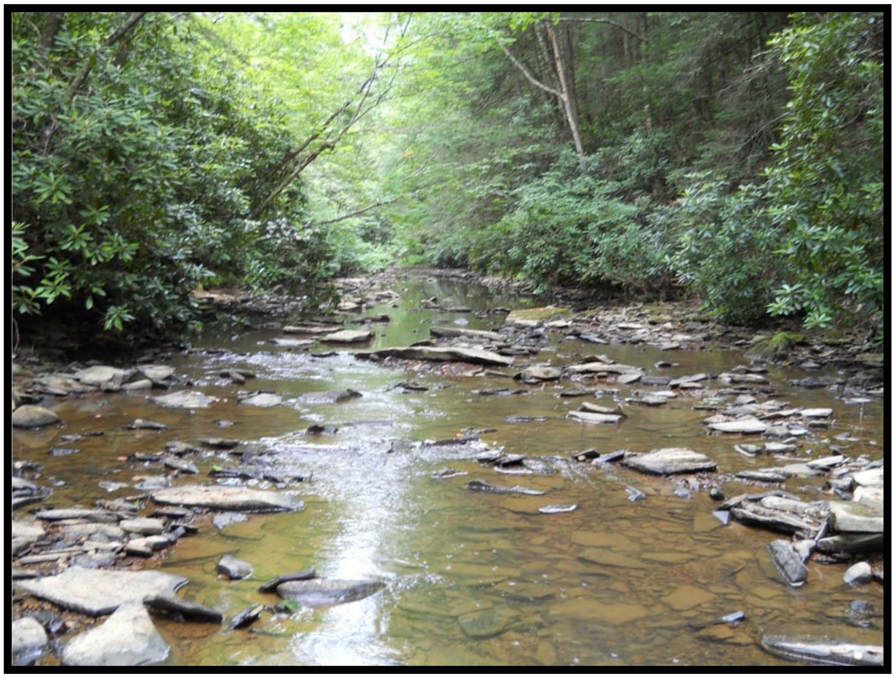


Photo 17. Thorny Creek during low flow conditions.
Photo by Paul W. Hughes.

Photo 18. Thorny Creek during base flow conditions.
Photo by Paul W. Hughes.

Photo 19. Thorny Creek during high flow conditions.
Photo by Paul W. Hughes.

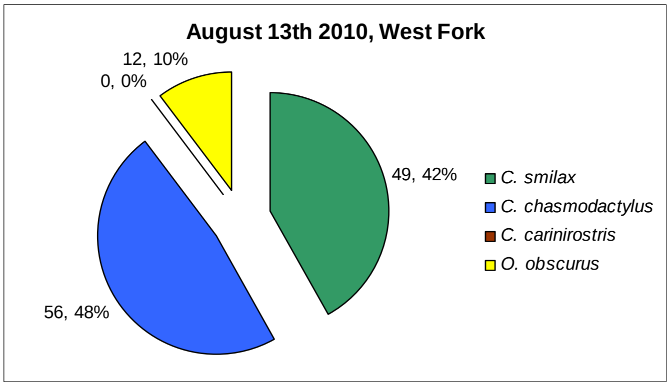
Photo 20. Thorny Creek completely froze over.
Photo by Paul W. Hughes.

Appendix C

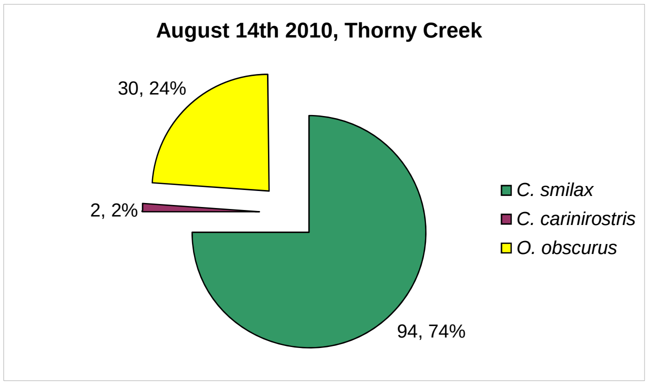
Monthly Collection Pie Charts

The following pie charts depict changes in the abundance and overall percentages of crayfish species collected by monthly sampling events.

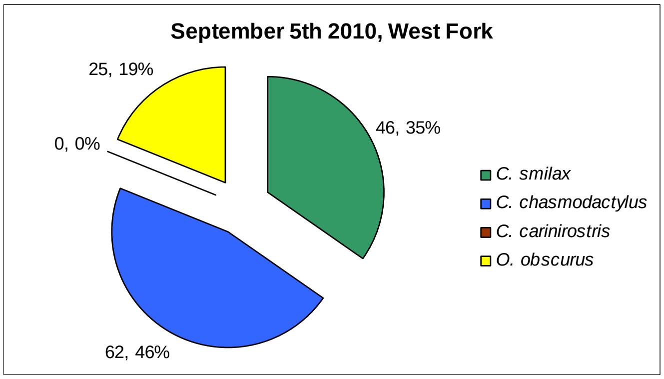
Trends observed: 1. Increase in abundance of *C. carinirostris* collected during the colder months.
2. Increase in abundance of *O. obscurus* collected during the warmer months.



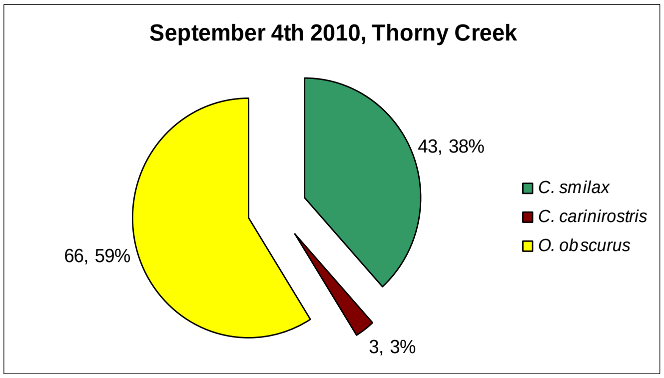
August West Fork Collections.



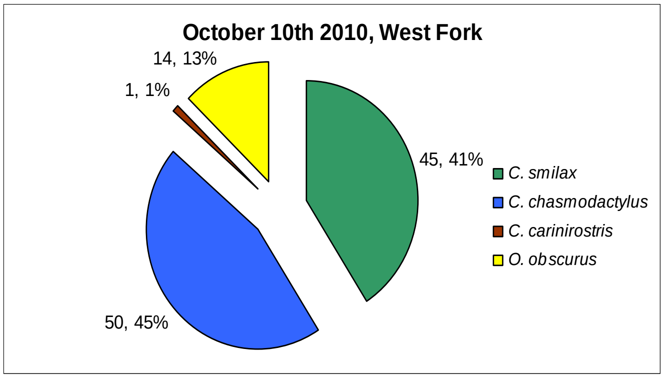
August Thorny Creek Collections.



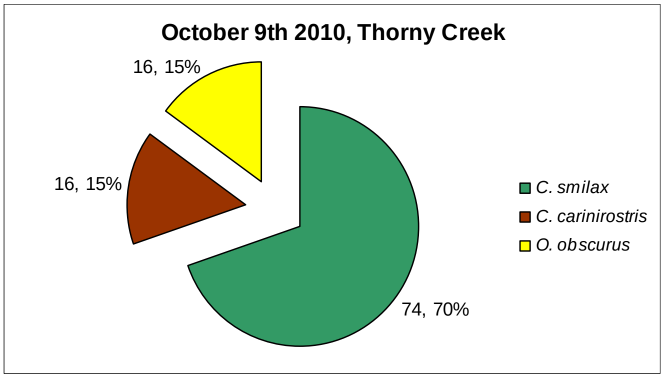
September West Fork Collections.



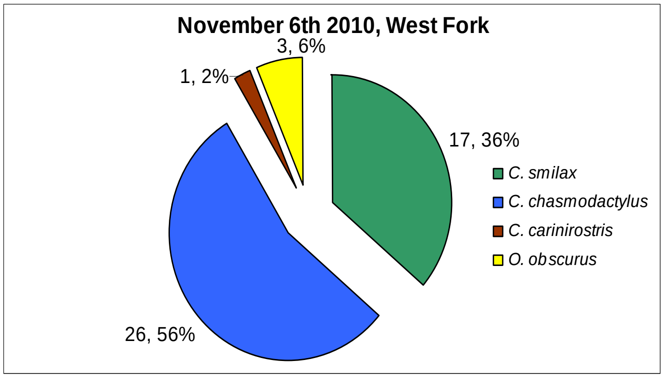
September Thorny Creek Collections.



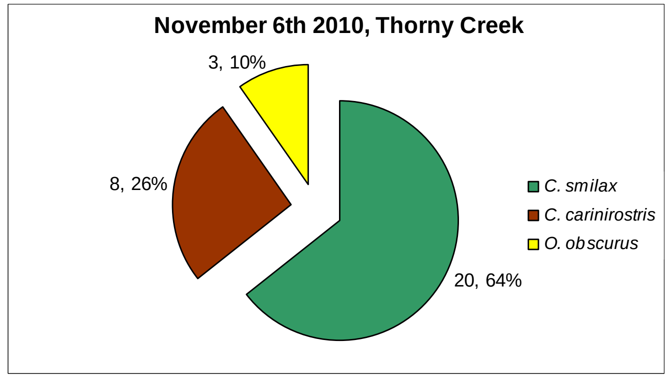
October West Fork Collections.



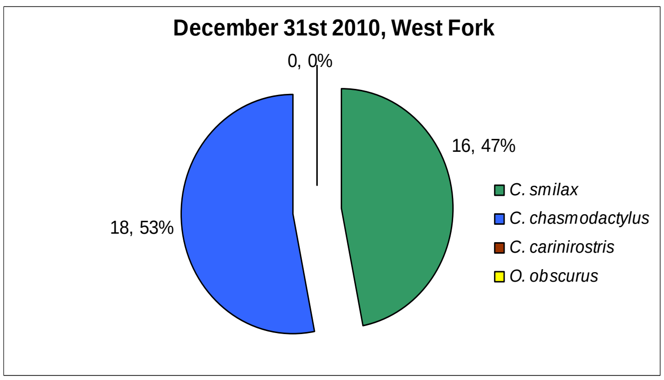
October Thorny Creek Collections.



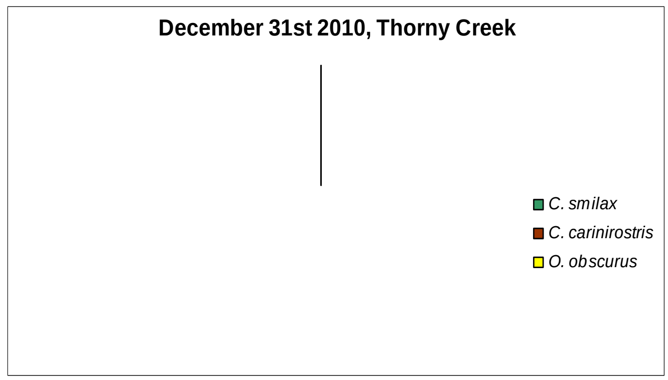
November West Fork Collections.



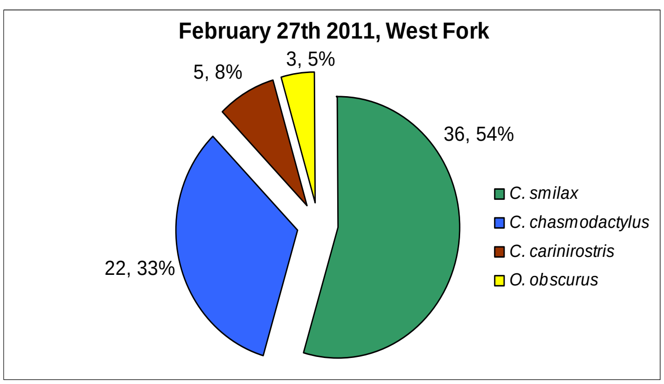
November Thorny Creek Collections.



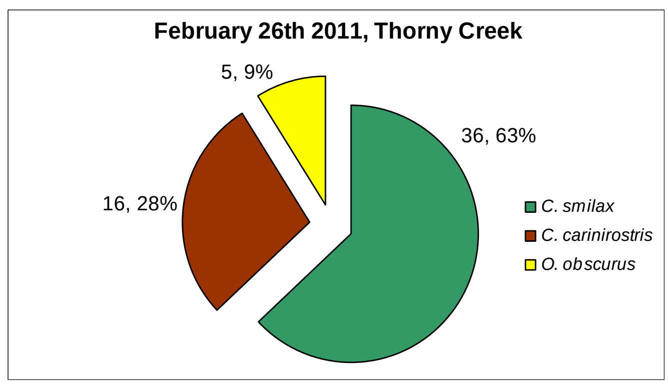
December West Fork Collections.



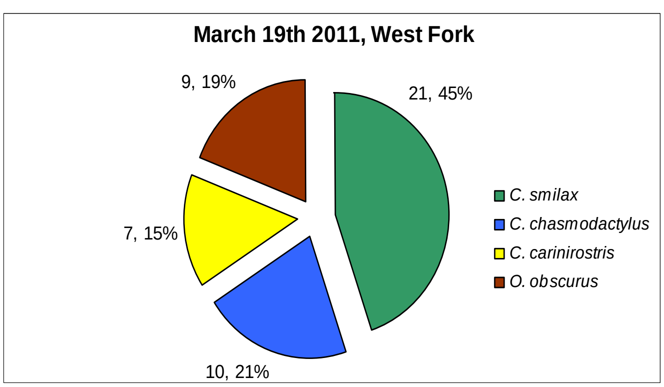
December Thorny Creek Collections.* Froze.



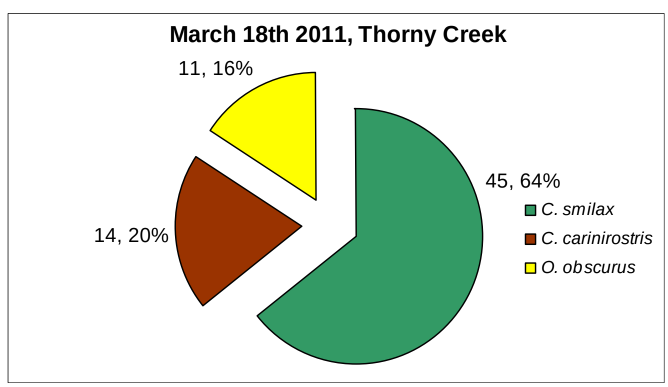
February West Fork Collections.



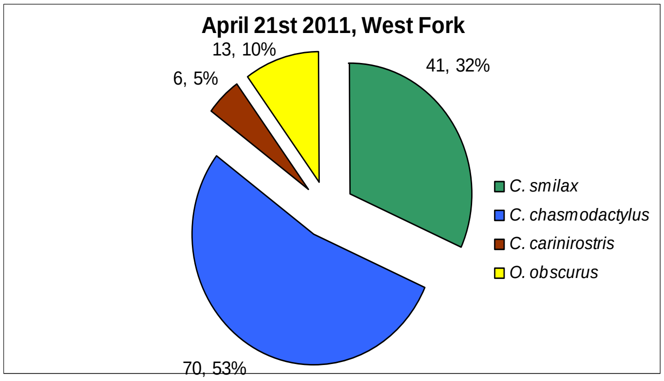
February Thorny Creek Collections.



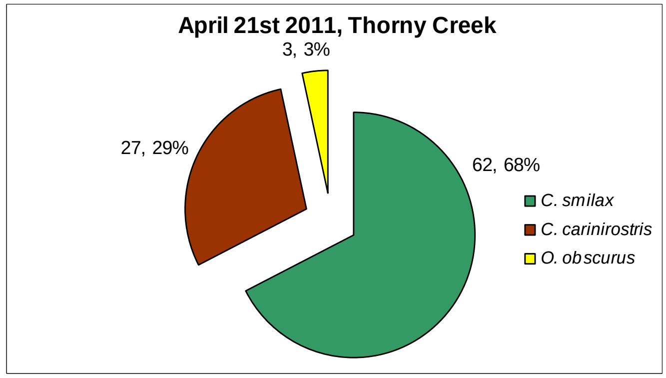
March West Fork Collections.



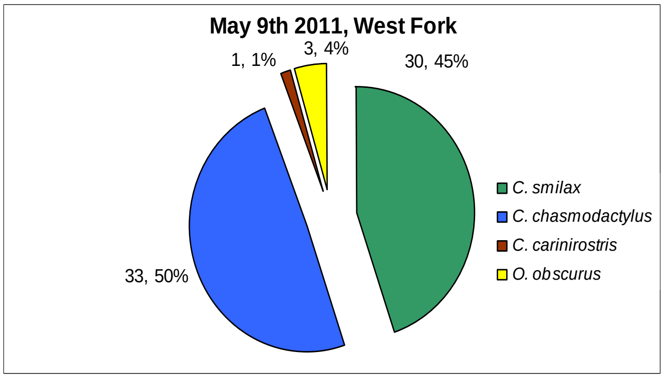
March Thorny Creek Collections.



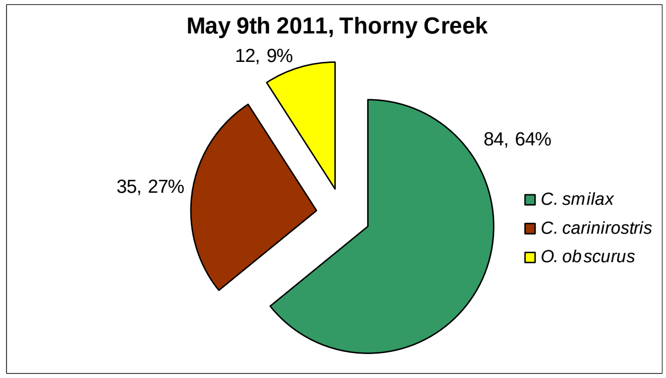
April West Fork Collections.



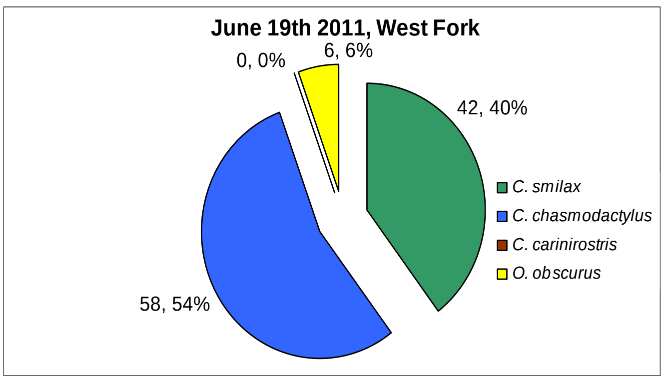
April Thorny Creek Collections.



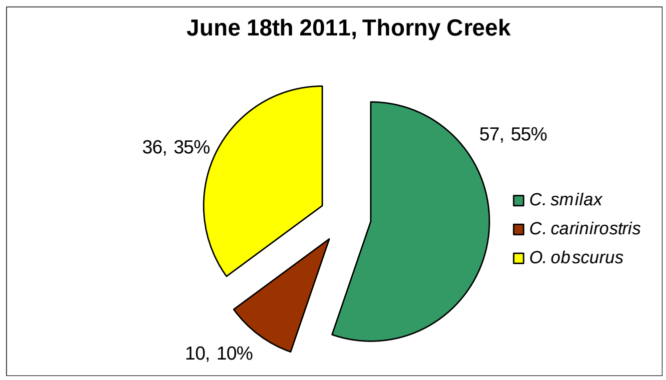
May West Fork Collections.



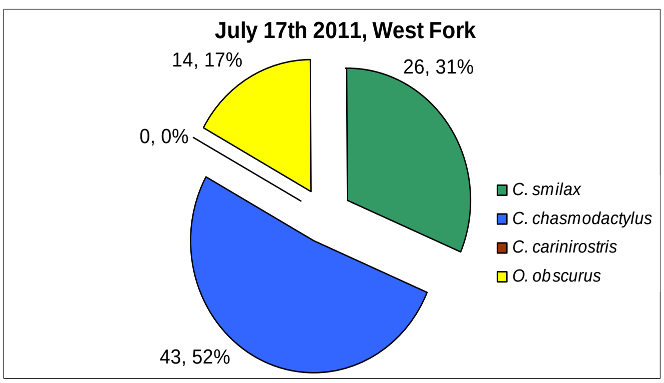
May Thorny Creek Collections.



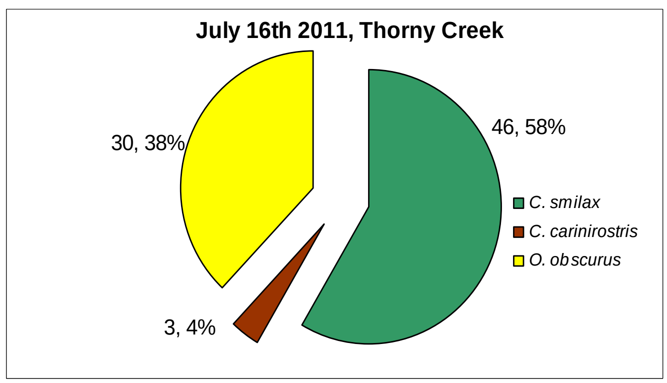
June West Fork Collections.



June Thorny Creek Collections.



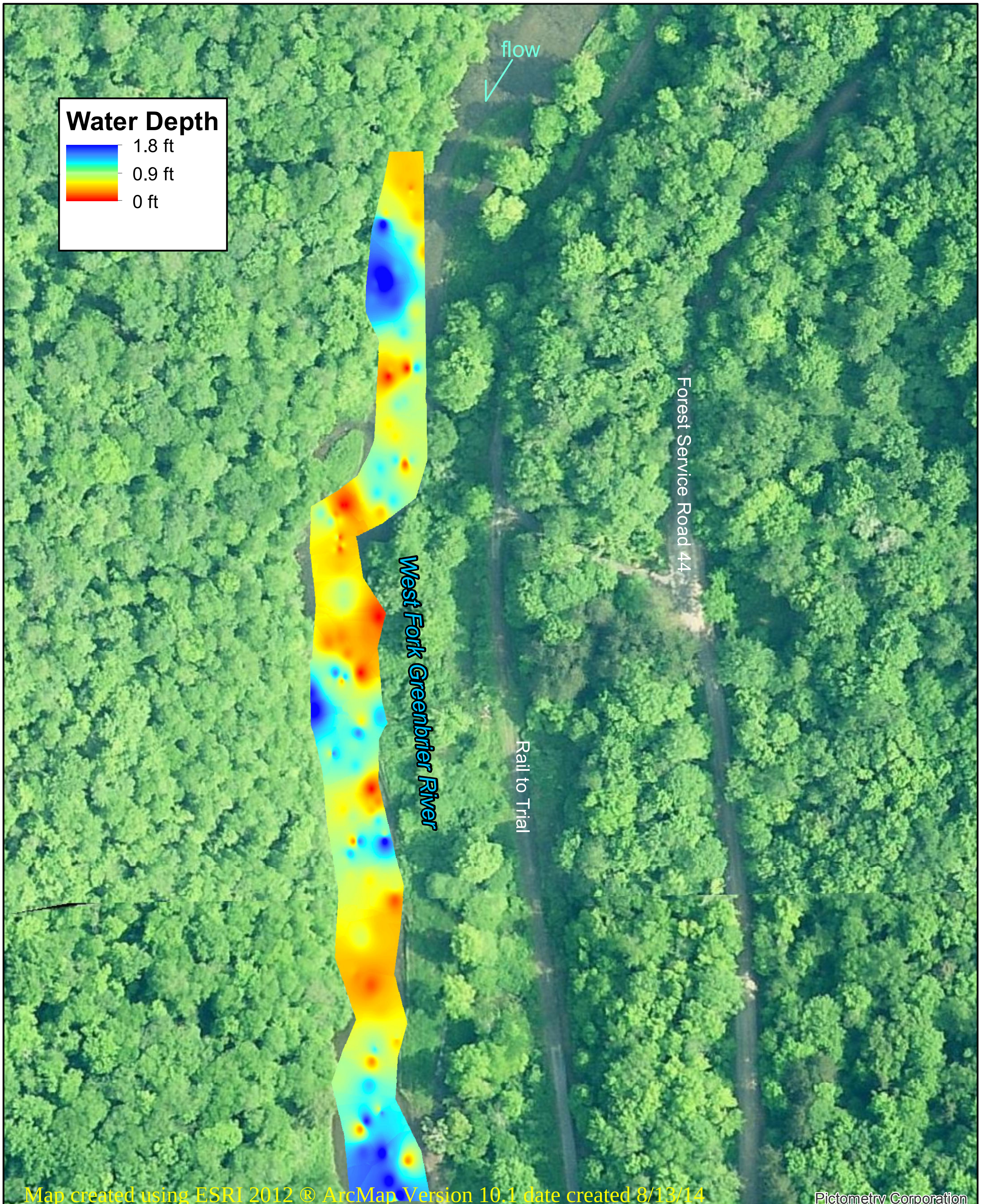
July West Fork Collections.



July Thorny Creek Collections.

Appendix D

GIS Stream Characterization Maps

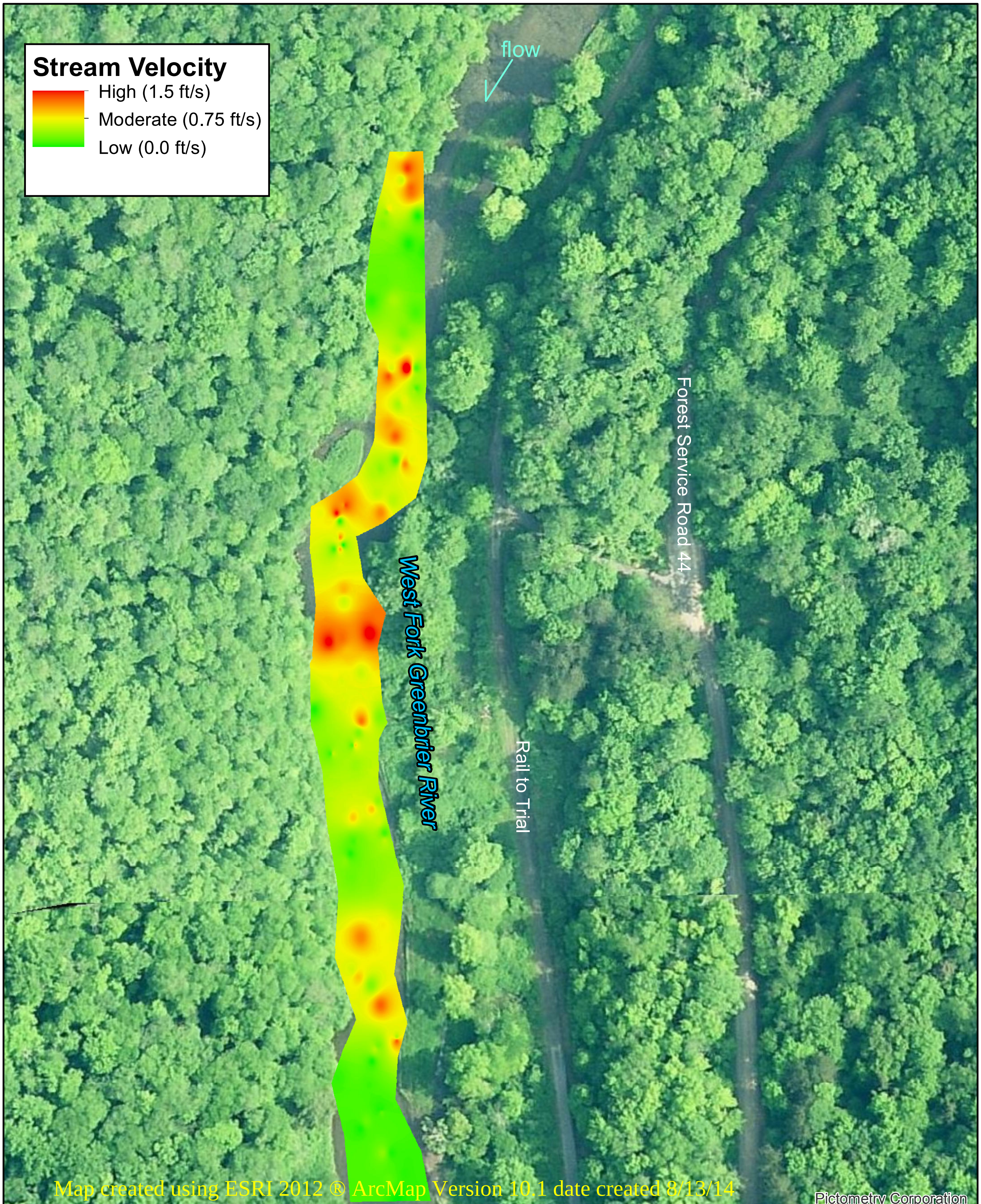


N
W E
S

Stream depth measured in feet along the West Fork Greenbrier River *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

0 30 60
Meters
Base Map: 2010-2011 Aerial Imagery

Watershed Resources



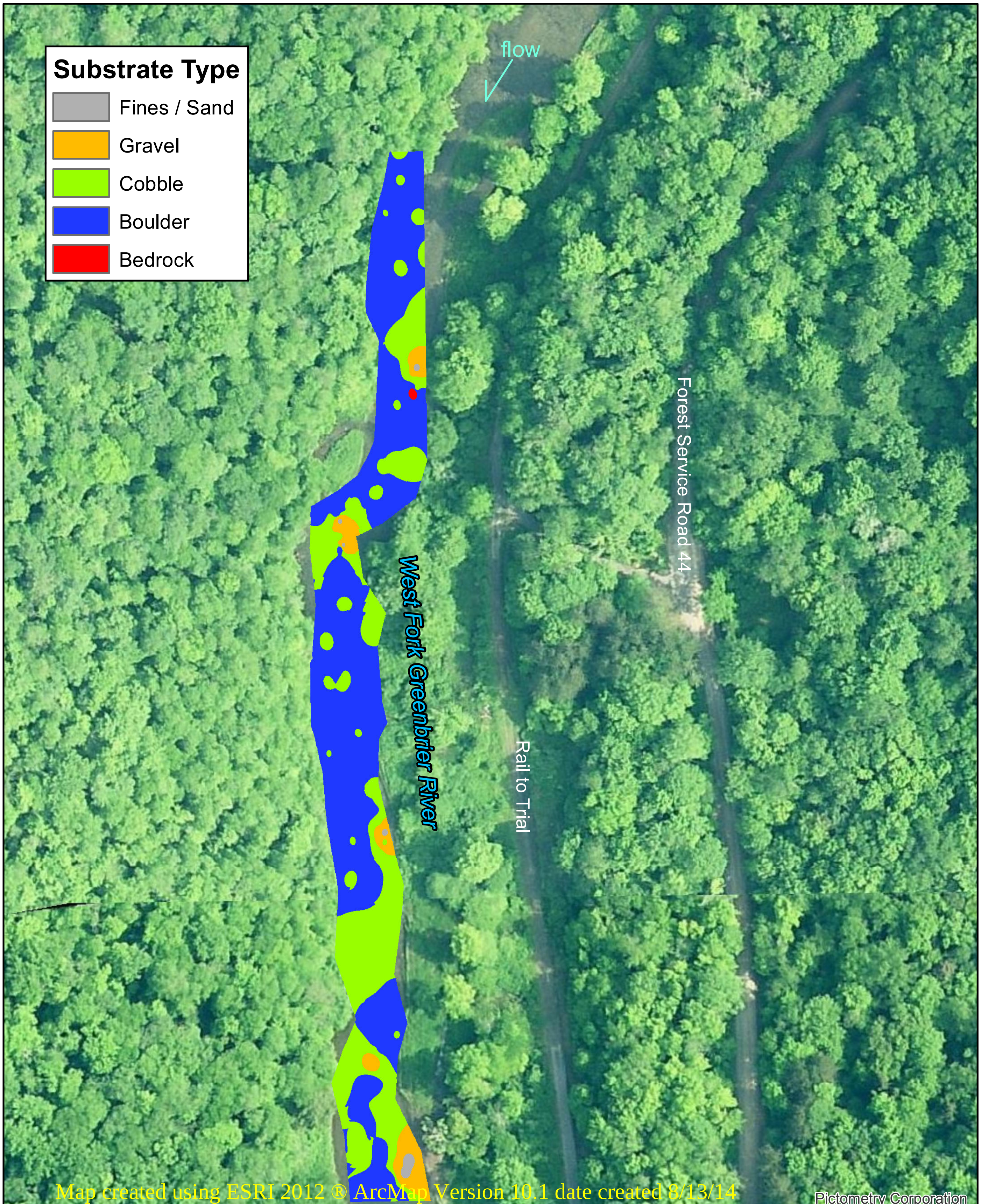
N
W E
S

Stream velocity measured in feet per second along the West Fork Greenbrier River *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

0 30 60 Meters

Base Map: 2010-2011 Aerial Imagery

Watershed Resources



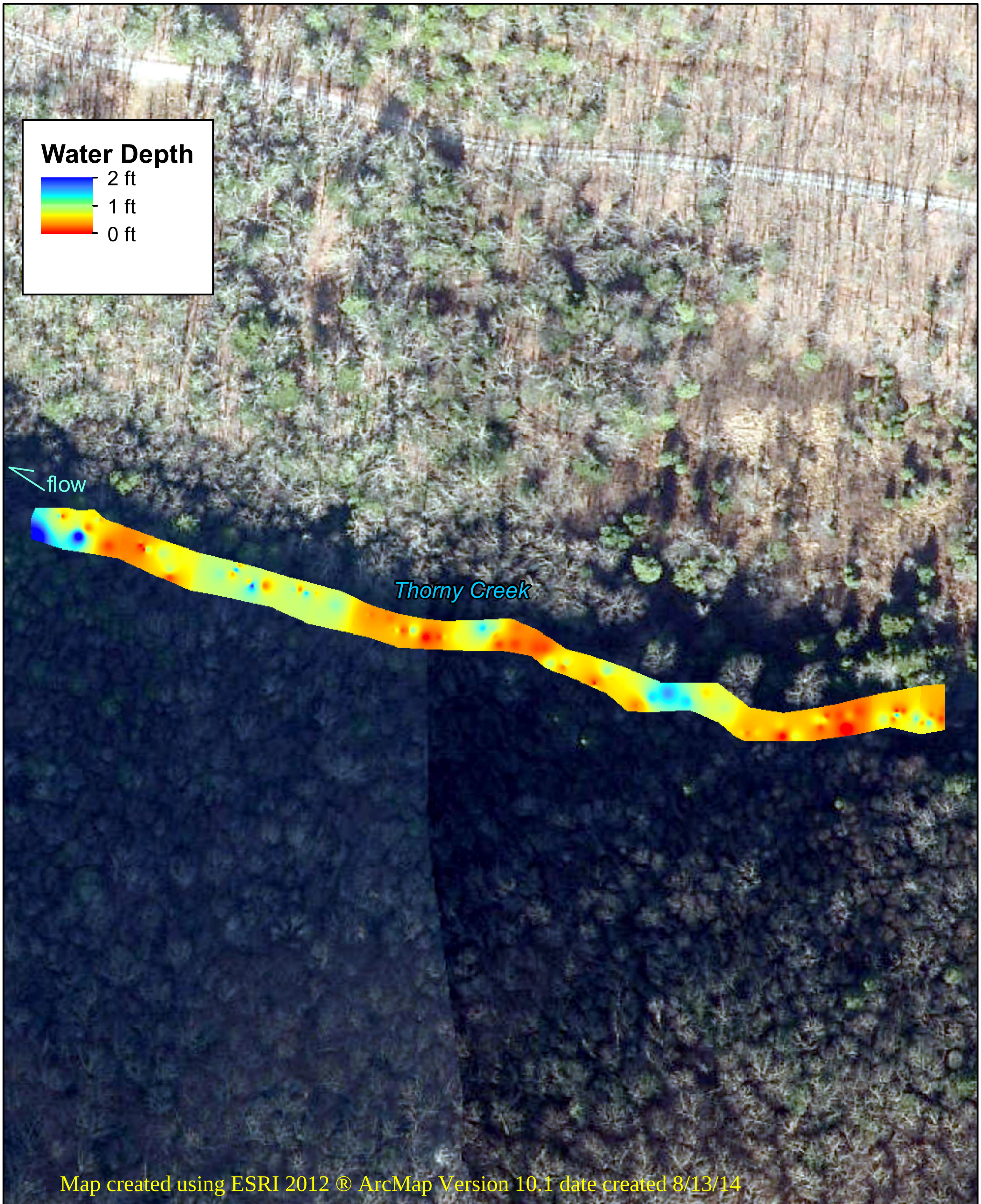
N W E S

Substrate type along the West Fork Greenbrier River *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

0 30 60 Meters

Base Map: 2010-2011 Aerial Imagery

Watershed Resources

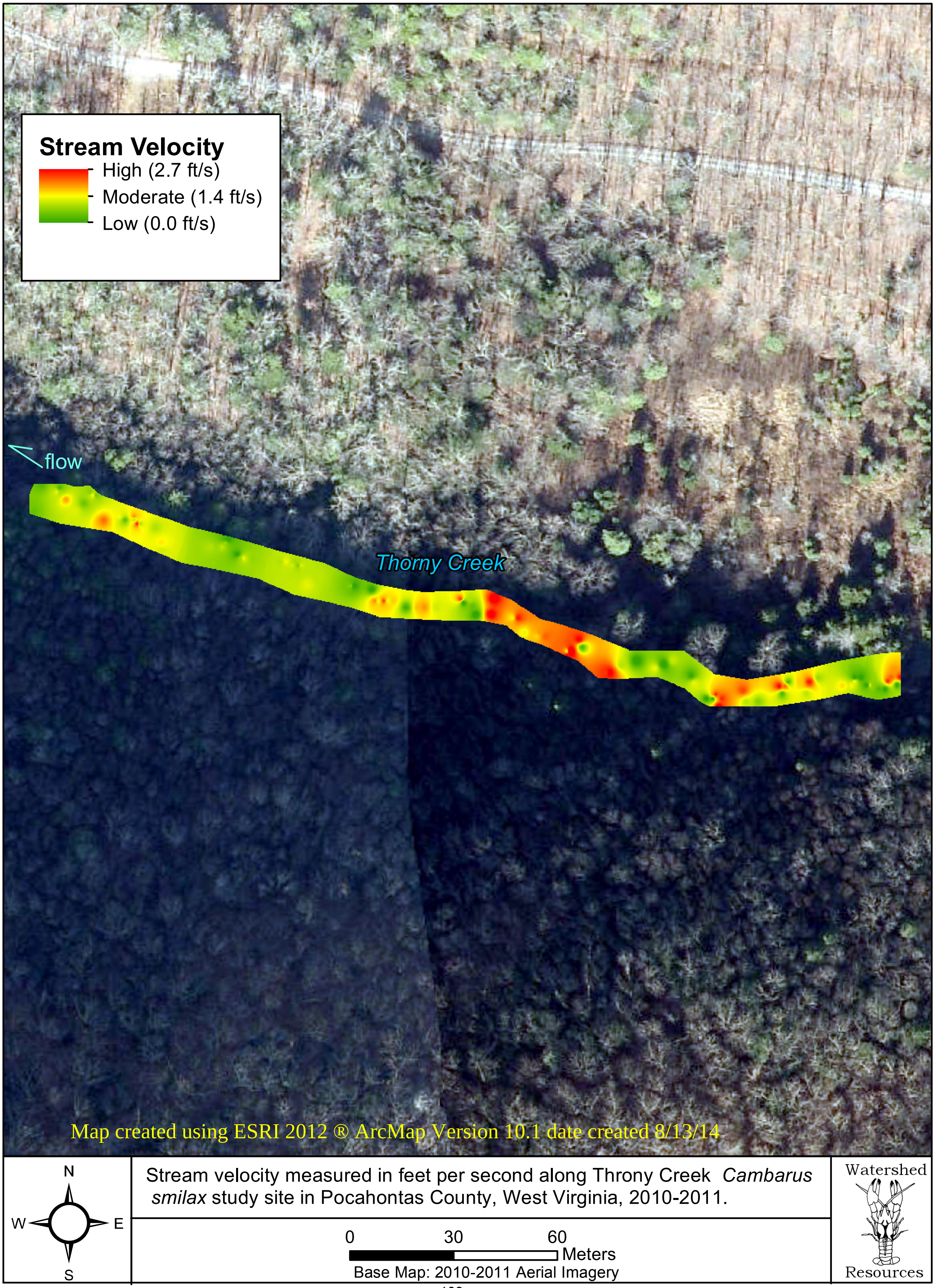


N
W
E
S

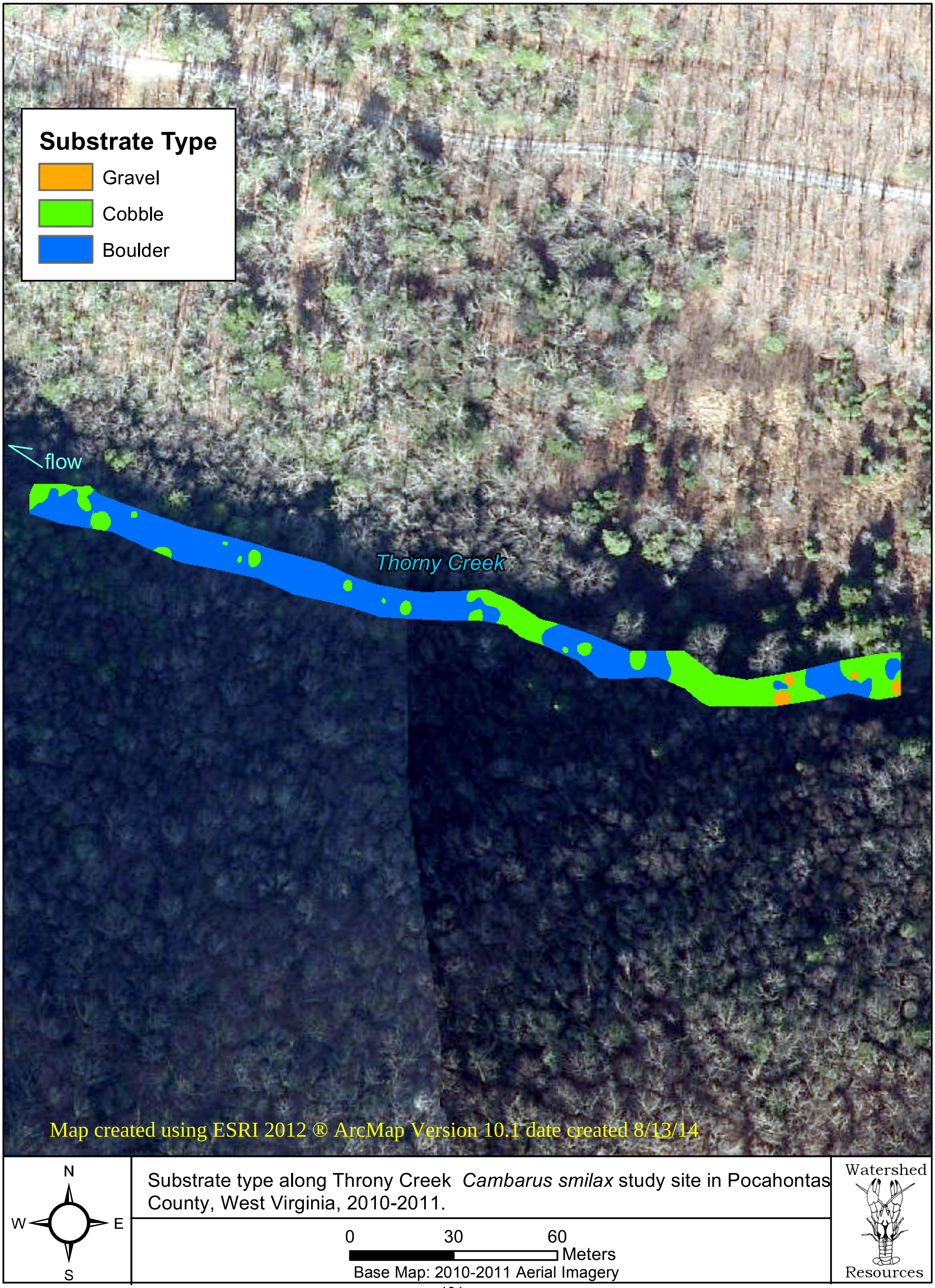
Stream depth measured in feet along Throny Creek *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

0 30 60
Meters
Base Map: 2010-2011 Aerial Imagery

Watershed Resources



Stream velocity measured in feet per second along Throny Creek *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

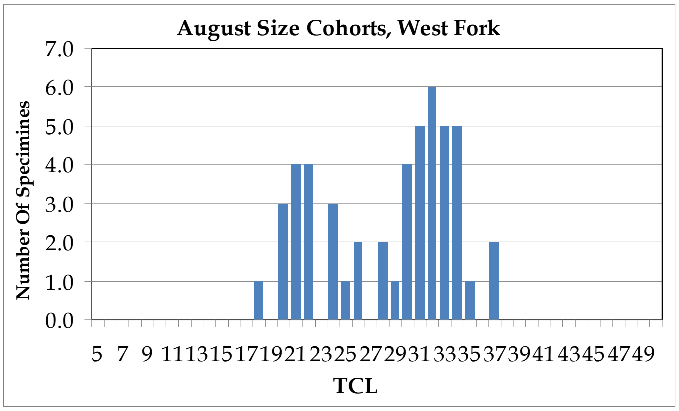


Substrate type along Throny Creek *Cambarus smilax* study site in Pocahontas County, West Virginia, 2010-2011.

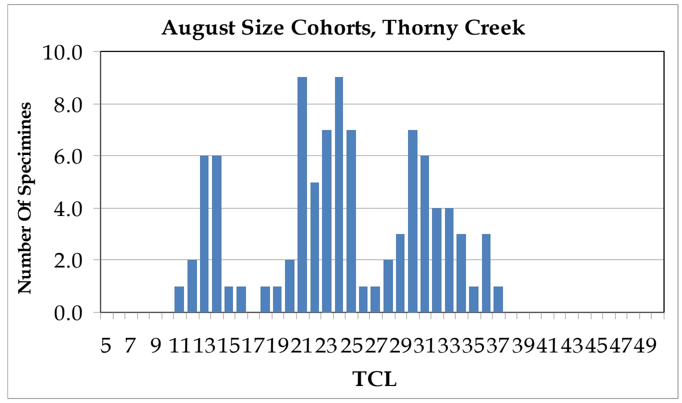
Appendix E

Size Cohorts

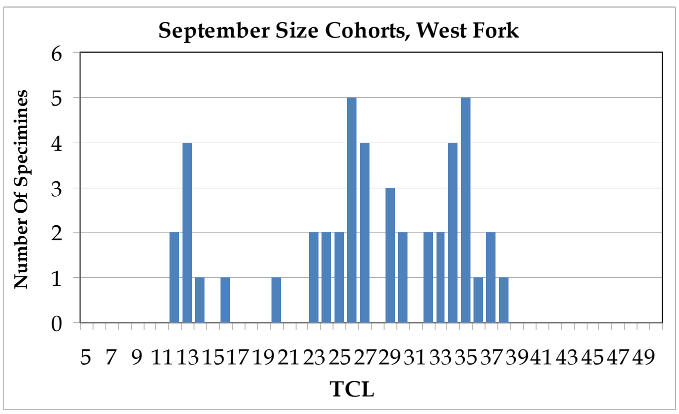
The following histograms depict the sizes of every *C. smilax* collected by monthly sampling events.



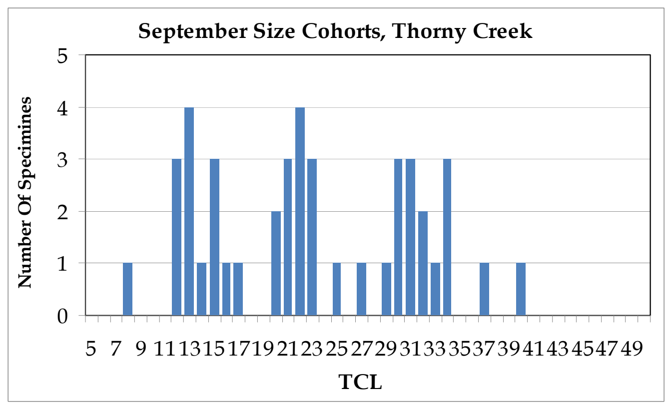
August West Fork Collections.



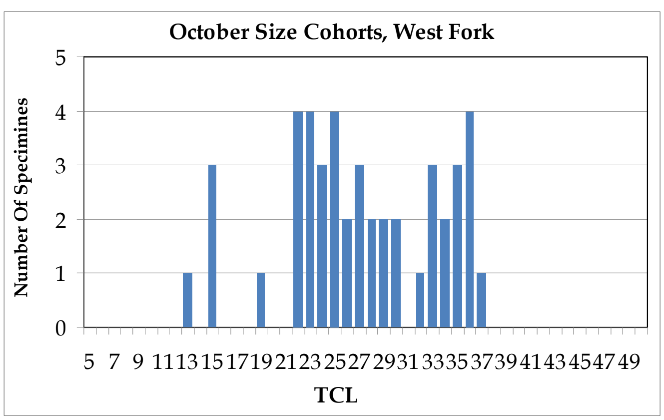
August Thorny Creek Collections.



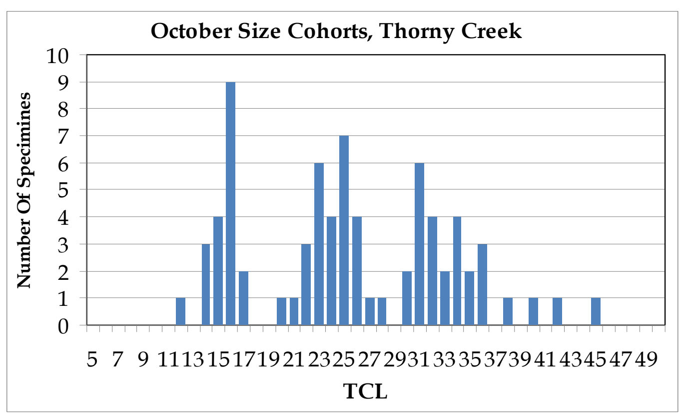
September West Fork Collections.



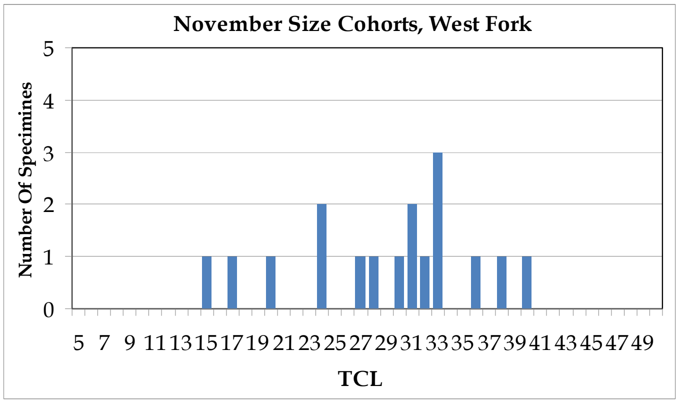
September Thorny Creek Collections.



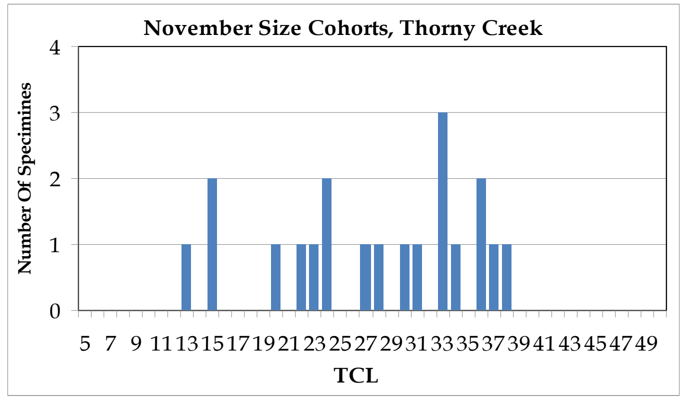
October West Fork Collections.



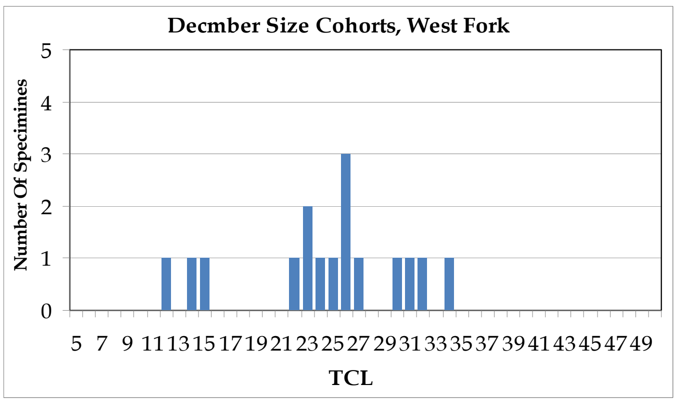
October Thorny Creek Collections.



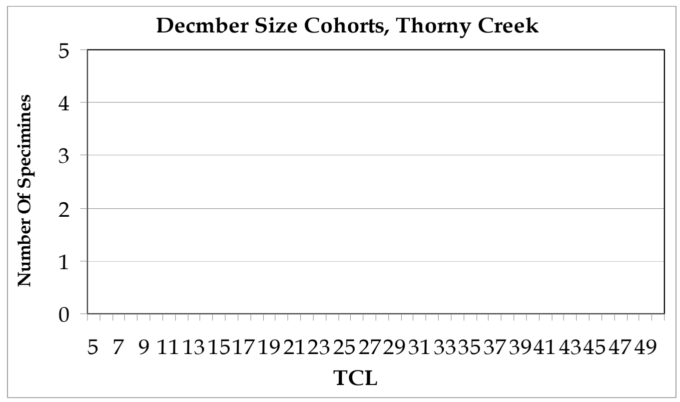
November West Fork Collections.



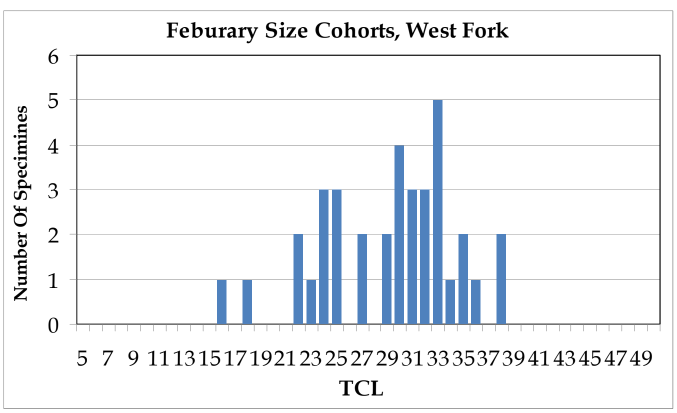
November Thorny Creek Collections.



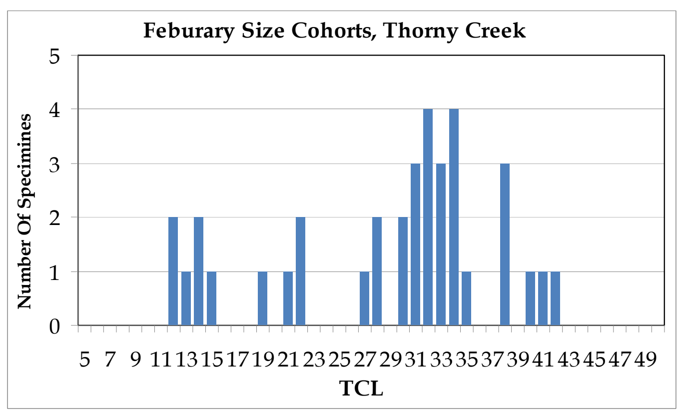
December West Fork Collections.



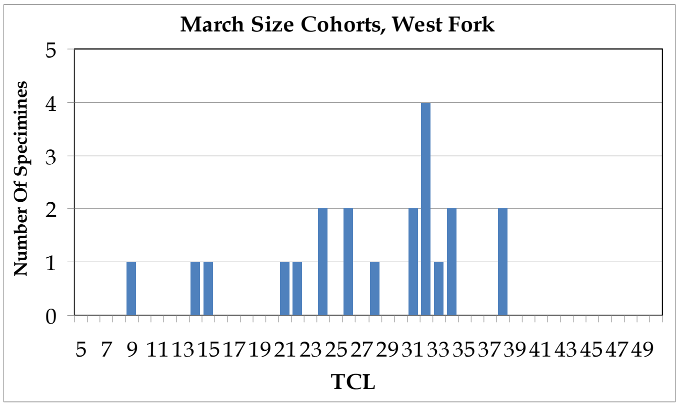
December Thorny Creek Collections.* Froze.



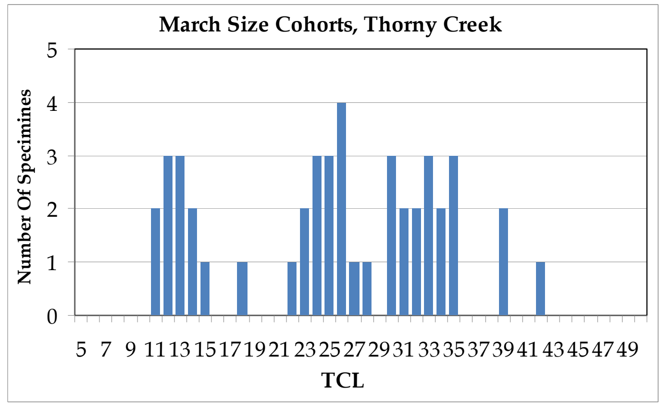
February West Fork Collections.



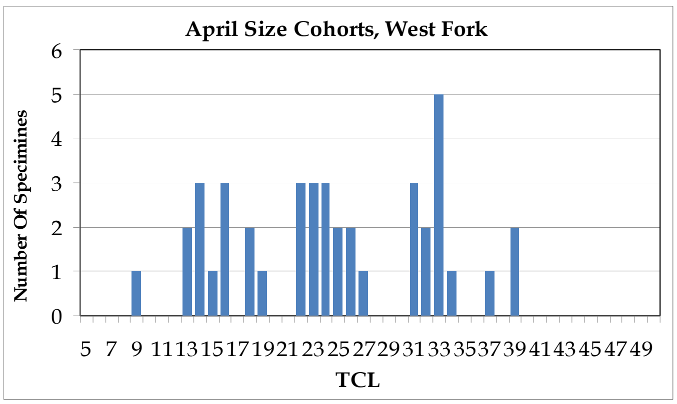
February Thorny Creek Collections.



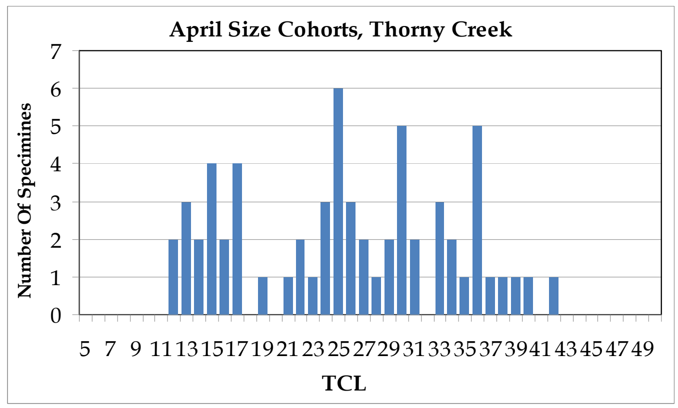
March West Fork Collections.



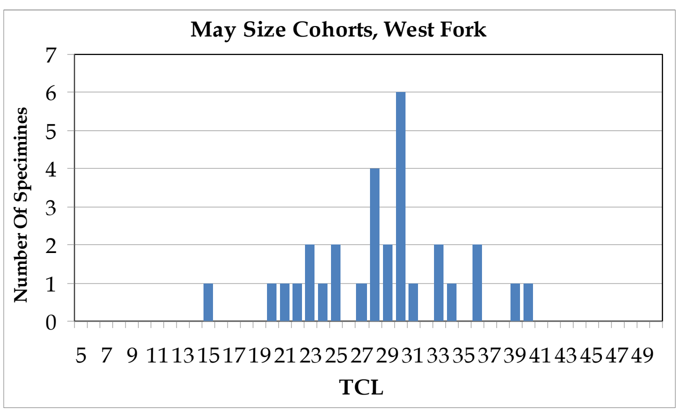
March Thorny Creek Collections.



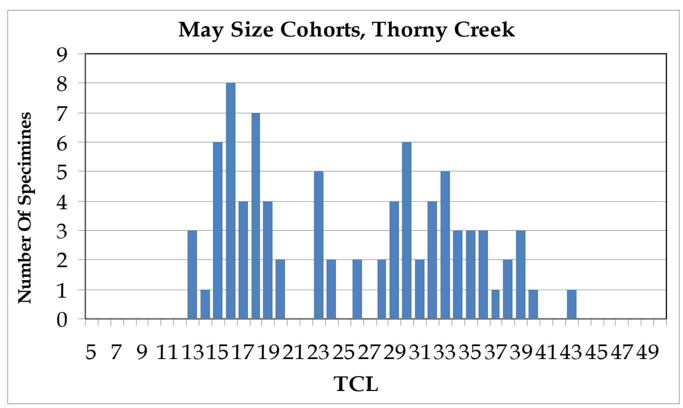
April West Fork Collections.



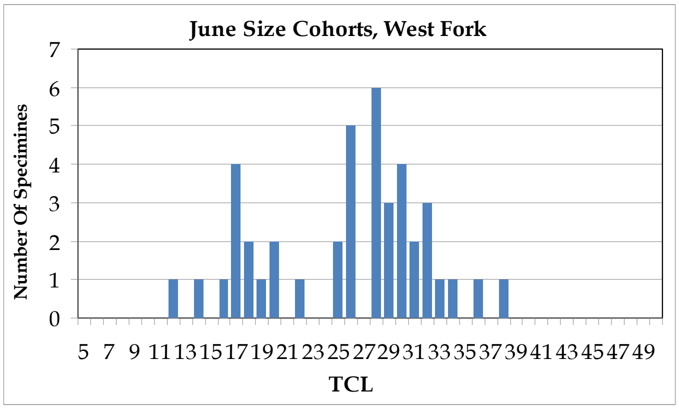
April Thorny Creek Collections.



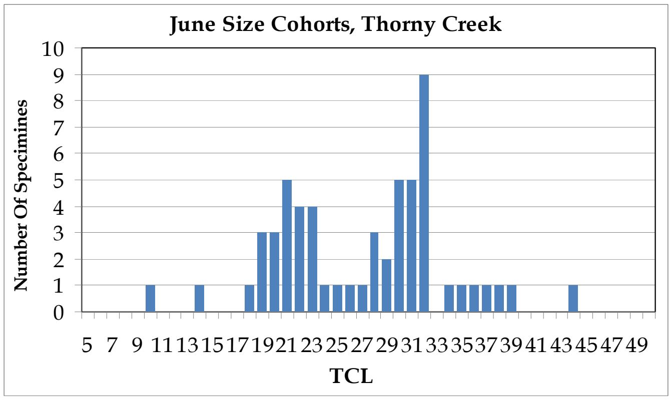
May West Fork Collections.



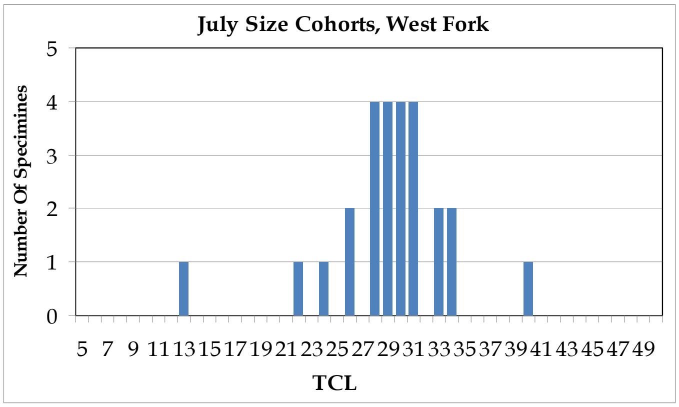
May Thorny Creek Collections.



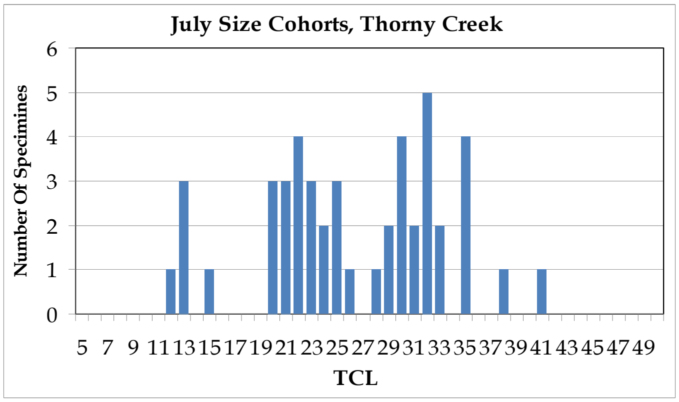
June West Fork Collections.



June Thorny Creek Collections.



July West Fork Collections.



July Thorny Creek Collections.

Appendix F

IBR Letter

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www.marshall.edu

Office of Research Integrity
Institutional Review Board

December 8, 2014

Paul Hughes
310 Echols Lane
Lewisburg, WV 24901

Dear Mr. Hughes:

This letter is in response to the submitted thesis abstract on the natural life history of Cambarus smilax, the Greenbrier Crayfish. 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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A State University of West Virginia • An Affirmative Action/Equal Opportunity Employer

Appendix G

Curriculum Vita

Paul W. Hughes

Curriculum Vitae

310 Echols Lane, Lewisburg, WV 24901
Tel: 304-992-1137
Email: pwhughes@suddenlink.net
Alternative Email:

EDUCATION

Finished Classes, Thesis is being completed
Master of Science: Biological Sciences
Area of Emphasis: Watershed Science Resources
Marshall University, Huntington, WV. August 2009 to Present
GPA: 3.84

Advisor: Thomas G. Jones, Ph.D.
Thesis: The Natural Life History of *Cambarus* (*Puncticambarus*) *smilax*, (The Greenbrier Crayfish)

Bachelor of Science: Major, Natural Resource Management
Concentration, Applied Science
Glenville State College, Glenville WV. May 2008
GPA: 3.78

Advisor: Milan Vavrek, Ph D.
Undergraduate Project: Examining Subspecies Designations for Timber Rattlesnakes *(Crotalus horridus)*

U.S.C.G. Electronics "A" School: Graduated Electronics Technician 3rd Class
United States Coast Guard Training Center, Petaluma California. August 2000
Made Electronics Technician 2nd Class. June 2001

SPECIALIZED SKILLS

- Fish Assemblage Identification (Specialty: Appalachian Ridge / Valley and Plateau, and Maryland Atlantic Slope)
- EPA, WVDEP, and Orsanco Bioassessment Protocols
- Longitudinal Stream and Cross Section survey design and assessment
- Stream Structure design, Bank Erosion Hazard Indexes, and Erosion Rate Calculations
- Freshwater Mussel Surveying and Identification (Specialty in WV, PA, MA, NJ, DE, and TN)

- High Gradient Stream Habitat Assessment for Kentucky and western West Virginia
- Stream Restoration, Assessment, and Analysis
- Aquatic Ecosystem Protection Plans (AEPP)
- Geographic Information Systems- ArcGIS 9.1-9.3.1 & 10
- Trimble, ArcPad, ArcView, ArcGlobe, ArcScene, DNRGarmin
- Sontek Sonar River Profiling, Hydrolab Multi-Parameter Water Instruments
- Electro fishing (Vessel/Backpack), Trawl Nets, Gill Nets, Kick Nets, Hoop nets, Surber, Seine
- SCUBA(adv., night, deep, hookah),
- Substrate Sampling and Analysis: Inverse Distance Weighting, Copper Pole, Wolman Pebble,
- Longitudinal and Cross Sectional River Profiling
- Scientific Writing Applications: Adobe Professional, Microsoft Access, Excel, Word, PowerPoint,

AFFILIATIONS

American Fisheries Society
Friends of the Lower Greenbrier River
Trout Unlimited
Freshwater Mollusk Conservation Society (FMCS)

GRANTS and AWARDS

Graduated Magna Cum-Laude with a Bachelor of Science
G. I. Bill - $36,000 towards college tuition for undergraduate.
2 Letters of Accommodation for exemplary work while serving in the U.S.C.G.

GRADUATE RESEARCH EXPERIENCE: Contact info – Dr. Tom Jones (jonest@marhall.edu – 304.389.5832); Dr. James Joy (joy@marshall.edu - 304 696-3639)

- **Caribbean Island Ecology** (May 2011)
    - Traveled to Bonaire, studied Coral Reef Ecology
    - Studied Bonaire's history and anthropology
    - Completed 23 scuba dives at various locations under various conditions
    - Assisted Senior Professors with directing students while scuba diving

- **Head Water Stream Assessment**
    - Assisted a student with High-Gradient Stream Assessments for grant work
    - Identified trees and plants along with Rapid Bioassessment Protocols EPA

- **Delaware River Mussels** (October 2010 – May 2011)
    - Assessed changes in a mussel population at Wallkill River NJ for the State
    - Worked with the State Park to ensure proper procedures were used
    - Completed monthly surveys under various conditions
    - Completed Dry-Suit dives while diving at just-above freezing conditions

- **Ohio River, WV Mussels Survey** (October 2010)
  - Scuba diving random areas to asses fresh water mussel populations
  - Compared protocols for collecting and assessing mussel populations
  - Scuba dove under low visibility, in colder water.

- **Delaware River, NJ Mussels Survey** (September 2010)
  - Scuba dove to assess mussels populations using Hookah type diving gear
  - Laid grids to strategically search for mussels
  - Completed over 20 dives in a week

- **Susquehanna River, MD Mussels Survey** (August 2010)
  - Scuba dove to assess mussels populations using Hookah type diving gear
  - Laid grids to strategically search for mussels
  - Completed over 45 dives in a week

- **Susquehanna River, MD Trawling, Electrofishing, Snorkeling** (July 2010)
  - Benthic trawling and electro-trawling to compare results
  - Backpack electro-fishing and seine netting for darters and other fish
  - Mapping velocity levels in tributaries to asses habitat
  - Led snorkeling surveys to asses biotic and Abiotic factors in a river

- **Allegheny River, PA Mussels Survey** (July 2010)
  - Scuba diving random areas to asses fresh water mussel populations
  - Using scuba and copper-pole assessments to determine bottom substrate
  - Running a boat and leading a team in proper diving procedures

- **Ohio River, WV Bank Surveying** (June 2010)
  - Assessing the habitat and structures associated with bank usage
  - Using photography and GPS to mark record habitat and structures

- **Seminar, MA Maryland Biological Stream Survey MBSS** (May 2010)
  - Seminar on local identification for mussels, fish, reptiles, and crayfish
  - Passed the mussels identification test

- **Lower Ohio River, KY Bioassessment** (December 2009)
  - Setting, collecting gill nets
  - Electro-trawling, using modified trawling methods
  - Collect scales of fish specimens for aging

- **Susquehanna River, MD Trawling/Electrofishing** (November 2009)
  - Benthic trawling for darters and other fishes
  - Electrofishing and seine netting for darters and other fishes
  - Specimen data and research collection

- **Allegheny River, PA Mussel Survey** (August-October 2009)
  - Assisted in driving and maintaining boats
  - Assisted SCUBA divers
  - Mussel identification and data logging
  - Substrate mapping using copper poling techniques

- **Kanawha River, WV Bioassessment** (March-September 2009)
  - Assisted in driving and maintaining boats
  - Night-time, boat electrofishing
  - Fish and benthic insect identification
  - Sediment accumulation (Booner tubes)

- **Parasitology Lab Assistant, Marshall University** (August 2009 -May 2010)
  - I worked as an assistant to Dr. James Joy for his parasitology classes
  - Performed necropsies on collected fish
  - Identified fish parasites and mounted slides of them
  - Learned different pathologic pathways for aquatic parasites

UNDERGRADUATE RESEARCH EXPERIENCE: Contact info – Dr. Lisa Castle (Lisa.Castle@glenville.edu – 304-462-6307); Dr. Milan Vavrek (Milan.Vavrek@glenville.edu -304-463-6375)

- **White Sulphur Springs Fish Hatchery** (Volunteer) (April 2008)
  - Learned and practiced sterile techniques in aquaculture
  - Sterilized and loaded stocking trucks
  - Counted, collected, and packed trout eggs to be sent to other fish hatcheries
  - Sterilized containers for algae growth, to feed unionid mussels
  - Inoculated algae strains for growth
  - Used a dairy centrifuge to concentrate algae
  - Collected host fish using back pack electrofishing for mussel inoculation
  - Inoculated larval mussels on host fish

- **Cyanobacteria Growth in the Greenbrier River** (August-November 2006)
  - Worked with the WVDEP for data collection and resources
  - Contacted Friends of the Lower Greenbrier River for resources
  - Collected water samples and tested the phosphorus and nitrogen levels
  - Collected algae and bacterial specimens for identification
  - Collected water quality data (pH, temp., dissolved oxygen, etc.)

- **Smallmouth Bass Tissue Collection (**Volunteer) (April-September 2006)
  - Collected smallmouth bass tissue sample in the Greenbrier River and James River

- Preserved the samples and sent them to Cal Borden for DNA analysis

WORK EXPERIENCE

Potesta and Associates Inc. May 2011 – April 2013 Charleston, WV
Currently working as a Staff Scientist and head Fish Biologist at a top environmental consulting firm in West Virginia. Responsibilities include fish identification, toxicology reporting, permitting, following proper DEP, DNR, EPA protocols and regulations, leading field crews.

Environmental Solutions & Innovations October 2010 –May 2011 Cincinnati, OH
Worked part-time as a biological field technical and environmental consultant. Worked mostly with freshwater mussel surveys in multiple States in the Northeast.

Teaching Assistant August 2008 – May 2011 Huntington, WV
Act as a teaching assistant for professors and instruct two to four weekly labs in basic biology.

Stony Brook Plantations August 2004–August 2006 Union, WV
Part-time hunting and fishing guide.
Guided fishing trips down rivers in southern West Virginia.

U.S.C.G. ESD Buxton August 2000–August 2003 Buxton, NC
Electronics' Technician 2nd Class
Worked to operate, maintain, and troubleshoot all electronics for Station Hatteras, Station Oregon-Inlet, Station Ocracoke and Group Cape Hatteras. Repaired and worked on a number of different marina electronics. Knowledgeable in basic electronic theory, GPS, DGPS, and GIS methods.

C.G. Electronics "A" School January 2000 – August 2000 Petaluma, CA
Graduated Electronics' Technician 3rd Class
Study and graduated from the six month electronics school. I learned the basic of electrical theory and how to operate, maintain, and troubleshoot a number of different electronics.

U.S.C.G Cutter Kennebec January 1999 – January 2000 Portsmouth, VA
Enlisted member- Non-rate
Built aids to navigation, worked pneumatic tools and winches, maintained large boat maintenance, painted, cleaned, and other military duties.

Enlisted, U.S. Coast Guard November 1998 – January 1999 Cape May, NJ
Enlisted member
Served as an enlisted member of the US Coast Guard.

Elmore's Plumbing May 1998 – November 1998 Lewisburg, WV
Plumber's Assistant

Worked on many projects that involved an overall understanding of water and gas lines and how to assemble them.

TEACHING EXPERIENCE Contact info – Mrs. Susan Weinstein (weinstei@marshall.edu – 304.696.2428)

Principles of Biology Lab (for majors) – August-January 2009; Marshall Univ.
Principles of Biology Lab (for non-majors) – January-May 2010; Marshall Univ.
Principles of Biology Lab (for non-majors) – August-January 2010; Marshall Univ.
Principles of Biology Lab (for non-majors) – January-May 2011; Marshall Univ.

RELEVANT COURSEWORK

Marshall University: Bioassessment, GIS and Data Systems, Wildlife Conservation, Career Planning in Scientific Fields

Glenville State College: Ecology, Plant Ecology, Plant Physiology, Dendrology, Forest Ecology, Remote Sensing and GIS, CAD Mapping, Microbiology, Cell Physiology, Environmental Law

FIELDWORK SKILLS

Large River Ecology: Open Water SCUBA certified, Trawling, boat electrofishing, fish identification, fish field preservation techniques, substrate surveying.

Stream Ecology: Seine netting, backpack electrofishing, riparian surveying, photography in the field.

Forest Ecology: Canopy surveying, soil profiling, tree and plant identification.

Aquaculture Techniques: Using sterile techniques, applications of up-dwelling and down-dwelling systems, knowledge of necessities of systems (food in-take, water movements, waste removal, UV light and filters for regulating bacteria).

Astacology: Crayfish field collecting, crayfish field preservation techniques, crayfish identification.