THE LIFE HISTORY OF FAXONIUS RUSTICUS (GIRARD, 1852) IN SUNFISH CREEK, MONROE COUNTY, OHIO

A thesis submitted to
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In partial fulfillment of
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Master of Science
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by
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Approved by
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Dr. Mindy Armstead
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APPROVAL OF THESIS

We, the faculty supervising the work of Kyle Thomas McGill, affirm that the thesis, *The Life History of Faxonius rusticus (Girard 1852) in Sunfish Creek, Monroe County, Ohio*, meets the high academic standards for original scholarship and creative work established by the Department of Biology and the College of Sciences. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ABSTRACT

Crayfish are among the most imperiled faunal groups globally. The continued spread of invasive species is a major impact to crayfish. Invasive species, much like the native species, are often understudied. As declines continue, invasive species spread, and new species are discovered; additional life history studies are as important as ever for crayfish conservation. Life history data was collected for rusty crayfish (*Faxonius rusticus*) from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio. *Faxonius rusticus* are native to the Ohio River drainage around Cincinnati, Ohio but have extensively expanded their range over the past 30 years. Sunfish Creek is a direct tributary to the Ohio River and known to be invaded by *F. rusticus*. Collections were conducted monthly obtaining morphometric data, representative photo vouchers, and population observations and trends. Evidence of reproduction was noted in winter to early spring with gravid females observed from March to May of 2018. Free living juveniles were first observed in June of 2018. Adult female total carapace length (TCL) ranged from 15.0 to 37.5mm with the smallest female observed with eggs at 18.7mm. Adult male TCL ranged from 15.0 to 41.9mm with Form I and Form II males observed during all months of data collection. Juveniles were observed during all months of data collection with TCL measurements that ranged from 4.2 to 14.9mm. Life history data observed for *Faxonius rusticus* in Sunfish Creek provided insight to an invasive population that has become well established 50 years post invasion.

CHAPTER I: INTRODUCTION TO *FAXONIUS RUSTICUS* (GIRARD, 1852)

TAXONOMY AND IDENTIFICATION

Crayfish are classified as members of the phylum Arthropoda, subphylum Crustacea, class Malacostraca, order Decapoda, infraorder Astacidea, and from there form three distinctive families Astacidae, Cambaridae, and Parastacidae. North American crayfish are within the family Cambaridae (Crandall and De Grave, 2017). Crayfish are identified by a hard exoskeleton which is regularly shed/molted then regrown and fortified as the individual grows (Taylor and Schuster, 2004). Additional metrices used for crayfish classification are genetic analysis and a variety of morphological characters including but not limited to: gonopod structure, rostrum size, chelae size, body and chelae coloration, size and shape of cephalothorax (body), orientation and number of tubercles (bumps), and shape and texture of mandibles (Jezerinac et al. 1995) (Figure 1). A gonopod is a modified structure designed for sperm transfer and is a character readily used in crayfish identification (Taylor and Schuster, 2004). The crayfish within the family Cambaridae have gonopods that observably change structure as the male molts between sexually reproductive (Form I) to sexually non-reproductive (Form II) (Taylor and Schuster, 2004). In 2017, Crandall and De Grave restructured the naming convention for several crayfish species world-wide. The species serving as a center point for this project was one of the groups Crandall and De Grave revised. Originally known as a member of the genus *Orconectes* (Cope, 1872) as *Orconectes rusticus* (Girard, 1852) is now a member of the genus *Faxonius* (Ortmann, 1905) as *Faxonius rusticus* (rusty crayfish). The change from *Orconectes* to *Faxonius* is based on a separation of cave dwelling (*Orconectes*) and non-cave dwelling (*Faxonius*) species that were originally organized under *Orconectes*.

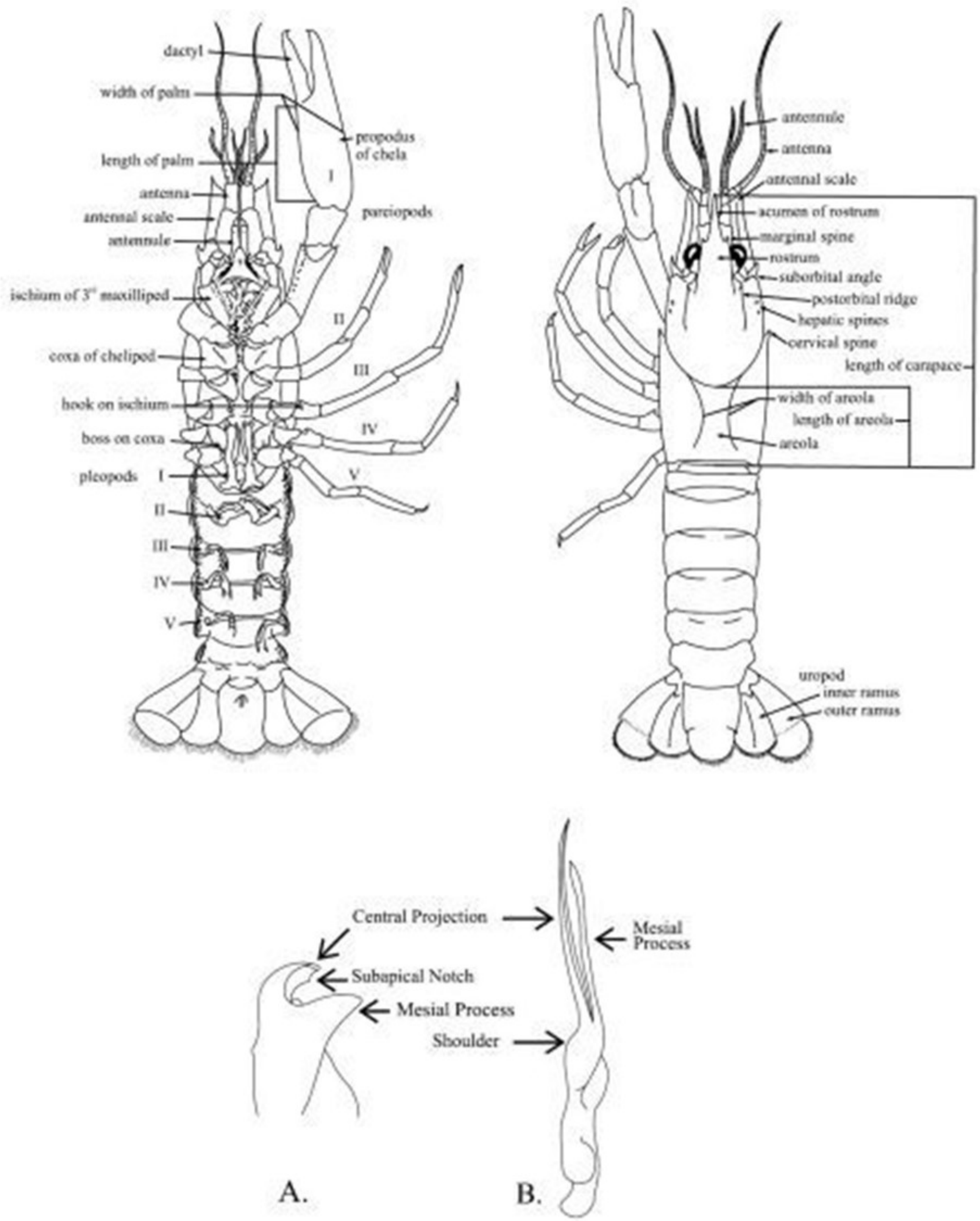

Figure 1. General diagram of male crayfish detailing characters used in morphometric processing. A. Represents a *Cambarus* gonopod. B Represents a *Faxonius* gonopod. Taken from Hobbs et al. 1989.

Faxonius rusticus can be identified by their carapace and lateral surface of the chelae displaying brown to green coloration always displaying a dark red (rusty) spot on either side of their carapace, where its common name the rusty crayfish was derived (Page, 1985; Jezerinac et al. 1995; Taylor and Schuster, 2004). The dorsal portions of their carapace typically display a U-shaped dark brown saddle that extends anteriorly, and abdomen segments often appear dark brown with W-shaped patches that are also dark brown (Taylor and Schuster, 2004; Gunderson, 1995). They have large chelae that are gapped at the base when closed and the distal most points of their chelae are often red tipped followed by black bands (Page, 1985; Taylor and Schuster, 2004; Gunderson, 1995). Other physical characteristics noted by Taylor and Schuster (2004) include: rostrum distal margins straight to slightly concave, rostrum proximal margins more convex, rostrum that is deeply trenched, lack of median carina, small spines near the junction of the base of acumen and termination of rostral margins, acumen often greater than or equal to the width of the rostrum at the marginal spines, dorsoventrally compressed carapace, presence of cervical spines, large chelae with elongated fingers, smooth palms sometimes with punctuations, weak ridges observed on dorsum of fingers, and two gentle rows, with several interspersed, of tubercles on the mesial margin of palm and dactyl. Sexual dimorphism is observed with males often having larger chelae and being slightly larger overall than females. Sexually reproductive males (Form I) are distinguished by the two corneous central projections each with a smaller non-corniculate median process and the rear surface displaying a strongly angled shoulder (Jezerinac et al. 1995). Sexually non-reproductive males (Form II) are distinguished by non-corniculate mesial process smaller than the central projections and without the rear surface displaying a strongly angled shoulder (Jezerinac et al. 1995). Females have a nearly rhomboidal annulus ventralis with a trench through the anterior portion and two posteriorly facing projections that extend over the central depression (Taylor and Schuster, 2004).

BACKGROUND

Faxonius rusticus is native to the Midwest United States and was originally described from the middle Ohio River at Cincinnati, Ohio and known to inhabit the Ohio River proper and its tributaries within northern Kentucky, Indiana, and Ohio (Creaser, 1931; Page, 1985) (Figure 2). The invasion front of *F. rusticus* has been expanding for several years as depicted in the USGS range map (Figure 2) with first observations outside of their natural range as early as the 1960s (DiDonato and Lodge, 1993). Primarily through bait bucket introductions (Hobbs et al. 1989; Page, 1985, Taylor et al. 1996), *F. rusticus* have become a widespread invasive species in many states within the United States (Taylor and Redmer, 1996) and even into Canada (Crocker and Barr, 1968; Berrill, 1978; Momot, 1992) (Figure 2). States with known invasive populations of *F. rusticus* include: New Mexico, North Dakota, South Dakota, Minnesota, Iowa, Illinois, Michigan, West Virginia, Tennessee, Pennsylvania, Wisconsin, Maine, New Hampsire, Vermont, Massachusetts, Rhode Island, New York, New Jersey, North Carolina, and Maryland (Figure 2). Europe has completed a risk assessment summary sheet for the potential invasion of *F. rusticus* based on a single known population of *Faxonius juvenilis* (Girard, 1852), a known similar species to *F. rusticus*, in France. The only known occurrences of *F. rusticus* in Europe are in aquaria (Rogers and Watson, 2016). This species is known to inhabit multiple freshwater waterbody forms (lakes, ponds, and streams) preferring clear well oxygenated waters with diverse habitat and available shelter/cover objects (Capelli, 1982 and Gunderson, 1995). Taylor and Redmer (1996) found that they showed preference for cobble habitat for available cover objects. *Faxonius rusticus* is known to inhabit low to high flow streams but Capelli (1982) and

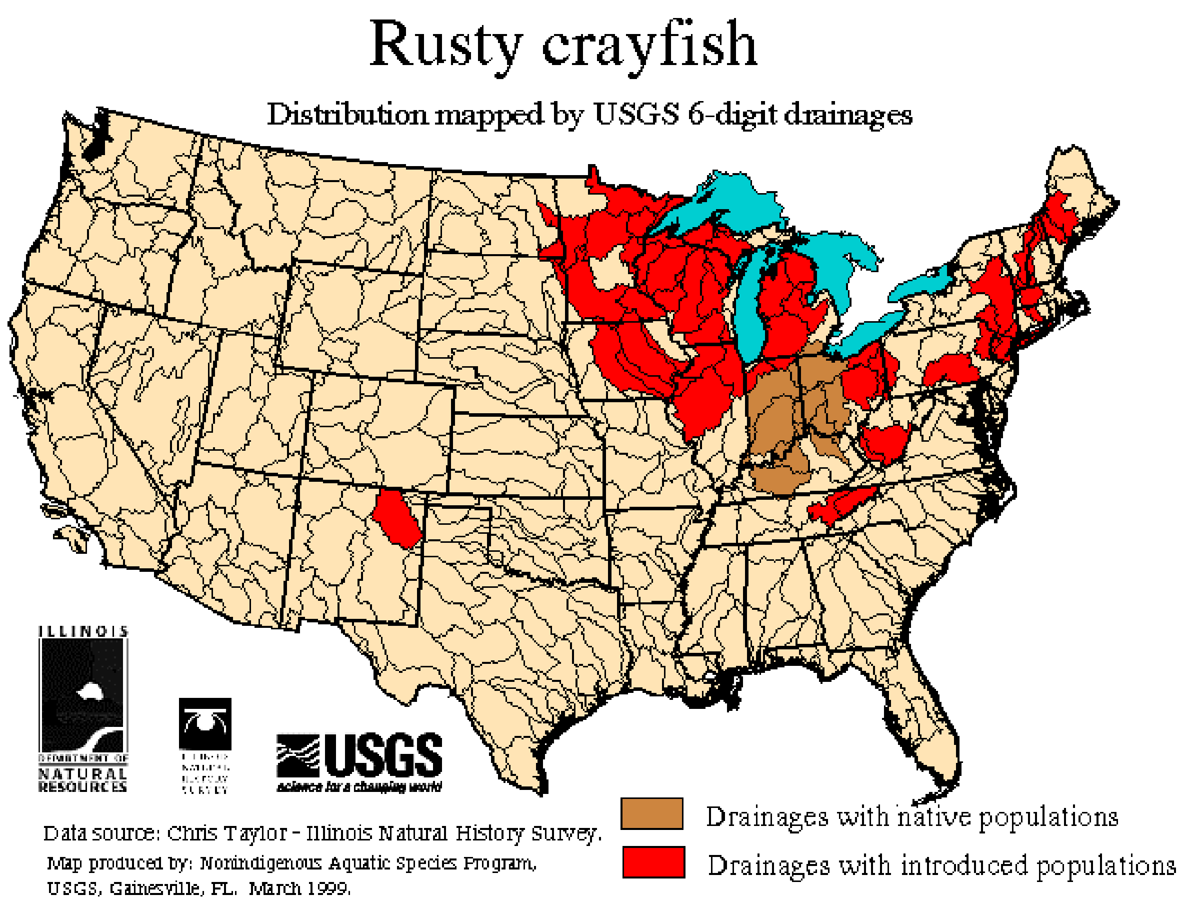

Figure 2. Distribution map for native and invasive populations of *Faxonius rusticus* in North America. Taken from ncwildlife.org 2018

Gunderson (1995) found they are less successful burrowers, compared to other species, when water conditions decline. An example of this is a comparison with the papershell crayfish *Orconectes immunis* (Hagen, 1870) that is known to burrow in lakes to avoid desiccation due to reduced water levels. *Faxonius rusticus* is not known to achieve such feats and need more permanent waterbodies (Gunderson, 1995). In the various waterbody types, *F. rusticus* was predominately found in less than one meter of water but has been found as deep as 14.6 meters (Taylor and Redmer, 1996). Butler and Stein (1985) found that juveniles were typically found in shallower habitats (less than 15cm) near edge habitat while adults were often found in the deeper habitats (greater than 20cm). This species is known to be a non-burrower (Taylor et al. 1996) and is noted as dominating perennial waterbodies investigating the benthos at night spending the majority of daylight hours under cover objects (Crocker and Barr, 1968; Stein, 1977; Taylor and Redmer, 1996). Personal observations in the field differed in seeing *F. rusticus* investigating the stream benthos at both day and night and using cover objects in the presence of potential predators. Personal observations during dive surveys in the Ohio River mainstem indicated that *F. rusticus* may create small burrows in clay banks of the river when no other structural habitat is available. Habitat complexity is known to be of strong importance to the dispersal patterns (Taylor and Redmer, 1996) and the species is typically observed in rock or rocky debris habitat. Although many habitat characteristics play a role in sustainability, Phillips et al. (2009) found the littoral zone of lakes and streams to be the most significant for *F. rusticus*. Observed differences in growth rate and aggression between native and non-native populations are theorized to contribute to invasion success and the impact invasive populations have on native ecosystems (Pintor et al. 2008; Pintor and Sih, 2009; Sargent and Lodge, 2014; Reisinger et al. 2017; and Glon et al. 2018)). This theory is based on the idea of how aggressive the invader needs to be in relation to the presence or absence of competition (Pintor et al. 2008). An example being the invading species may be less novel than the native species and therefore would need to be more active, increase feeding rates, overcome native species defenses to aid in their success, and be less bold to minimize predation risk (Bondar et al. 2006; Turner et al. 2006; Pintor et al. 2008). Mather and Stein (1993) found that *F. rusticus* typically exhibited the same activity level to that of other *Faxonius* of similar size in the presence of predatory fish but exhibited increased activity to that of other *Faxonius* of differing size in the presence of predatory fish. They also found similar results when predatory fish were absent, but the activity difference of differing size groups favored *F. rusticus*.

Mating of *F. rusticus* appears to be temperature driven as they have been observed mating in early spring, late summer, and early fall. Females are capable of storing sperm from multiple males until water temperatures are appropriate for egg fertilization (Berrill and Arsenault, 1984) and Gunderson (1995) summarized that female egg counts can range from 80 to 575 eggs. Berrill (1978) found that upon hatching, juveniles will stay near the mother for several weeks (8 to 10 instars). Once maturity is reached, growth rates slow considerably. Prins (1968) found that fecundity appears strongly correlated to the size of sexually mature females. Males have been known to molt multiple times per year going from Form II (sexually inactive) in the spring molting back into Form I (sexually active) in the summer and females typically molt once a year following the release of their young (Gunderson, 1995). Lorman (1980) found that *F. rusticus,* in northern Wisconsin, were found to live up to four years and adults had a total carapace length (TCL) ranging from 18.5mm to 58.0mm. Jezerinac et al. (1995) documented information on 141 specimens of *F. rusticus* from six collection locations within two counties (Cabell and Putnam) in West Virginia. Of the total individuals analyzed 39 were Form I males, 36 were Form II males, and the remaining 66 were females. Total carapace length for all individuals observed ranged from 22.7mm to 36.5mm. Palm length for all individuals observed ranged from 3.7mm to 9.4mm. Propodus length for all individuals observed ranged from 14.1mm to 35.0mm. The sex ratio for individuals observed was 1.1:1 which was not statistically different (χ^2=0.57, p>0.3) from a 1:1 ratio. The relationship between number of eggs and carapace length was NE= 8.31 x TCL-91.49 (r=0.93, n=49). Jezerinac et al. (1995) noted the presence of smooth edges on *F. rusticus* mandibles within West Virginia populations.

Previous studies have shown that chelae size is an important component of multiple interactions including dominance between males, predatory defense, and foraging (Bovbjerg, 1956; Stein, 1976; Berrill and Arsenault, 1982 and 1984; and Bruski and Dunham, 1987). Chelae size appears to be an important factor in sexual dimorphism, dominance behaviors, foraging, and defensive behaviors favoring larger chelae as a male selected trait, further suggesting the role chelae play in interactions between males and females (Snedden, 1990). *Faxonius rusticus* juveniles were recorded having greatest survivability between 20-25 degrees Celsius but exhibit optimal growth between 26-28 degrees Celsius (Mundahl and Benton, 1990). Juvenile and adult *F. rusticus* are known to favor similar water temperatures favoring survivability; however, they are typically not observed in similar habitats. Adults are believed to displace juveniles into warmer habitats to maximize growth, facilitating competitive ability and fecundity (Mundahl and Benton, 1990). This behavior favors an R-selected reproductive strategy by focusing on maximizing growth rate over initial survivability in efforts to gain the competitive edge and increased chance of continued fecundity (Mundahl and Benton, 1990).

Historically, crayfish have been classified as detritivores, herbivores, and predators (Lorman and Magnuson, 1978; Webster and Patten, 1979; Huryn and Wallace, 1987) known to feed on detritus, periphyton, macrophytes, eggs of aquatic species, and several benthic macroinvertebrates (Lorman and Magnuson, 1978; Lorman, 1980; Jones and Momot, 1983). Hanson et al. (1990) and Momot (1992) found juveniles show preference toward stoneflies, mayflies, midges, and other similar benthic macroinvertebrates while Lodge and Lorman (1987) found adults preferred snails. Lodge and Lorman (1987) also found that *F. rusticus* was capable of reducing total macrophyte biomass within two months.

INVASIVE IMPACTS

The need for understanding invasive species characteristics and traits for success has gained much research attention over the past thirty years as the invasion front continues to expand (Kolar and Lodge, 2001; Hulme et al. 2008; Blackburn et al. 2011) (Figure 2). Invasive species have been deemed responsible for the eradication and endangerment of many species of fish, mollusks, crayfish, and other aquatic invertebrates (Miller et al. 1989; Pitcher and Hart, 1995; Dobson et al. 1997; Lodge et al. 1998). One of the leading causes of decline in native species with small natural ranges (i.e. crayfish) is invasive species (Lodge and Hill, 1994; Lodge et al. 1998). Small native ranges in North American crayfish are exemplified by 11 species that are endemic to a single location and an additional 20 species known from two to five locations (Taylor et al. 1996). Some North American crayfish species, approximately 43%, are restricted to the boundary of a single state (Taylor et al. 1996; Lodge et al. 1998) and several species may inhabit multiple states but are only found in drainages that cross those state boundaries (Lodge et al. 2000). Populations with small natural ranges will exemplify invasive impacts because a small area inundated by an invasive species could impact a larger portion of a native population with range restrictions (Gilpin and Soule, 1986; Rabinowitz et al. 1986). While understanding the mechanisms driving the dispersal of invasive species is important, it is also imperative to track the interactions and outcome of invasive species impacts to biotic and abiotic characteristics of the environment (Shea and Chesson, 2002; Blackburn et al. 2011). This need for a broader understanding is due to the variety of approaches for which invasive species are analyzed such as invader characteristics, natural predation, characters of the ecosystem being invaded, and available resources (Lonsdale, 1999; Sher and Hyatt, 1999; Davis et al. 2000; Kolar and Lodge, 2001; Keane and Crawley, 2002). The various viewpoints are typically assessed independently to determine potential issues when they are likely interconnected and should be analyzed concurrently (Shea and Chesson, 2002). The changes to native crayfish and other members of aquatic ecosystems associated with the introduction of invasive crayfish are a driving force of the current and future studies in efforts to understand the invasion mechanisms (Capelli and Munjal, 1982; Berrill, 1985). As far as invasive species are concerned, *F. rusticus* are just a single example of the global invasive species problem currently posing substantial impacts to native biodiversity in aquatic ecosystems (Lodge, 1993; Vitousek et al. 1996; Williamson and Fitter, 1996; Hill and Lodge, 1999; and Lodge et al. 2000). It has been noted that most invasive species are extremely difficult to nearly impossible to eradicate once established. With that in mind, strong efforts have been and are continually being made to find ways to reduce and contain the spread of these invasive species (NISC, 2001; Lodge et al. 2006).

Hill et al. (1993) noted the *F. rusticus* has proven to be an avid invader by almost always outcompeting the native species as it expands its range, and often times out competes other invasive species such as virile crayfish, *Faxonius virilis* (previously *Orconectes virilis* [Hagen, 1870]; Crandall and De Grave, 2017). The invasion of *F. rusticus* has led to the extirpation of *F. virilis* in many of Canada’s watersheds (Davies, 1989; Heneberry et al. 1992; France and Collins, 1993). A contributing factor to this interaction could be derived from *F. rusticus* and *F. virilis* occupying similar habitat associated with the littoral zone of lakes and streams commonly occurring in complex rocky habitat providing structure for predation evasion (Crocker and Barr, 1968; Stein, 1977; Taylor et al. 1996). Other observations in Canada from Crocker and Barr (1968) found native species presence in waterbodies do not deter the *F. rusticus* from continuing their ever-expansive invasion. *Faxonius rusticus* have eliminated Allegheny crayfish, *Faxonius obscurus* (previously *Orconectes obscurus* [Hagen, 1870]; Crandall and De Grave, 2017); a native species, from the Sunfish Creek drainage (Thoma, 2007). It has been theorized that the aptitude for which *F. rusticus* invade new systems is partially due to 1) a higher metabolic rate than many of its congeners resulting in increased consumption rates (Jones and Momot, 1983), 2) being reproductively categorized as an R selected species emphasizing maximum growth (Soderback, 1991 and 1992; Lindqvist and Huner, 1999), 3) having a higher intrinsic competitive dominance as both a native and invasive when compared to other species (Hill et al. 1993; Gioria and Osborne, 2014; Byers, 2000; Sanders et al. 2003; Crandell and De Grave, 2017), and 4) having differing growth, survival, and olfaction abilities from native species (Hill et al. 1993; Willman et al. 1994). Reproducing at colder temperatures (i.e. earlier) gives *F. rusticus* an additional advantage over other R-selected crayfish (Mundahl and Benton, 1990; Soderback, 1991 and 1992; Lindqvist and Huner, 1999). Earlier reproduction and focus on maximum growth provide a competitive advantage over K-selected crayfish that focus on survivability. An example of higher competitive dominance was noted by Lodge et al. (1994), where *F. rusticus* out competed native species for cover objects effectively increasing potential predation rates of the crayfish displaced from cover objects. Glon et al. (2018) found that *F. rusticus*, as both native and non-native populations, were more aggressive and dominant over *F. virilis*, a known avid invader. Dresser et al. (2016) found that native crayfish will spend more time exhibiting agnostic behaviors in the presence of *F. rusticus* and the extent of the agnostic behavior varied based on each interaction in duration, aggression, and sex difference of each species. These agnostic behaviors often relate to competitive behaviors over food resources, structural habitat, and fighting/retreating for establishment of dominance. Some studies have shown that increased metabolic rates of *F. rusticus* can be linked to notable reductions in available macrophytes and benthic macroinvertebrate biomass (Olsen et al. 1991; Momot, 1992; Lodge et al. 1994). Nystrom (1999) found the, often times, large scale reduction in macrophyte biomass from grazing has been linked to reduction of benthic macroinvertebrates through predation and the alteration/destabilization of aquatic habitats resulting in the decline of other aquatic species. Reid and Nocera (2015) noted the potential they have to negatively impact recovery efforts for sensitive aquatic species by out competition of native crayfishes, which are an established food source. Impacts to native fauna that are established food sources have the potential to negatively affect other aquatic species as it is unknown how suitable *F. rusticus* are as dietary replacements even considering their relative abundances (Reid and Nocera, 2015). Because crayfish are known as ecosystem engineers (Statzner et al. 2000; Creed and Reed, 2004), it is not a stretch for negative impacts, to the environment and aquatic community, to be expected with invading crayfish populations (Phillips et al. 2009). *Faxonius rusticus* are known to be a dominant invading species outcompeting native species and are known for overexploitation of resources (Hill et al. 1999; Nystrom, 1999). Phillips et al. (2009) noted that crayfish diminish habitat complexity through vegetation reduction, re-suspension of sediment, and bioturbation when altering habitat which can alter habitat for many other species within the ecosystem (i.e. fish and other benthic macroinvertebrates). In laboratory experiments Welch (2014) found that they increase turbidity. Reproductive events have been found happening earlier in the year (at cooler water temperatures) than multiple other crayfish species (members of *Cambarus* and *Faxonius*) inhabiting the same waters, giving their young an advantage on development and foraging of available resources (Mundahl and Benton, 1990). Perry et al. (2001 and 2002) noted hybridizing with the northern clearwater crayfish *Faxonius propinquus* (previously *Orconectes propinquus* [Girard, 1852]; Crandall and De Grave, 2017); which appeared to be a mechanism that interrupted reproduction efforts of the native species.

PROJECT JUSTIFICATION AND HYPOTHESES

It is well documented that crayfish play an integral role to both aquatic and terrestrial ecosystems by functioning as predator, prey, and continually altering habitat (Roell and Orth, 1993; Lodge and Hill, 1994; Dorn and Mittelbach, 1999; Statzner et al. 2000; Swecker, 2012). Crayfish are among the most imperiled faunal groups globally and continue to decline from invasive species and anthropogenic impacts (Capelli and Munjal, 1982; Berrill, 1985; Nystrom, 1999; Reid and Nocera, 2015). The roles they fill coupled with their continual decline warrants additional studies to slow and hopefully prevent further eradication. Invasive species play a role in the decline of many species (Miller et al. 1989; Pitcher and Hart, 1995; Dobson et al. 1997; Lodge et al. 1998); therefore, understanding the mechanisms behind their success could provide insight on potential methods for reducing the invasion success and associated impacts. The severity of documented impacts of the invasive *Faxonius rusticus* (Girard, 1852) or rusty crayfish has been the driving force behind many studies.

The primary reason behind selecting Sunfish Creek as a study location is based on known occurrence data for *F. rusticus* within this stream, which is outside of the native range for *F. rusticus* (Figure 2). The main goal is to conduct a life history study on a population long after it was known to invade a stream. Few life history studies have been completed at the expansion edge of invasive species and fewer, if any, life history studies have been completed several years post invasion. A study conducted by the Ohio Environmental Protection Agency (OEPA) from 2010 detailed a biological assessment of the Sunfish Creek Watershed. Their efforts deemed Sunfish Creek as a High Quality Water supporting healthy fish and macroinvertebrate populations (OEPA 2010). Sunfish Creek was even stated to have comparable fauna to Captina Creek, a state renowned stream of exceptional biological integrity (OEPA 2009). A survey was conducted by Roger Thoma (2007) detailing the status of *Faxonius obscurus* in the presence of *F. rusticus* within the Flushing Escarpment. The Flushing Escarpment is a series of direct tributaries to the Ohio River along the eastern edge of Ohio. This area has been geographically isolated and contains the original Allegheny River fauna from before the Illinoisan and Wisconsin glacial invasion (Thoma, 2007). The study completed by Thoma (2007) also provided supplemental information on *F. rusticus* densities and extent upstream at six sites within the Sunfish Creek drainage.

The main hypothesis of this study is that *F. rusticus* will be established within Sunfish Creek in higher densities than its potential native congeners *Faxonius obscurus*, Rock crayfish *Cambarus carinirostris* (Hay, 1914), and Bigwater crayfish *Cambarus robustus* (Girard, 1852), based on previously documented information from Thoma (2007). Upstream movement of the *F. rusticus* population is theorized to be displayed by oscillating collection numbers within the habitat over the course of the study. Based on literature review, *F. rusticus* is expected to dominate the littoral zone of the stream (Crocker and Barr, 1968; Stein, 1977; Taylor et al. 1996). Flow rates are theorized to negatively change the number of individuals observed as flow increases. The assumption is that individuals will look more to cover objects during increased flows but also survey efficiency will also likely diminish during times of elevated flows. Sexual presence within the population is expected to favor males as they are known to have larger chelae which are known to aid in encounters with congeners species, reproduction, obtaining and maintaining shelter, and obtaining resources (Bovbjerg, 1956; Stein, 1976; Berrill and Arsenault, 1982 and 1984; and Bruski and Dunham, 1987). The establishment of a sexual maturity threshold will categorize adult size for the population and allow for comparison to other known adult population sizes. Observations are expected to increase with water temperature. Significant differences are expected to be observed between morphometric measures between Form I and Form II males. Observable differences between densities of Form I and Form II males are expected to coincide with reproductive season. The potential for multiple molting events within the survey duration are expected. Variation between repetitive observations of morphometric measurements are expected to be minimal.

Additional goals are to increase awareness of life history information as well as pertinent observations of invasive populations. This project details locally specific life history information on the invasive *F. rusticus* as they function in Sunfish Creek, Monroe County Ohio. This project is a partial fulfillment of the requirements for completion of a Master's Thesis.

CHAPTER II: LIFE HISTORY OF *FAXONIUS RUSTICUS* (GIRARD, 1852) IN SUNFISH CREEK, MONROE COUNTY, OHIO

INTRODUCTION

Crayfish are a critical component of aquatic ecosystems as they typically function as the largest aquatic invertebrate in biomass, are capable of immense habitat alteration from overturn during feeding, are generally more aggressive feeders, are capable of range expansions, and play important roles in both terrestrial and aquatic food assemblages. In an ecosystem food assemblage crayfish function as both predator and prey by feeding on plants, animals, and detritus as well as being a food source for many aquatic, terrestrial, and avian species (Corey, 1988). Because of the many functions/roles they fulfill (i.e. predator, prey, and ecosystem engineer), crayfish are frequently categorized as keystone species (Momot et al. 1978; Lorman, 1980; Momot, 1984; Lodge and Lorman, 1987; Olsen et al. 1991; Creed, 1994; Momot, 1995; Rabeni et al. 1995; Simberloff, 1998; Wilson, 2002; Swecker, 2012; Distefano et al. 2013). Similar to many aquatic and invertebrate species, crayfish are currently declining and are ranked among the top three most imperiled fauna closely trailing freshwater snails and Unionid Mussels (Taylor et al. 2007). Even though new species are still being described yearly, life history studies are lacking for many described species (Distefano et al. 2013).

One of the leading threats to freshwater ecosystems are invasive species with impacts including over exploitation of available resources, shifts in ecosystem roles for native species, an alteration of existing food web dynamics, and in some cases complete extirpation of native species (Simon and Townsend, 2003; Eby et al. 2006; Cucherouset and Olden, 2011). Invasive species in North America have been labeled the culprit for the endangerment or extinction of multiple aquatic invertebrates and native fishes (Miller et al. 1989; Dobson et al. 1997). In most cases, invasive species are only successful when the ecosystem niche they fulfill is unoccupied or poorly occupied in the ecosystem they are invading. For the rusty crayfish *Faxonius rusticus* (Girard, 1852), restructured by Crandell and De Grave (2017) from the former *Orconectes rusticus* (Girard, 1852), a vacant ecosystem niche is not necessary due to their exceptional proficiency at invading and outcompeting native congeners (Momot et al. 1978; Capelli and Munjal, 1982).

SUNFISH CREEK

Sunfish Creek in Monroe County Ohio, a direct tributary to the Ohio River, falls within the Round bottom USGS 7.5 quadrangle at the following coordinates Latitude: 39.749526, Longitude: -80.907209. Sunfish Creek is one of the streams categorized under the Flushing Escarpment and is considered to be within the Western Allegheny Plateau ecoregion. A series of direct tributaries to the Ohio River from Monroe to Columbiana County along the Eastern edge of Ohio form the Flushing Escarpment. The Sardis Coll, a geographical separation of the pre-glacial Allegheny and Teays River systems, forms the southern extent of the Flushing Escarpment (Tight, 1903; Trautman, 1981). These streams are dominated by unglaciated Pennsylvanian shale bedrock, are high gradient, and have multiple historical industrial and urban impacts (Thoma, 2007). Slucher et al. (2006) noted that substrates for the Flushing Escarpment are a mix of sandstone, shale, coal, and limestone layers. Sunfish Creek is known to have a higher bedrock composition of limestone (Thoma, 2007). Sunfish Creek has a 103 square mile drainage area calculated at the project location. Land use within the Sunfish Creek watershed is dominated by forest (62.2%) with only 7.49% of land use within the drainage basin developed land (urban); the area receives an average of 98 centimeters of rain annually (USGS, 2018).

In 2007 Roger Thoma completed surveys at multiple locations within the Flushing escarpment to assess the status of the Allegheny crayfish *Faxonius obscurus* (Hagen, 1870) (previously *Orconectes obscurus* [Hagen, 1870]; Crandall and De Grave, 2017) in the presence of the invasive *F. rusticus*. *Faxonius rusticus* was first observed in the Sunfish Creek drainage by Dr. D.H. Stansbery in 1967 (Thoma, 2007). *Faxonius obscurus* is a known native species to this drainage (Thoma, 2007) and the potential interactions with *F. rusticus* were the reasoning for the study. Surveys were completed in other streams within the Flushing Escarpment, but for the purposes of this project the results for Sunfish Creek will be the only information detailed herein. Surveys were completed at a total of six sites within the Sunfish Creek drainage area with results representing two species *F. rusticus* and the Rock crayfish *Cambarus carinirostris* (Hay, 1914). *Faxonius rusticus* were observed at all six survey sites with an average observed density of 0.75 per meter squared. *Cambarus carinirostris* were observed at five of the six survey sites with an average observed density of 0.93 per meter squared. The presence of *F. obscurus* was not observed at any of the six survey sites and confirmed the observation of extirpation. The last known collections of *F. obscurus* from Sunfish Creek were recorded in 1988 by R.F. Jezerinac and G.W. Stocker (Thoma, 2007). The results of this study show *F. rusticus* populations were thriving at the expense of *F. obscurus*. The results were concluded detailing that some time within 30 to 40 years, *F. rusticus* moved in and extirpated *F. obscurus* from a basin in which it once thrived (Thoma, 2007).

The Ohio Environmental Protection Agency (OEPA) completed a Biological Assessment of Sunfish Creek watershed in 2009 encompassing 18 sites of which nine were in Sunfish Creek proper (OEPA, 2009) (Appendix B). Dense and diverse fish and macroinvertebrate communities, including fish species known as intolerant to water pollution, were observed at all nine sites earning the recommendation for Exceptional Warmwater Habitat (EHW) designation between Negro Run and Standingstone Run as well as being designated as a Superior High Quality Waters (SHQW) along with three of its tributaries (Piney Fork, Opossum Creek, and Leith Run). Of the nine Sunfish Creek sites: one site was classified excellent (score of ≥75), six were classified good (score of 55 to 69), and two were classified fair (score of 43 to 54) according to the Qualitative Habitat Evaluation Index (QHEI) scores. The QHEI is a method designed to evaluate the quality of habitat within a stream. The evaluations covered in this method are designed to measure the habitat factors that affect fish communities, which are typically the same habitat factors that are important to other aquatic species (Rankin, 1989). An Index of Biological Integrity (IBI) is a method used to evaluate water pollution presence within a stream. The IBI for Sunfish Creek has increased from 45.0 (1983) to 48.6 (2009) due to increased observations of fish species deemed pollution intolerant in the population. The OEPA found that less than half of the sites on Sunfish Creek displayed heavy to moderate substrate embeddedness. Observed bedrock composition was similar to that found by Thoma (2007) and was predominately limestone. The macroinvertebrate community observed within Sunfish Creek was considered to be comparable to the community observed in Captina Creek, a stream known for exceptional biological quality (OEPA, 2010).

MATERIALS AND METHODS

Site

This stream was selected as the locality for the project because it is known as a High Quality Water according to the OEPA (2009) and known to contain an invasive population of *F. rusticus* (Thoma, 2007). The study reach was selected after completing spot checks for areas containing riffle, run, and pool stream morphology as well as diverse substrate composition likely to support crayfish. Two sites on Sunfish Creek were selected for the study location and only one was used for the duration of the study. Site selection was based on habitat availability and suitability. Sunfish Creek at the survey location flows along the periphery of open bottom land at the base of a hillside (Appendix B). The left descending bank (LDB) of the stream is bordered by a gravel road (Gibbs Road) with residential development just beyond the road. The entirety of the right descending bank is directly adjoined by forested hillside proximal to the survey area. Upstream of the survey location approximately 0.80 kilometers resides a cattle farm where the cattle do not appear to have direct access to the stream. Approximately 2.4 kilometers down-stream of the survey location Sunfish Creek becomes a wider slack water stream with a defined channel deep enough for small boat traffic. Bedrock was not observed in any of the surveyed habitat although it is likely more prevalent upstream of the survey location.

Life History

Surveys were completed from October 2017 to October 2018 preferentially during the first half of each month. The survey reach was extended to include additional run habitat found directly upstream of the riffle (March 2018) due to severe habitat alteration from multiple high water events in late 2017 and early 2018. Each month a total of 30 seine hauls were completed within the survey reach and were divided into 10 hauls in the riffle, run, and pool. Seine hauls were completed using a 2.44 meter by 1.22 meter seine net and served as the primary collection method. Hand collection was completed during supplemental searches and surveys during times of low water/flow. Seine hauls were completed by placing the net facing upstream (slightly angled downstream) below the habitat to be surveyed. One person held the net while another flipped available cover objects and lightly disturbed the stream bottom pushing the water and any crayfish downstream into the seine. Survey efforts allowed for the calculation of catch per unit effort (CPUE) for each morphology type based on the replicates of the seine hauls (fixed size) and total specimens observed. The CPUE measures the number of individuals collected per seine haul. Biotic and abiotic factors were recorded each month including: substrate percent composition, wetted width, bankfull width, air and water temperature, current weather conditions, recent rainfall, time, date, embeddedness changes to stream bank or instream habitat, current water level (high, normal, low), and average and max depth for each morphology. Embeddedness assesses the extent of which silt and sand have filled the interstitial space around gravel, cobble, and boulder habitat. Flow data was based on the nearest and most similar surrogate site Captina Creek as there are no known stream gauges on Sunfish Creek. The Captina Creek gauge provided flow estimates for Sunfish Creek at the time of survey efforts.

A goal of 60 specimens per month was selected in effort to obtain enough data for statistical analysis. All individuals collected during survey efforts were retained in minnow buckets for identification and morphometric processing. During times of reproduction, females with eggs and juveniles were placed in separate minnow buckets in order to minimize stress and predation. Processing was completed along the stream bank under shade directly following completion survey efforts. Measurements were taken using SPI dial calipers to the nearest 0.1 millimeter and photographs were taken using an Olympus TG-4 camera. Morphometric processing included: sex, juvenile or adult, reproductive status for males and females (Form I, Form II, gravid, in-berry, and free-swimming juveniles), total carapace length (TCL), palm length (PaL), propodus length (PrL), abdomen length (AL), abdomen width (AW) (Figure 1). Other observations were recorded including: abnormalities (dents, pre-molt, fresh molt, and missing or regenerated chelae), and representative photographs. Molt state was judged based on softness, an overtly clean and slippery exoskeleton, and a separation at the junction between the carapace and most anterior abdominal segment. Gravid females are described as having extruded eggs (or in the process of extrusion) that appear very dull greenish brown color, while females that are in berry are described as having extruded eggs that appear vibrate to deep red or maroon colored eggs (similar to that of red berries). The measurements entail the following: TCL was measured from the anterior tip of the rostrum to the posterior most point on the cephalothorax, PaL from the most distal to the most proximal ends on the palm of the chelae, PrL from the most distal to the most proximal points on the propodus on the chelae, AL from the most anterior point of the abdomen to the most posterior point of the telson, and the AW was measured across the most anterior tergal plate of the abdomen (Figure 1). Crayfish were hand released back to the water following morphometric processing. Due to permit discrepancies, voucher specimens were not awarded for this project. Photographic vouchers were collected for ovigerous female each month so minimum egg counts could be calculated; however, size measurements for eggs were not feasible.

Statistical Analysis

For this dataset, the sexual maturity threshold was established based on the smallest male and female displaying signs of sexual maturity (i.e. glare, eggs, and Form I gonopods); similar to the work of Payne and Price (1983) and Distefano et al. (2013). This threshold was used as a basis for separating adults from juveniles because the statistical analysis primarily focused on characteristics and measurements of adults. Individuals with deformed/damaged abdomens, carapace, rostrum, or chelae and double missing or double regenerated chelae were eliminated from the dataset to ensure uniformity in the parameters being analyzed.

Analysis of covariance (ANCOVA) was used to compare male Form I and Form II reproductive state PaL, PrL, AW, and AL in order to determine whether significant differences between the morphometrics exist. Differences in reproductive state PaL, PrL, AW, and AL adjusted for TCL were detected using students t-test after testing for slope homogeneity. Additionally, standard error, range, and mean, were calculated for each measurement (TCL, PaL, PrL, AL, and AW) which establish population characteristics and to compare with other populations. Male to female ratios were calculated each month and for the entirety of the project. Histograms were created for each individual month detailing the size range and class of all collected crayfish demonstrating age classes within the population and tracking growth rates of the population. The number of bins was generated using Rice's rule stating that the cube root of the observations multiplied by two will calculate the appropriate number of bins. Rice's Rule was selected in efforts not to artificially smooth the histogram. The bin range was calculated by dividing the dataset range by the number of bins. Chi squared analysis were generated using RStudio to look for significance in habitat preference for individuals observed. Chi squared analysis was also used to evaluate any significance for the dominate sex observed month to month and for the entire project.

RESULTS

Site

Representative photos of stream morphology are provided in Appendix B. Representative hydrographs from the gauge on Captina Creek are compiled in Appendix C to represent an estimate for the flow conditions at the time of surveys. The upstream drainage area for the project location was calculated at 103 square miles and the upstream drainage area for the gauge location on Captina Creek was calculated at 128 square miles (USGS, 2018). Based on the similarity in gradient and habitat (Thoma, 2007; OEPA, 2009 and 2010) flows for Sunfish Creek should be approximately 81% of the observed flow at Captina Creek. Flows in Captina Creek were recorded around the time that surveys were conducted in Sunfish Creek. Date ranges for most months were set to cover three days on either side of the survey date with February being the exception where the whole month was included. This exception is due to not being able to complete surveys during February due to elevated water levels and the stream being frozen over. Flows ranged from five to 3300 cubic feet per second (cfs) between October 2017 to September 2018. The flow variation differed between months with the lowest flows recorded in October 2017 ranging from five to 35 cfs and the highest flows recorded in February 2018 (when surveys were not completed) ranging from 130 to 3300 cfs. Nine of the 12 graphs displayed maximum flows that were less than or equal to 500 cfs (Appendix C). The remaining months had maximum flows of 1300 cfs (September 2018), 3000 cfs (April 2018), and 3300 cfs (February 2018). With the exception of February, the month displaying the largest range in flow was April 2018 ranging from 105 to 3000 cfs (Appendix C).

During the final two months of survey efforts increased turbidity levels were observed in the stream. Turbidity was noted even at times of low flow stage in Sunfish Creek; visibility was nearly non-existent (less than 10cm) where it had been moderately consistent throughout the duration of the project (ranging from 40cm to 80cm) depending on recent rainfall. The site and upstream for approximately 1.6 kilometers were scouted at various times and flow conditions in the last two months to determine any changes in visibility, yet no difference was observed during multiple attempts. Following the final collection event in September, the Sunfish Creek watershed received heavy rainfall in a short amount of time resulting in immediate flooding as well as delayed back up from the Ohio River, consistent with fall conditions from 2017 (Appendix C).

Riffle

During the first four months of the study the riffle was dominated by gravel with a small presence of cobble and lacked a discernable thalweg. The riffle habitat ended as it dropped into pool habitat at the bend in the stream (Appendix B). In the lower extent of the riffle as depth increased, the thalweg began to favor the right descending bank (RDB) which was the outside bend of Sunfish Creek. The left descending bank (LDB) was bordered by a gravel bar adjacent to the riparian buffer. Average and maximum depth of the riffle were 16 centimeters and 27 centimeters, respectively. The habitat changed slightly each time there was a high water event and by spring the substrate had become more diverse and a defined thalweg was more obvious after each event. The embeddedness within the riffle was moderate at the beginning of survey efforts but steadily decreased as the habitat changed with each high water event. The average percent composition of substrate found within the riffle habitat were 2.3% fines, 17.7% sand, 55.4% gravel, 23.1% cobble, and 1.5% boulder at the conclusion of field efforts.

Run

The initial run habitat (October 2017 to March 2018) was directly downstream of the pool habitat and was dominated by cobble interspersed with boulder along the RDB transitioning to gravel along the LDB (Appendix B). Average and maximum depth of the initial run were 45 centimeters and 60 centimeters, respectively. The average (n=2) percent composition of substrate found within the run habitats were 9% fines, 10% sand, 28% gravel, 42% cobble, and 11% boulder. Embeddedness within the run was moderate and remained moderate as surveys progressed throughout the year. The habitat changed slightly each time there was a high water event and by March 2018 had diminished to the point where capture rates were suffering and supplemental searches were being conducted each month. The run was changed during the following months of surveys. The run habitat for the remainder of the project (March 2018 to September 2018) was directly upstream of the riffle habitat and was dominated by gravel and cobble interspersed with boulder along the LDB. Average and maximum depth of the run were 32 centimeters and 49 centimeters, respectively. The degree of embeddedness observed within the run habitat was still deemed moderate but appeared a slight degree higher than that of the original run habitat. The habitat in the center of the channel changed slightly each time there was a high water event but the habitat along either bank remained relatively unchanged. The average percent composition of substrate found within the riffle habitat were 7.5% fines, 10.6% sand, 41.9% gravel, 32.5% cobble, and 7.5% boulder at the conclusion of field efforts.

Pool

The pool habitat began in the bend at the base of the riffle and was dominated by cobble interspersed with boulder within the thalweg transitioning to gravel along the LDB (Appendix B). A shallow flat existed along the RDB the length of the pool which was dominated by macrophytes during late spring and summer. Average and maximum depth of the pool were 51 centimeters and 82 centimeters, respectively. Over the course of survey efforts embeddedness within the pool oscillated as it began at a moderate level and decreased with each high water event during the winter and spring but began to increase again in late summer as flows consistently decreased. The average percent composition of substrate found within the riffle habitat were 11.5% fines, 8.1% sand, 23.1% gravel, 32.7% cobble, and 24.6% boulder at the conclusion of field efforts. Habitat within the pool remained constant throughout the duration of survey efforts with only slight downstream shifts in some of the smaller cobble and increased presence of gravel along the LDB following the high water events.

Life History

The initial survey efforts (October 2017) were conducted at two sites on Sunfish Creek. Following the initial survey month, the first site failed to produce capture rates near the 60 individual goal initially established, due to unknown reasons. The second site was used for the remainder of the survey period (November 2017 to September 2018). Surveys were completed over the course of October 2017 and September 2018 during which time a total of 1184 crayfish were collected. Five individuals of the 1184 total observed were hand collected as opposed to the remainder (n=1179) being collected via seine hauls. Species composition included two species: *F. rusticus* (rusty crayfish) and *C. carinirostris* (rock crayfish). *Faxonius rusticus* dominated the captured species with 99.7% (n=1181) and *C. carinirostris* only made up 0.3% (n=3) of the total crayfish collected. June of 2018 yielded the highest capture rates for any given month of surveys (n=121) and November 2017 yielded the lowest capture rates (n=26). Supplemental data from Dr. Zach Loughman, collected in November 2017, was used to augment the low observations of November, bringing the collection total to 324 individuals, in efforts to strengthen the dataset (Table 1). The data for October and November were standardized for habitat and count based analyses to prevent bias in the dataset. For all other analysis all October and November data were used as the increased numbers appeared proportional to the other months. Throughout the surveys, high water events altered the habitat and thus specific habitat at the site level varied in order to reach the capture rate initially established. February 2018 was the only month where collections were not completed due to Sunfish Creek being frozen over the first part of the month and constant elevated water levels during the remainder of the month (Appendix C). The month to month ratio of males to females for adults favored males which were observed in higher densities 64% (n=7) of the collection events (Table 1). Chi squared analysis indicated that there

Month	Female	Male	Total	M:F
October 2017	64	68	132	1.0625:1
November 2017	159	165	324	1.0377:1
December 2017	37	42	79	1.1351:
January 2018	39	46	85	1.1795:1
February 2018	No collection	No collection	No collection	No collection
March 2018	26	43	69	1.6538:1
April 2018	29	41	70	1.4138:1
May 2018	23	29	52	1.2609:1
June 2018	65	56	121	0.8615:1
July 2018	46	41	87	0.8913:1
August 2018	51	40	91	0.7843:1
September 2018	42	32	74	0.7619:1
Total	581	603	1184	1.0378:1

Table 1. Monthly totals and ratio of observed males and females *Faxonius rusticus* collected from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

was no significant difference between males and females from month to month (χ^2= 11.296, df=10, p=0.3349). Adult males were also the dominant gender collected over the duration of the project with observation rates of 53.6% (n=505). In general, adult females were nearly as abundant but were only observed in higher densities from June 2018 to September 2018 (Table 1). When juveniles were included in the calculation, more males were still observed in overall collections at 50.9% (n=603) (Table 1). Presence of glare, eggs, and juveniles were noted monthly and are summarized in Table 2. The presence of glare and eggs were first noticed in March of 2018 and lasted until May 2018. Glare was observed in conjunction with the presence of eggs in March 2018 and is presumed to have been present in February 2018 when collections were not feasible. The presence of glare reached its highest density in March 2018 and pre-glare was observed in September 2018. Egg presence was first observed in March 2018 and reached highest density during April 2018 (Table 2, Figure 3). Attached instars were not observed during any month. Of the 25 females collected in March 16% were observed as adults with no sign of reproduction, 60% (n=15) were observed with glare, 8% (n=2) were observed with eggs, and the remaining 16% were juveniles. April 2018 resulted in an increased observation of gravid females. Of the 27 females collected 54% (n=16) were gravid, 25% (n=6) were observed with glare, 13% (n=3) were juveniles, and the remaining 8% (n=2) showed no signs of reproduction. May 2018 was the final month where gravid females were observed yielding similar results to the previous month. Of the 23 females collected 65% (n=15) were gravid/in berry, 4% (n=1) were observed showing signs of glare, 4% (n=1) were juvenile, and the remaining 26% (n=6) showed no signs of reproduction. Following the collections in May 2018 free living juveniles were observed and all observations of gravid females ceased (Figure 3). Minimum egg counts were determined for gravid females with photo vouchers by counting visible eggs. Of the 33

Month	Glare	Eggs	Juveniles
October 2017			X
November 2017	X		X
December 2018			X
January 2018			X
February 2018	No collections	No collections	No collections
March 2018	X	X	X
April 2018	X	X	X
May 2018	X	X	X
June 2018			X
July 2018			X
August 2018			X
September 2018	X		X

Table 2. Monthly presence of glare, eggs, and juveniles for *Faxonius rusticus* in Sunfish Creek, Monroe County, Ohio from October 2017 to September 2018

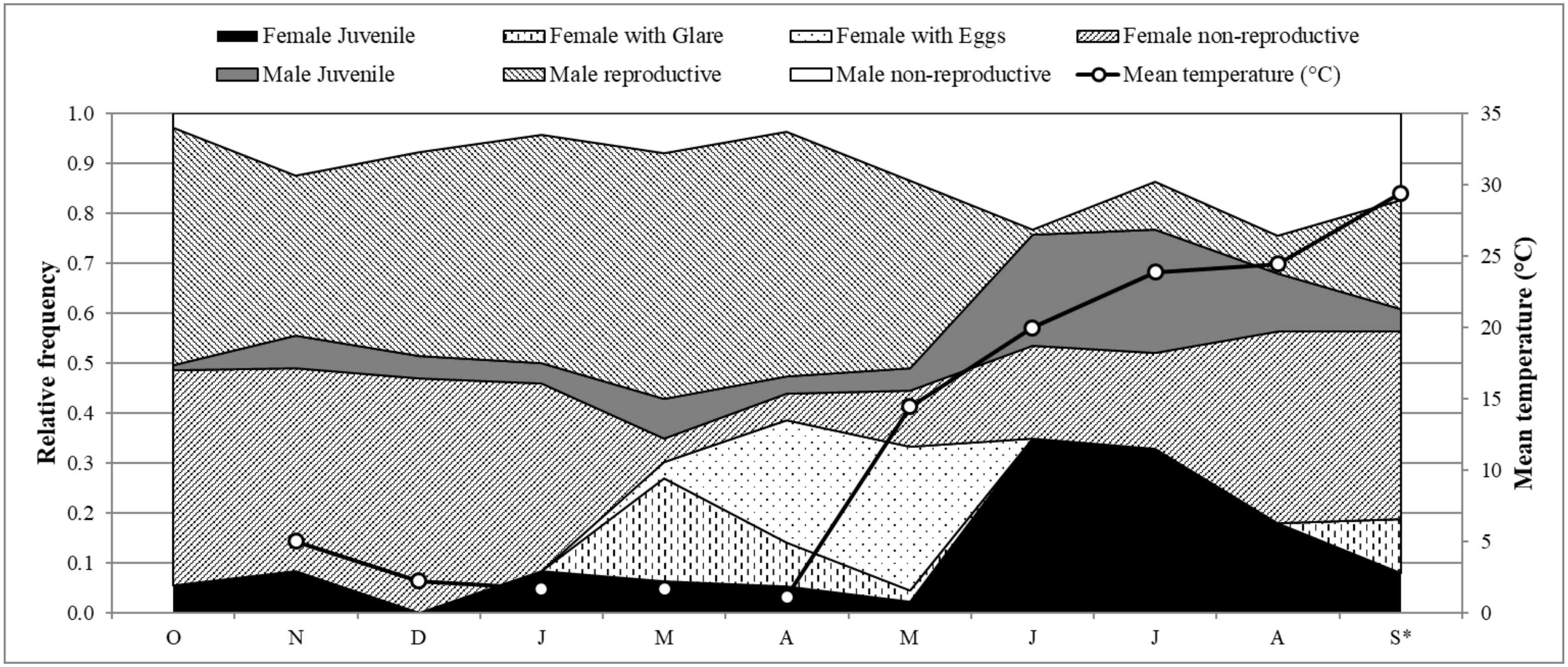

Figure 3. Relative frequency of *Faxonius rusticus* monthly from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

*Female with Glare representative of pre-glare females for September 2018 ovigerous females collected, photographs suitable for minimum egg counts were only present for 29 of the observed individuals. Minimum egg counts for the 29 ovigerous females with useable photo vouchers ranged from five to 93. Minimum egg counts for females in March 2018 ranged from 61 to 93 (n=2). Minimum egg counts for females in April 2018 ranged from 41 to 75 (n=13). Minimum egg counts for females in May 2018 ranged from 5 to 75 (n=14). Estimated egg counts appeared to increase with an increase in TCL; however, the r-squared value of 0.205 indicates variance in trendline (Figure 4). A single reproduction event was observed over the duration of the survey occurring from March 2018 to May 2018.

Form I male *F. rusticus* were observed for the entire duration of the study. Male *F. rusticus* were predominantly Form I throughout each month with Form II only being observed in higher numbers for the months of June, July, and August of 2018. Densities of Form I males reaches their highest from October 2017 to March 2018, peaking in January 2018. Form II were collected each of the 12 months of sampling but peaked in August 2018 (Figure 3). Sexual maturity in males and females were observed at identical size classes. The smallest male observed with Form I gonopods had a TCL of 15.0mm and the smallest female observed with glare present also had a TCL of 15.0mm. The smallest female observed with eggs had a TCL of 18.7mm (Table 3). Adult male rusty crayfish collected between had an observed TCL range of 15.0 to 41.9mm where adult females ranged from 15.0 to 37.5mm (Table 3, Figure 5). Juvenile *F. rusticus* ranged from 4.2 to 14.9mm with the smallest male and female measured at 4.2mm and 4.3mm, respectively (Table 3, Figure 6). Juveniles total carapace length increased from the smallest observed individual at 4.2mm (June 2018) to 14.8mm (September 2018) within a three month period (Figure 7). The TCL size class ranging from 18.0 to 26.0mm was the most prevalent observed over the 11 month survey period (Figure 8). The main variation from this was

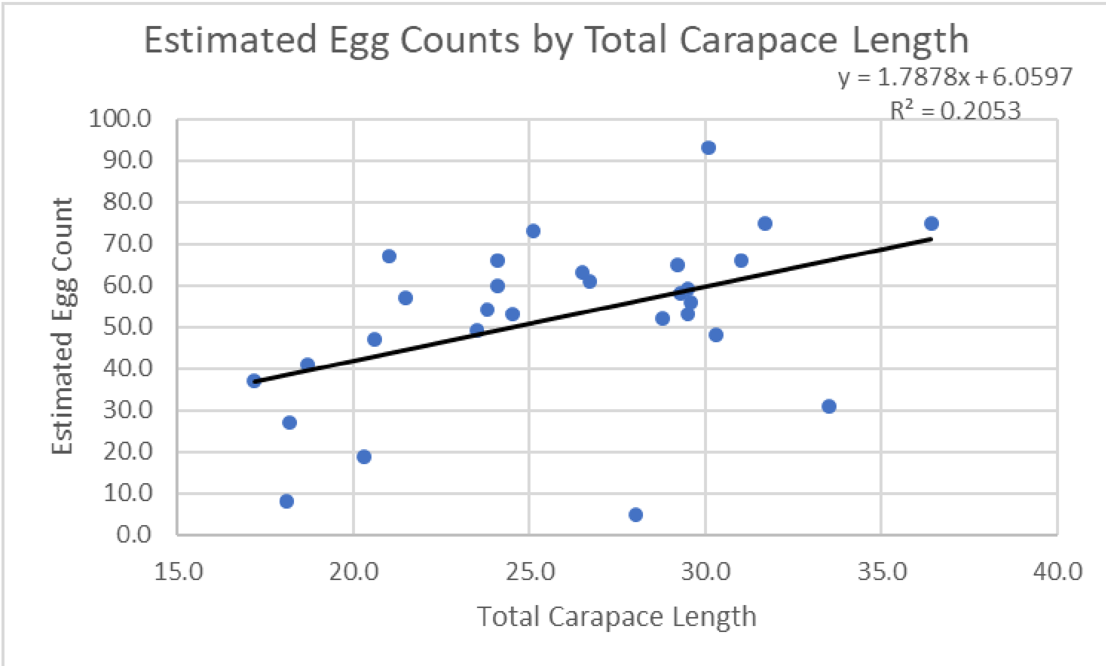

Figure 4. Estimated egg counts of ovigerous female *Faxonius rusticus* trended with total carapace length of individuals observed from March to May 2018 in Sunfish Creek, Monroe County, Ohio

	Male Form I	Male Form II	Male Juvenile	Female Adult	Female Juvenile
Mean TCL ± SE(mm)	25.0 ± 0.06(15.0-41.9)	21.3 ± 0.10 (15.0-41.6)	10.7 ± 0.10 (4.2-14.9)	21.9 ± 0.05 (15.0-37.5)	10.6 ± 0.09 (4.3-14.9)
Mean PaL ± SE(mm)	6.5 ± 0.04 (2.8-13.1)	4.7 ± 0.05 (2.2-12.1)	2.0 ± 0.04 (0.7-3.5)	4.3 ± 0.02 (1.9-9.9)	1.9 ± 0.04 (0.6-3.1)
Mean PrL ± SE(mm)	23.4 ± 0.08 (6.1-50.5)	15.2 ± 0.11 (6.4-44.0)	6.6 ± 0.07 (2.3-12.0)	14.3 ± 0.05 (2.4-32.6)	6.7 ± 0.07 (2.1-10.1)
Mean AL ± SE(mm)	26.3 ± 0.06 (15.4-41.0)	22.3 ± 0.10 (15.1-40.4)	11.4 ± 0.10 (4.0-18.5)	23.6 ± 0.05 (8.6-41.3)	11.4 ± 0.10 (4.0-17.8)
Mean AW ± SE(mm)	10.7 ± 0.04 (5.9-17.8)	8.8 ± 0.06 (5.4-16.6)	4.2 ± 0.06 (1.5-7.6)	10.2 ± 0.04 (5.4-19.0)	4.3 ± 0.06 (1.4-7.4)

Table 3. Mean standard error and range for TCL, PaL, PrL, AL, and AW for Form I and Form II male, adult female, and male and female juvenile *Faxonius rusticus* collected from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

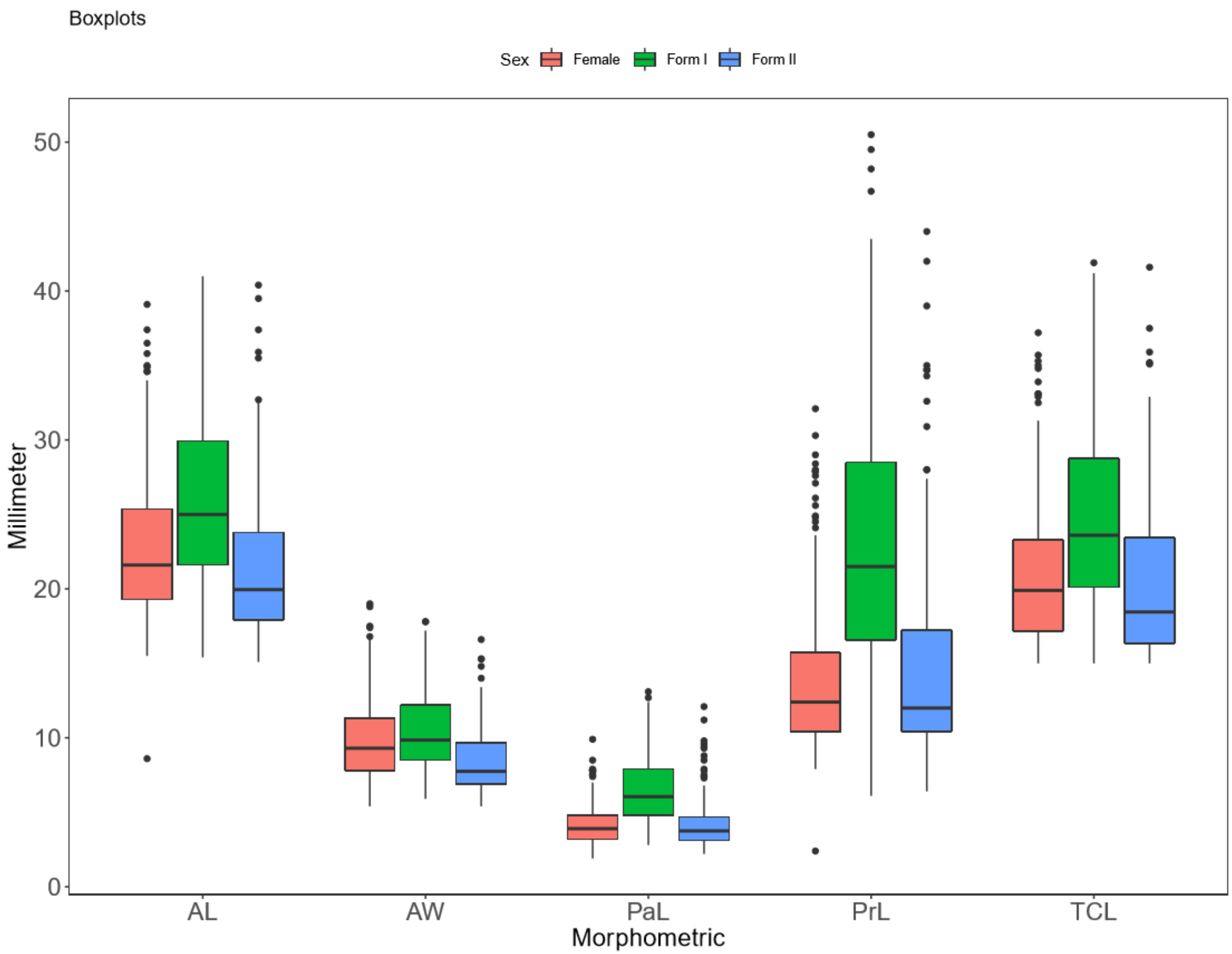

Figure 5. Boxplots displaying morphometric measurements of female, Form I male, and Form II male *Faxonius rusticus* collected from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

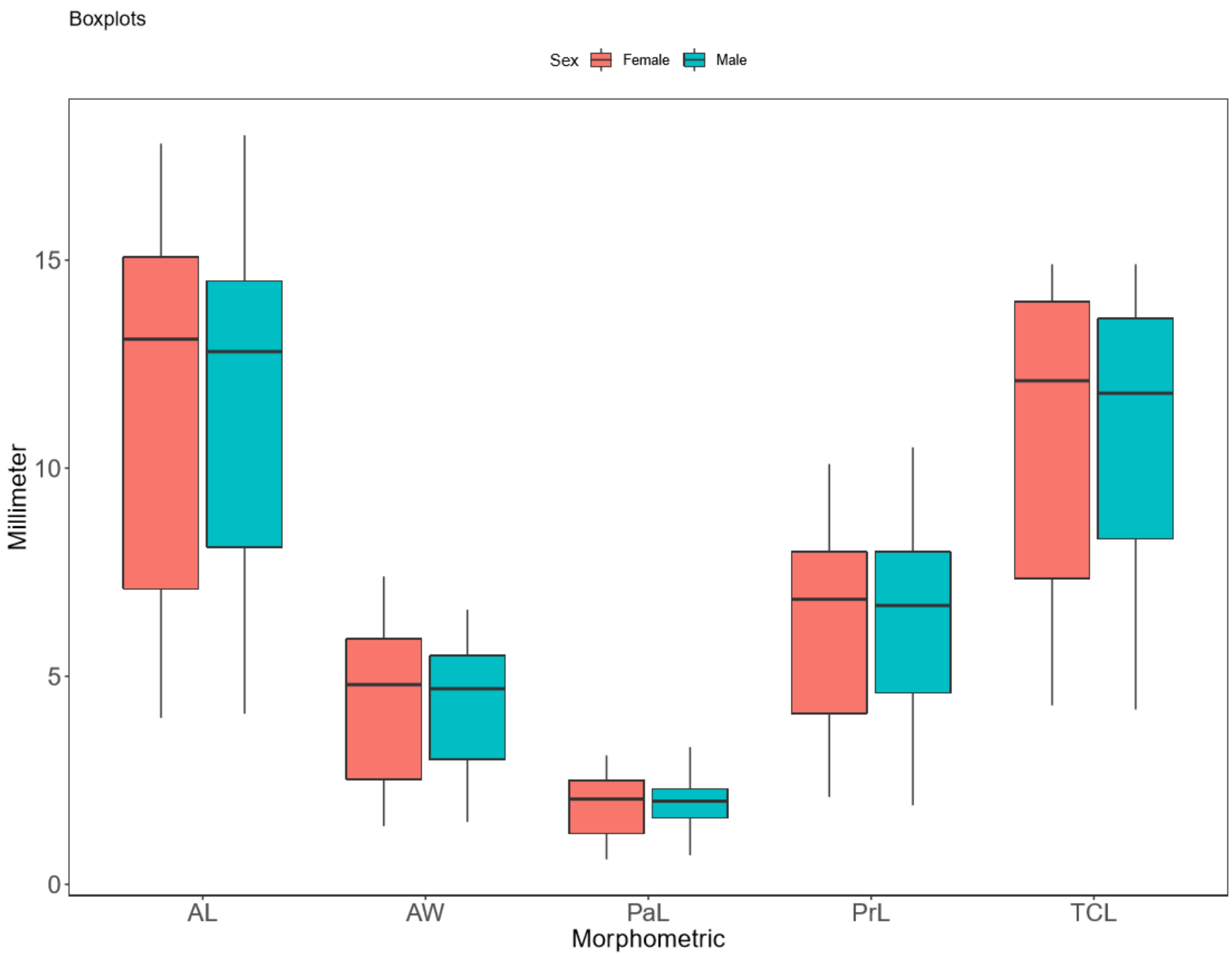

Figure 6. Boxplots displaying morphometric measurements of juvenile *Faxonius rusticus* collected from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

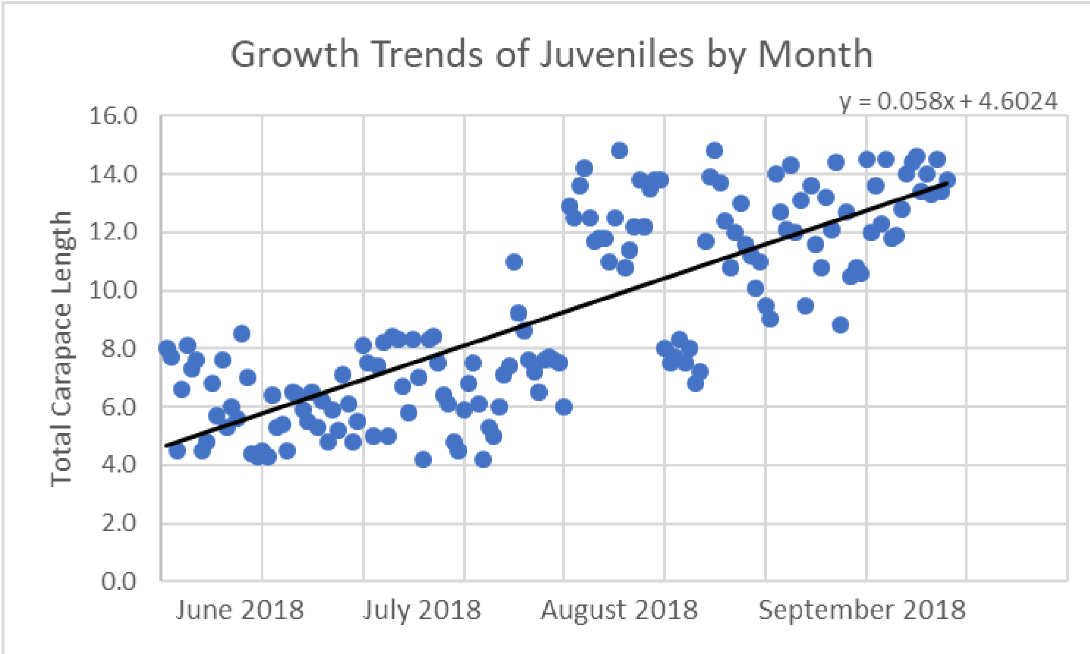

Figure 7. Growth trend in juvenile *Faxonius rusticus* total carapace length from July to September 2018 in Sunfish Creek, Monroe County, Ohio

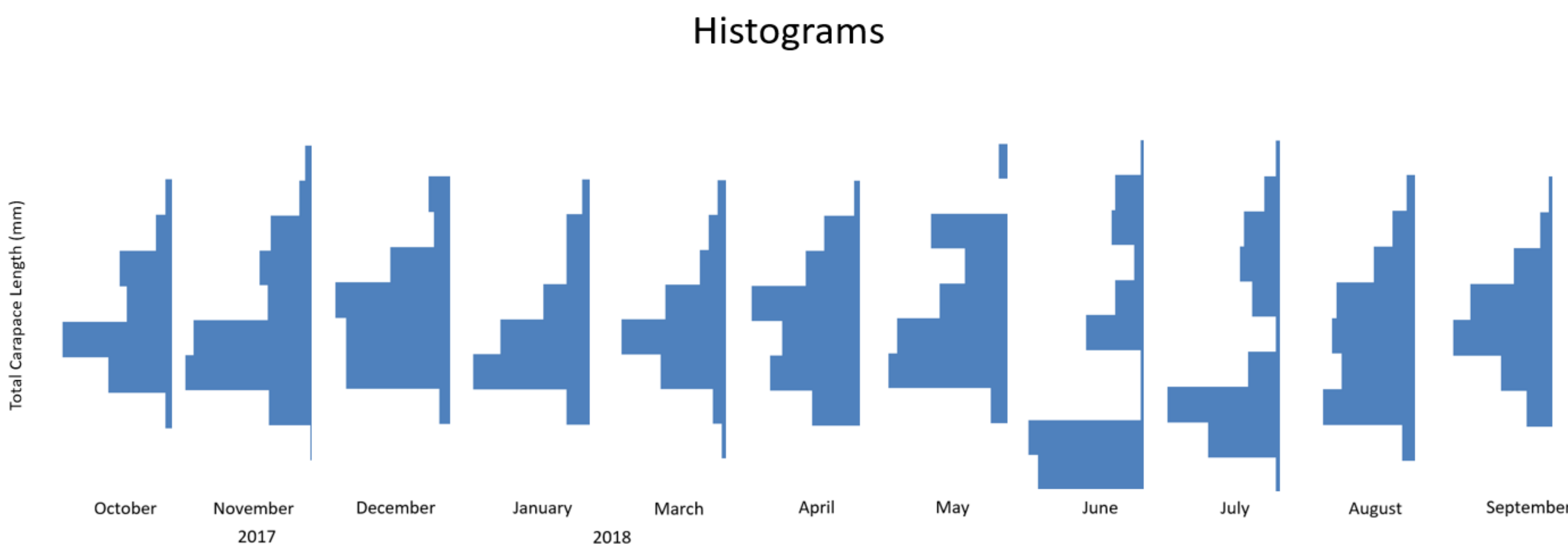

Figure 8. Histogram plotting frequency of total carapace length by month for *Faxonius rusticus* observed from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

observed from June 2018 to July 2018 which was the time frame when juveniles hatched. ANCOVA was conducted on the morphometrics collected on Form I and Form II males. Significant differences were detected between Form I and Form II male PrL ($F=269.0$, $P< 2.2e-16$), PaL ($F=18.70$, $P=2.2e-16$), and AL ($F=16.3$, $P=2.2e-16$) adjusted for TCL. Form I and Form II male AW adjusted for TCL ($F=5.44$, $P= 0.05029$) showed no significance. Analysis was only completed on Form I and Form II males as females were not separated into Form I and Form II. Females were not separated based on those with eggs and those without due to not enough females with eggs being observed for a strong comparison; therefore, females were not analyzed using ANCOVA.

Collections each month totaled the crayfish observed and noted how many were collected from each of the three habitat types (riffle, run, and pool). For this analysis, the supplemental data from November 2017 was omitted as the habitat in which the individuals were collected was not identified. Of the available habitat, 73.6% (n=649) of individuals observed were collected from the run habitat. Individuals were homogenously observed between the other two habitats at 12.9% (n=114) for riffle and 13.5% (n=119) for pool (Figure 9). Chi squared analysis indicated that there was a significant difference between observations between habitat types ($\chi^2=119.45$, $df=20$, $p=3.612e-16$). A permutation based two-way ANOVA was conducted looking at observations between habitats and months. Significant differences were not detected for the months ($F=271.3$, $P=0.4102$) but significant differences were detected for the habitat type ($F=8593.2$, $P<2e-16$).

Cambarus carinirostris made up only three of the 1184 individuals collected, all of which were female, over the duration of the project. A maximum TCL was observed at 18.7mm and a

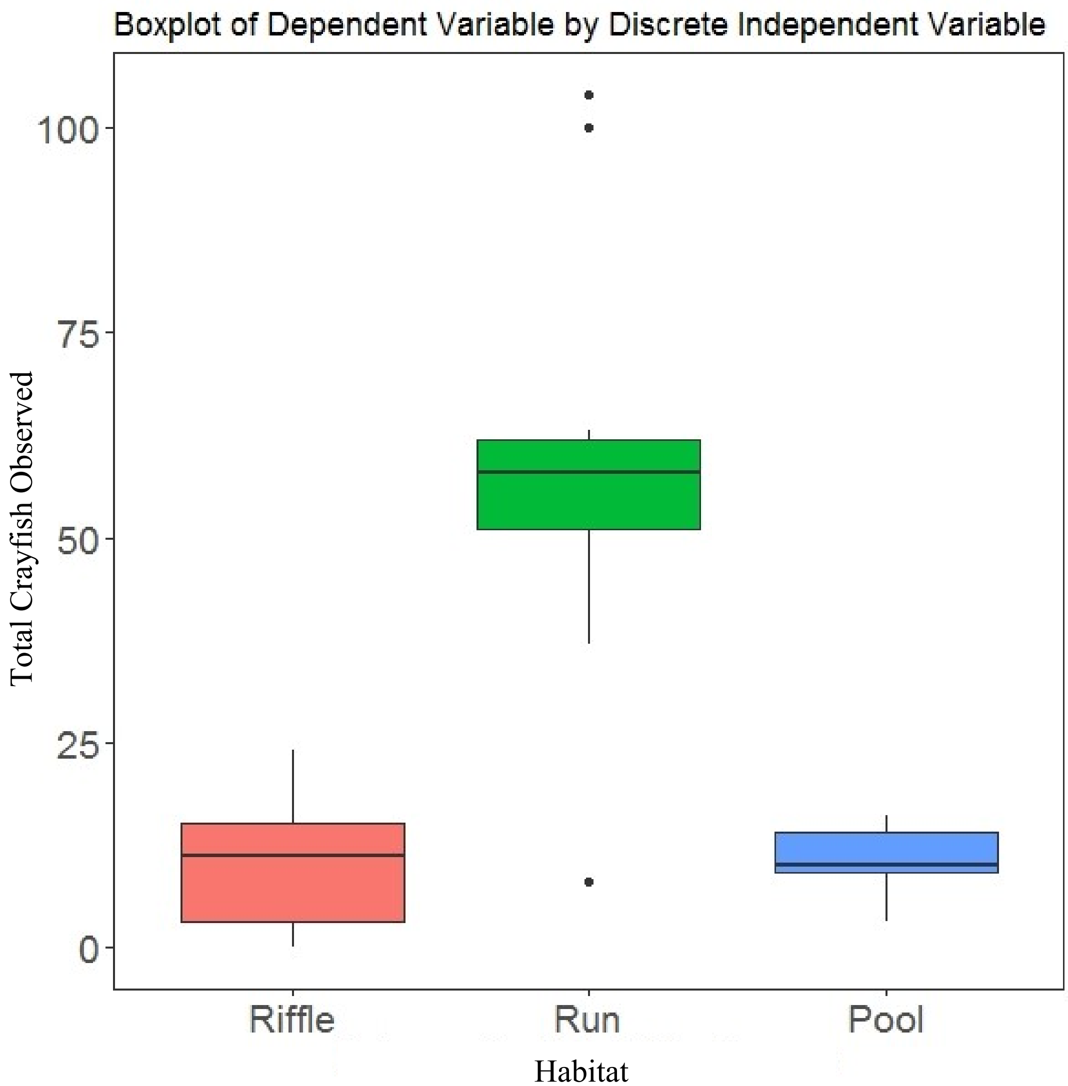

Figure 9. Boxplots of percent *Faxonius rusticus* observed within the riffle, run, and pool habitats from October 2017 to September 2018 in Sunfish Creek, Monroe County, Ohio

minimum at 8.4mm. Observations of *C. carinirostris* were sporadic with single individuals being collected in November of 2017, January of 2018, and March of 2018.

DISCUSSION

The main hypothesis that *F. rusticus* would be well established within the stream was supported by the collections of this project. *Faxonius rusticus* was by far the dominant species observed (n=1181), accompanied by only a single other species *C. carinirostris* (n=3)*,* within Sunfish Creek during the 11 months of collections. All habitats surveyed were inundated with *F. rusticus*. The majority (73.6%) of total individuals observed were collected from the run habitat. Statistical significance was found in habitat preference through the use of Chi squared analysis and supported the collection numbers that heavily favored the run habitat. Initial theories were that upstream movement would be evident within the population over the survey period. Upstream movement was not supported by the consistent monthly observations. Collection numbers were consistent with the exception of November 2017. It is unclear what caused the reduction in observation for this single month but observation numbers returned the following month.

Flow rates were theorized to compromise sampling efficiency and diminish observed individuals. Although actual flow rates were not calculated for Sunfish Creek during survey efforts, estimates were calculated based on the USGS hydrograph on Captina Creek. The drainage areas were calculated for each and the flows were proportioned (81% of the flows at Captina Creek) to be approximations of flow rates. Flow rates did not appear to impact observations as collection numbers remained relatively homogenous from January 2018 to May 2018 where the flows during collections ranged from approximately 86 to 3000 cfs. Neither survey efficiency nor observable activity appeared to diminish with increased water velocity.

The highest collection densities were observed in mid-late summer and early fall when water levels were low and water temperatures were higher. Observable differences in behavior throughout the year were very minimal. *Faxonius rusticus* were regularly out cruising the stream benthos throughout the day. One observed change was that as water temperature increased, more crayfish were observed in the riffle and pool habitats. Survey efficiency was expected to increase with water temperatures and this was supported by a single collection event in June of 2018 where 121 individuals were observed within 30 seine hauls.

Sex ratio within the population was theorized to favor males and the results supported this hypothesis. Differences were expected to be observable between Form I and Form II. Form I were expected to be larger as chelae and overall size are known to aid in encounters with congeners species, reproduction, obtaining and maintaining shelter, and obtaining resources (Bovbjerg, 1956; Stein, 1976; Berrill and Arsenault, 1982 and 1984; and Bruski and Dunham, 1987). Statistically significant differences were observed favoring Form I males for PaL, PrL, and AL. The significance is that these measurements relate to chelae and overall size providing Form I males with a competitive advantage. Variation between the monthly morphometric measurements were minimal as initially expected.

The sexual maturity threshold was established for the site at 15.0mm for both males and females. This sexual maturity threshold is smaller than 18.5mm and greater than found by previous investigators (Lorman, 1980; Jezerinac et al. 1995; Taylor and Schuster, 2004). Adult morphometrics ranges coincided with what Jezerinac et al. (1995) found in West Virginia. Sex ratios observed during this project of 1.0378:1 in favor of males reflected what many others have observed with a ratio of near 1:1 but tending to favor males (Hobbs and Jass, 1988; Page, 1985).

The measurements and ratios observed during this project roughly matched the results detailed by other authors (Taylor and Schuster, 2004; Gunderson, 1995).

Mating season for *F. rusticus* in Sunfish Creek appears to take place in early spring, similar to what Fielder (1972) found for other *Faxonius* species, as water temperatures begin to rise evidenced by the increased presence of females with active glare glands as well as the presence of gravid females. Glare was observed four to five months before any evidence of females with eggs were observed. For the most part, behavior did not appear to change during periods of gravidity and live young. The main observed differences were that gravid females were only collected under cover objects (to be expected) and gravid females were not observed within the riffle habitat within the duration of this study. It is possible that other riffle habitats within Sunfish Creek contained gravid females during the reproductive season of *F. rusticus*. A period of formed juveniles living attached to the mothers was expected. A lack in observation of this stage in reproduction proposes questions 1) is this step in the reproductive process streamlined as a mechanism for invasive success and 2) was the time between collection events too great to observe this reproductive stage? In the event one of these questions holds an answer regarding the transition from eggs to instars in *F. rusticus*, that answer may also shed some light on frequency and speed at which molting events take place. It is well established through literature that the process of molting puts crayfish in a vulnerable state. Looking at the process from the standpoint of an invasive mechanism, it would make sense to shorten the timeline of this process as greatly as possible providing a competitive edge through reduction of time spent vulnerable.

Faxonius rusticus remained Form I and Form II the entirety of the study with only three months (June, July, and August 2018) when Form II were more prevalent. Maintaining both forms year round differs from the idea of synchronous molting events. The near absence of Form I males in June (n=1) closely mirrors what Fielder (1972) found with *F. obscurus*. With a continual presence of sexually reproductive males throughout the year additional studies are warranted to identify the possibility of a second mating event in the same year or if maintaining a larger size and competitive drive aids in their invasive success. Snedden (1990) theorized that males with similar TCL but larger chelae should be more dominant over males of similar and smaller TCL as well as increase reproductive success and frequency. This theory of dominance could explain why the invasive population of *F. rusticus* in Sunfish Creek maintained Form I for the majority of the year in efforts to maintain dominance and continually advance the invasion front. A portion of this theory was verified by Berrill and Arsenault (1982 and 1984) where they found males with larger chelae successfully interrupted mating pairs, copulated, and emerged victorious more frequently when compared to interactions with smaller chelae males.

Gunderson (1995) noted *F. rusticus* being capable of molting more than once in a single year leading to the expectation of observing multiple molt events during this study. This hypothesis was not supported by the results with only a single molt event being observed following the reproductive period for *F. rusticus*. It is possible that molt events were missed due to time between survey events each month. Based on how few individuals showed signs of recently molting and how pre-molt was not readily observed the month prior, it is likely that molting events happen very quickly in *F. rusticus*.

The results of this study coincided with the results detailed by Thoma (2007) where *F. rusticus* was the dominant species and with only *C. carinirostris* being observed. An additional species *F. obscurus* is known from this drainage area but was not observed during either survey.

Thoma (2007) concluded that *F. rusticus* has extirpated *F. obscurus* from the Sunfish Creek drainage and this project further supports that conclusion.

Habitat observed during surveys coincided with that found from the Biological Assessment completed by OEPA in 2009. The habitat within the survey reach was high quality complex substrate with moderate or less than moderate embeddedness being observed. Actual counts and identifications of the fish and benthic macroinvertebrate community were not completed; however, person observations yielded a dense and diverse assemblage of aquatic species. The exception to this would be a diverse crayfish population that was observed to primarily consist of *F. rusticus*. While QHEI metrics were not calculated, the observed percent composition of substrate supported the presence of complex substrate similar to what was noted by the OEPA.

Invasive species warrant the same level of research and monitoring effort that native species receive as they are often first identified by researchers or those recreationally (i.e. fishing) using the waterbody. These initial observations typically lead to more in-depth studies in efforts to verify or identify the extent of invasion. Typically, studies are based on specific localities (i.e. streams and lakes) or to answer a specific question rather than assessing the species across the extent of its invasive and/or native range. Life history studies conducted at various locations within a species range are few and far between but could help provide a greater understanding of the species being studied and how they interact with each system they inhabit. Data collected across a species range or invasion extent could provide insight to dispersal mechanisms, competition with other species, and could potentially detect early onset of invading species (Glon et al. 2018). If we ever hope to slow down and optimistically stop the spread of invasive species, we first have to increase our background knowledge.

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APPENDIX A: IRB LETTER

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Office of Research Integrity

August 10, 2018

Kyle McGill
5559 Fairmont Pike Road
Wheeling, WV 26003

Dear Mr. McGill:

This letter is in response to the submitted thesis abstract entitled *"The Life History of An Invasive Species of Crayfish Orconectes rusticus (rusty crayfish) as it invades the tributaries of the Ohio River."* 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 Code of Federal Regulations (45CFR46) has set forth the criteria utilized in making this determination. Since the information in this study does not involve human subjects as defined in the above referenced instruction, it is not considered human subject research. 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 a 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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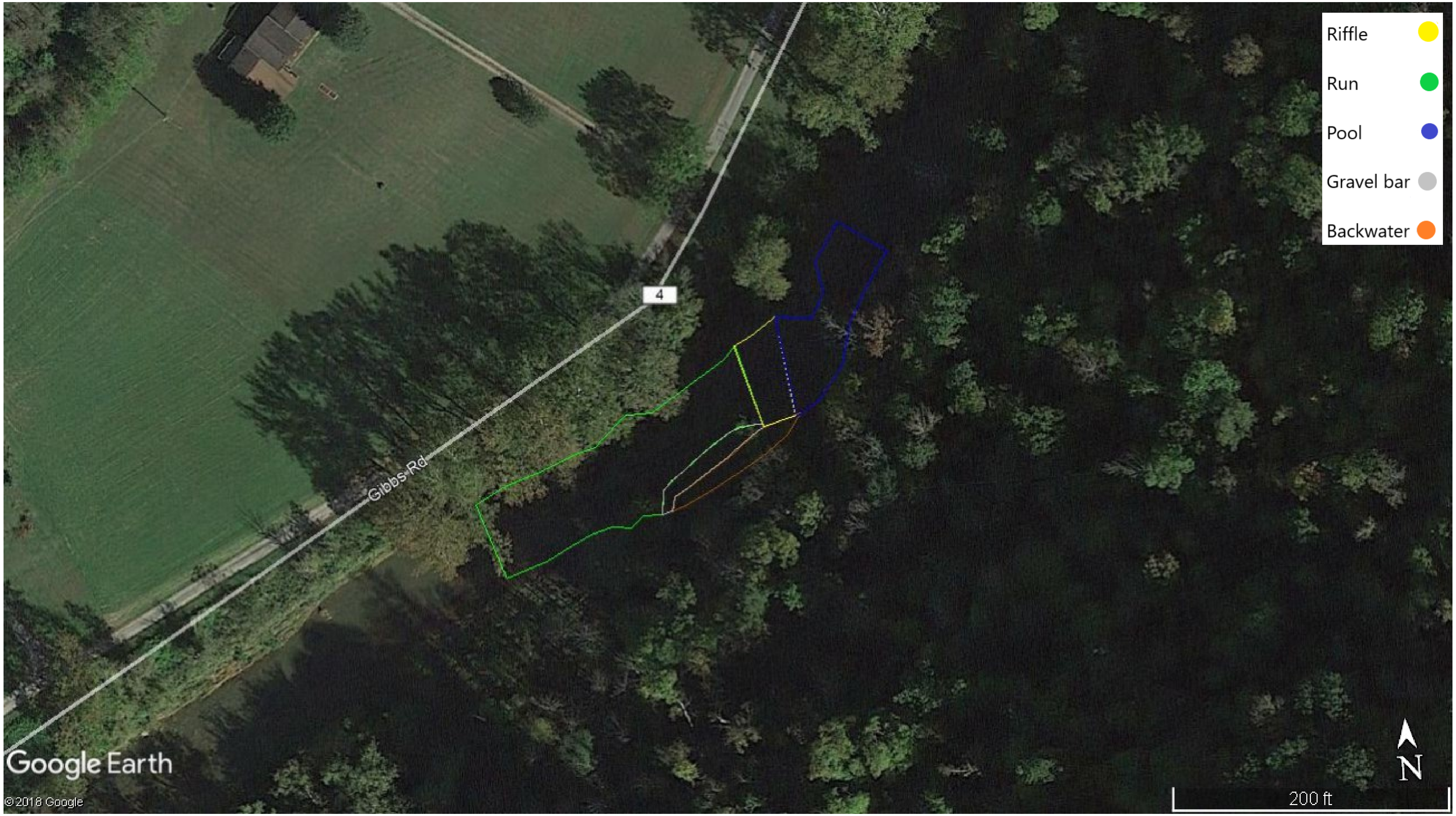
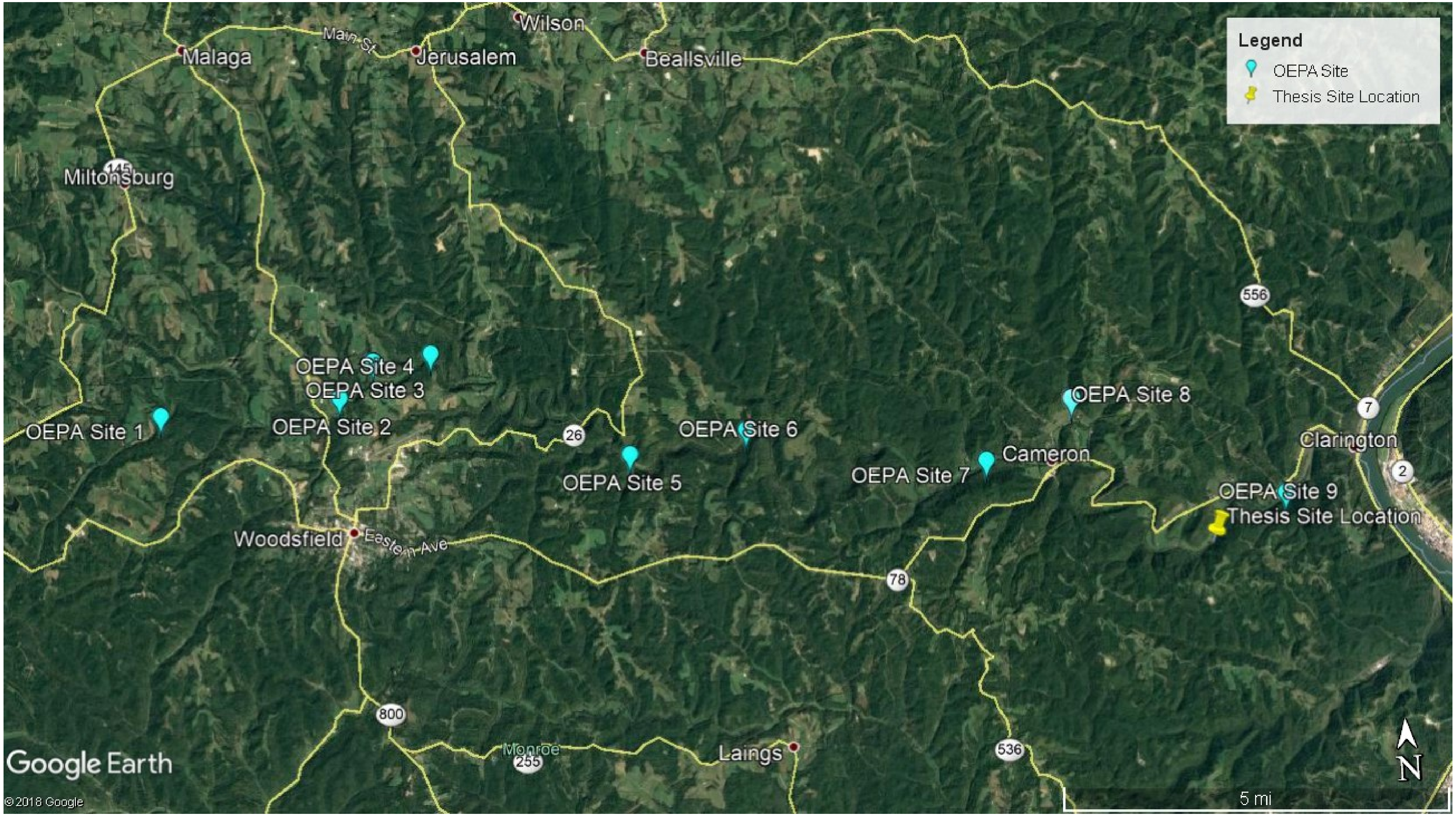
APPENDIX B: PHOTOGRAPHS

Site Location on Sunfish Creek in Monroe County, Ohio



Survey extent on Sunfish Creek in Monroe County, Ohio



Site Location in Relation to the Ohio Environmental Protection Agency Biological Assessment Sites on Sunfish Creek in Monroe County, Ohio

Representative photo of riffle habitat

Representative photo of run habitat

Representative photo of pool habitat

Representative photo of *Cambarus carinirostris* (rock crayfish), adult

Representative photo of *Cambarus carinirostris* (rock crayfish), juvenile

Representative photo of *Faxonius rusticus* (rusty crayfish), adult

Representative photo of *Faxonius rusticus* (rusty crayfish), form I (sexually reproductive) male

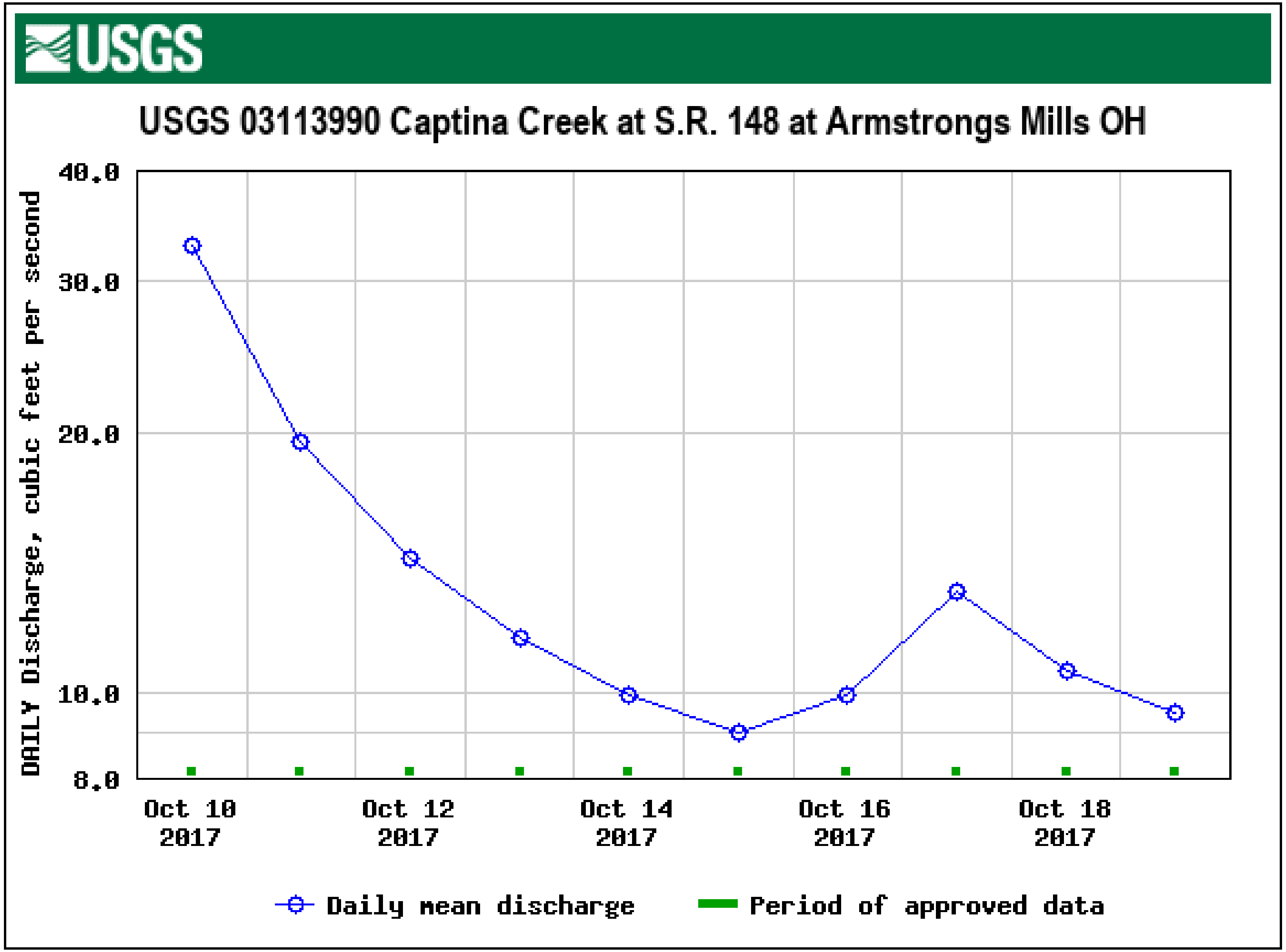
Representative photo of *Faxonius rusticus* (rusty crayfish), form II (sexually non-reproductive) male

Representative photo of *Faxonius rusticus* (rusty crayfish), adult female

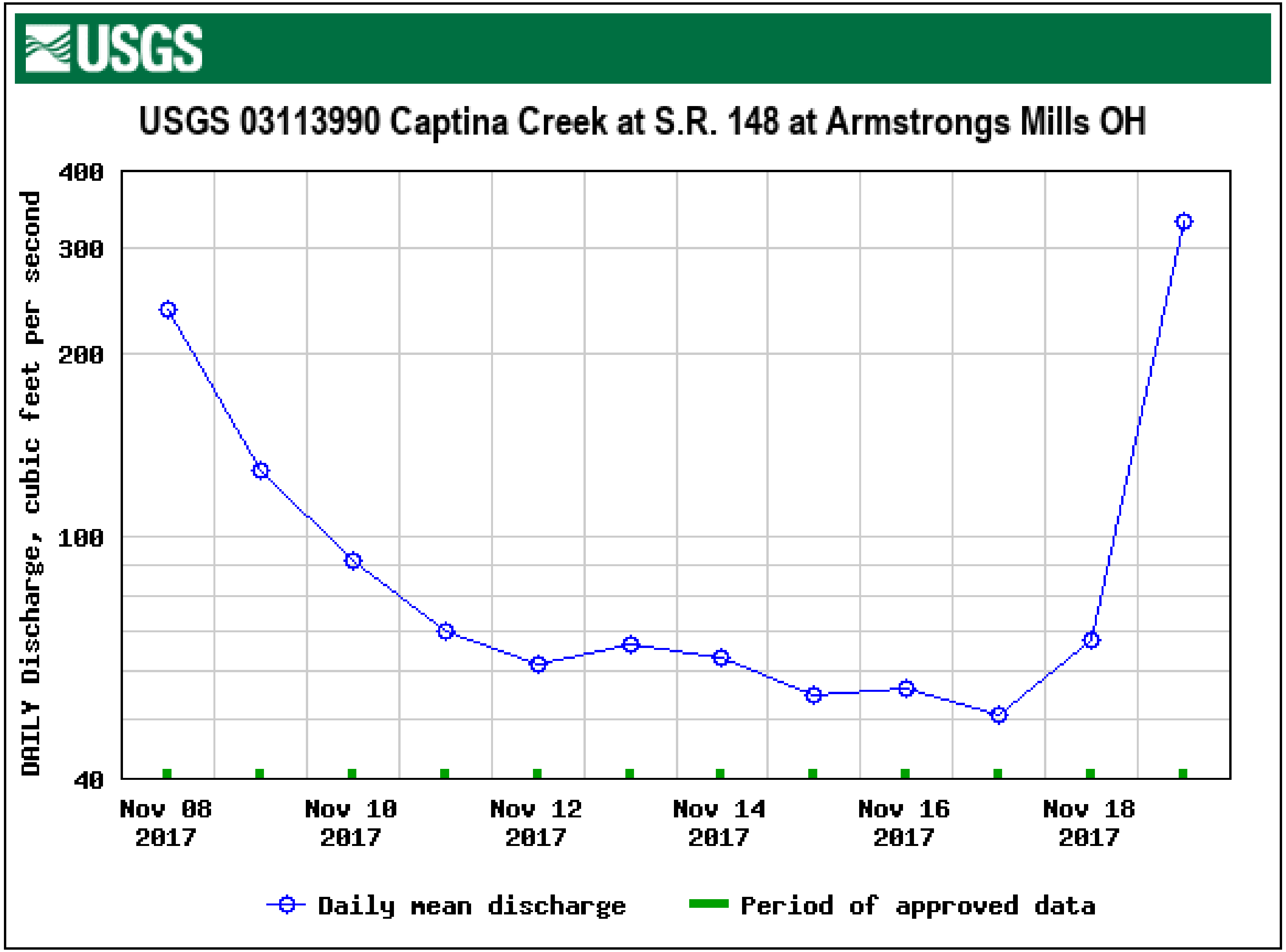
Representative photo of *Faxonius rusticus* (rusty crayfish), adult female with glare

Representative photo of *Faxonius rusticus* (rusty crayfish), adult chelae deformity

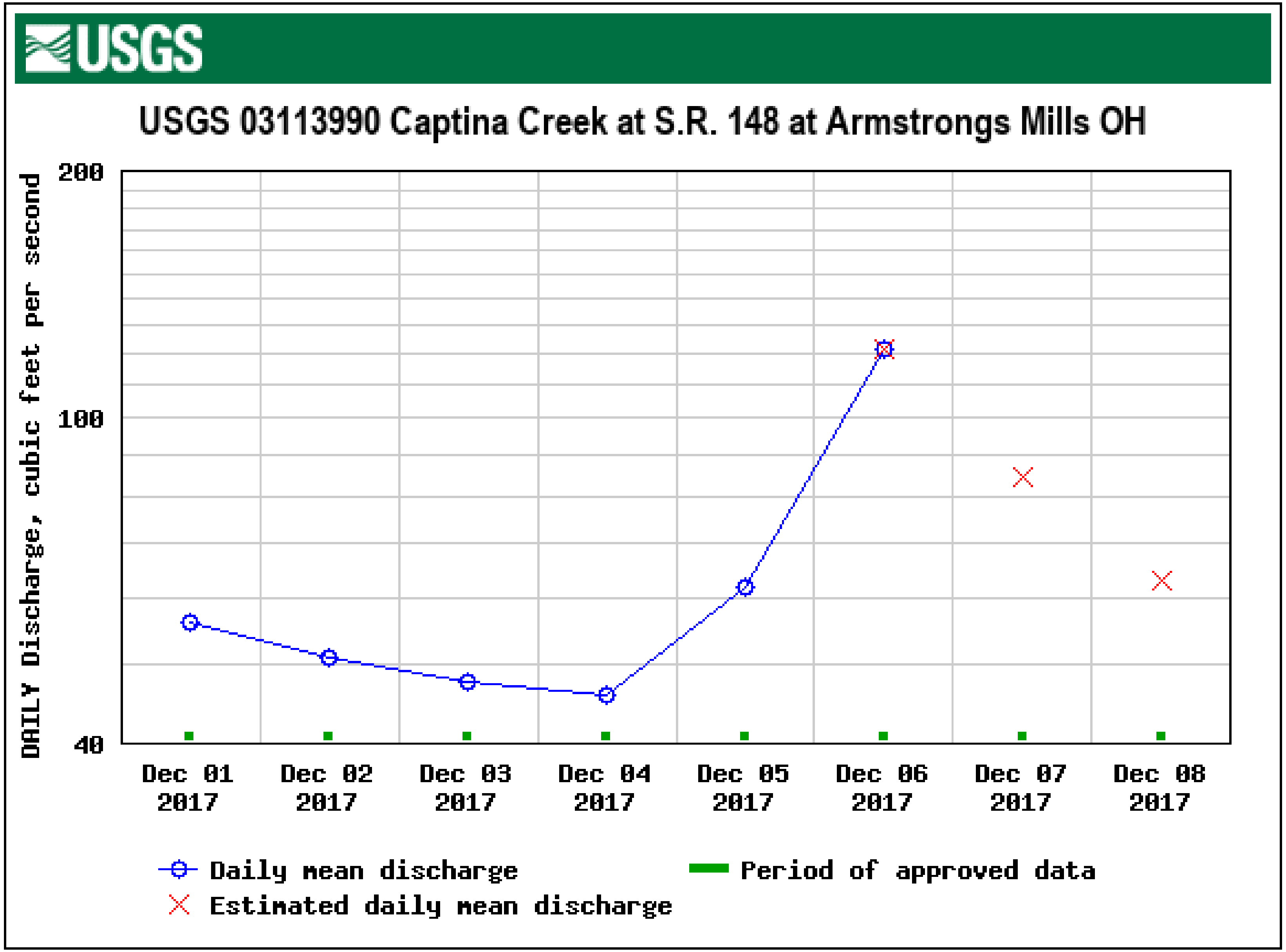
APPENDIX C: HYDROGRAPHS



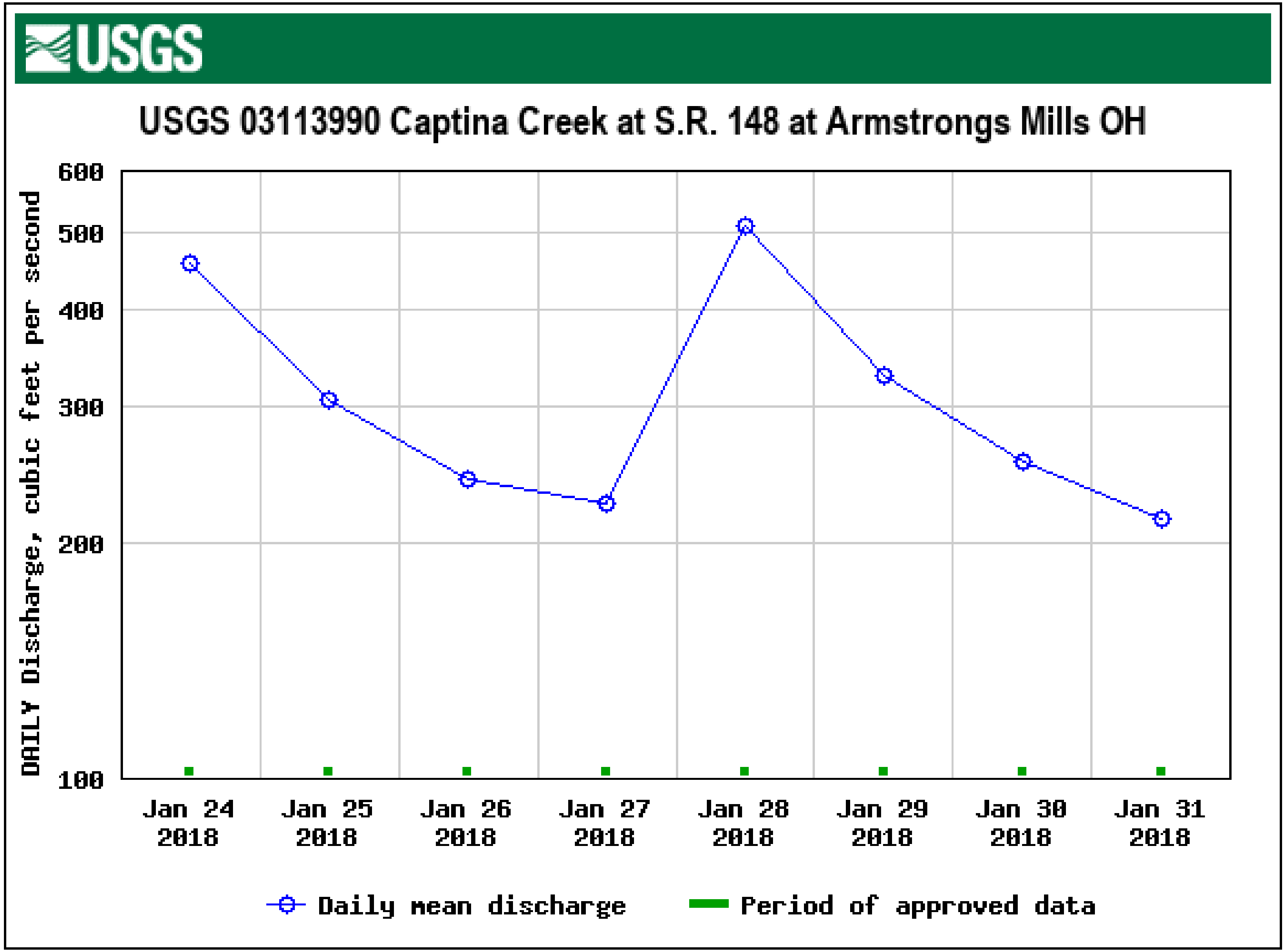
Hydrograph for Captina Creek October 2017



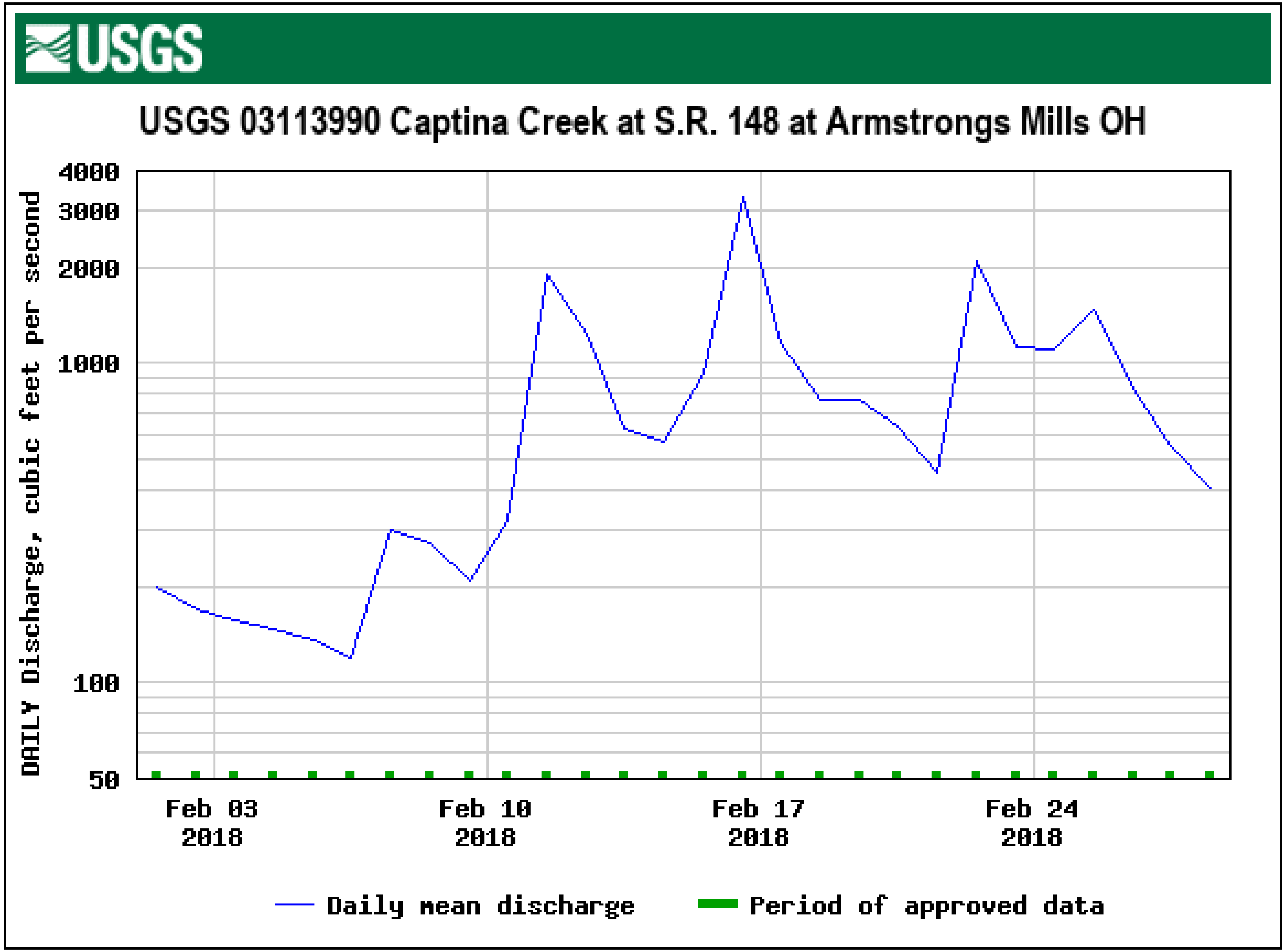
Hydrograph for Captina Creek November 2017



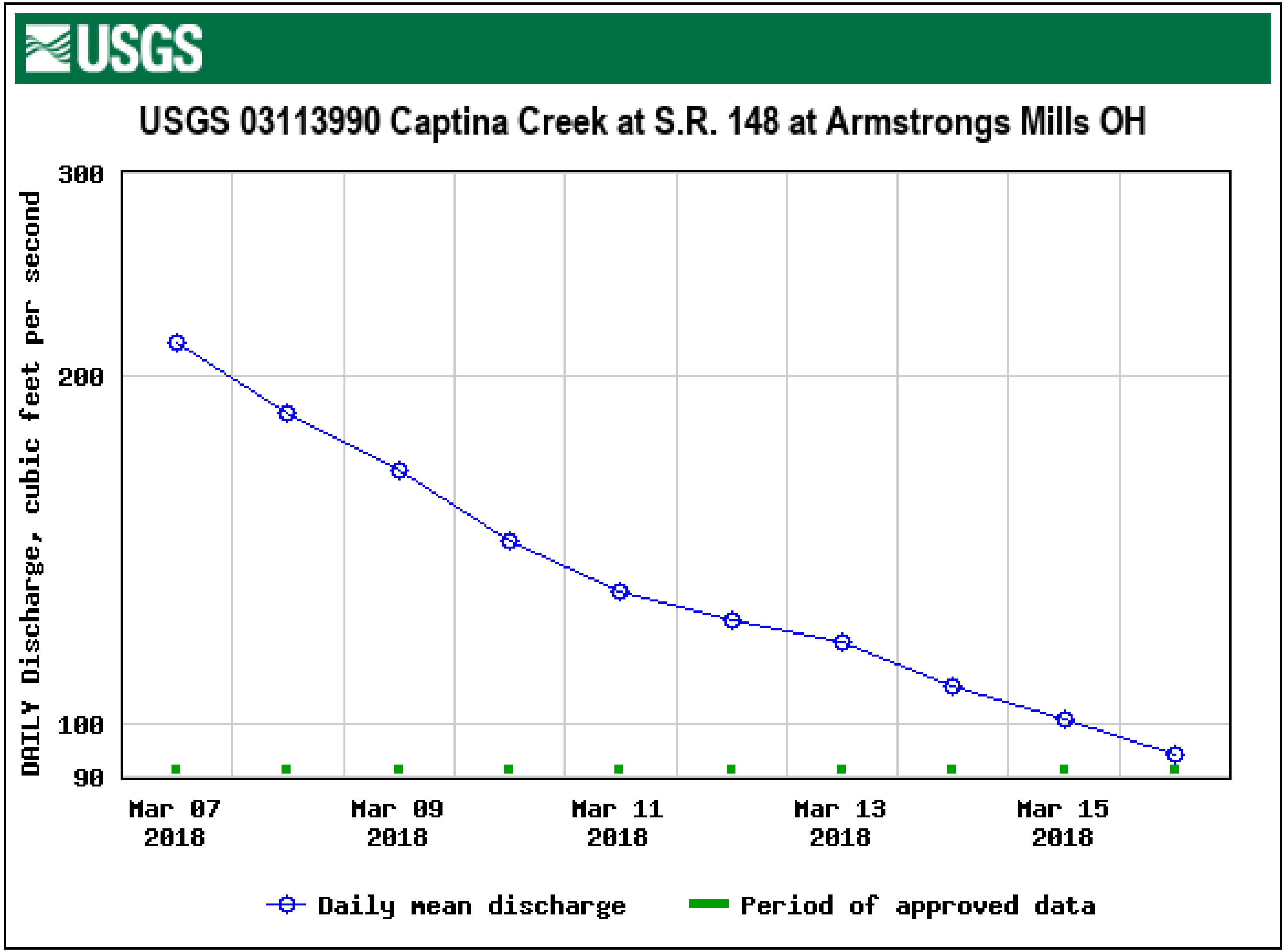
Hydrograph for Captina Creek December 2017



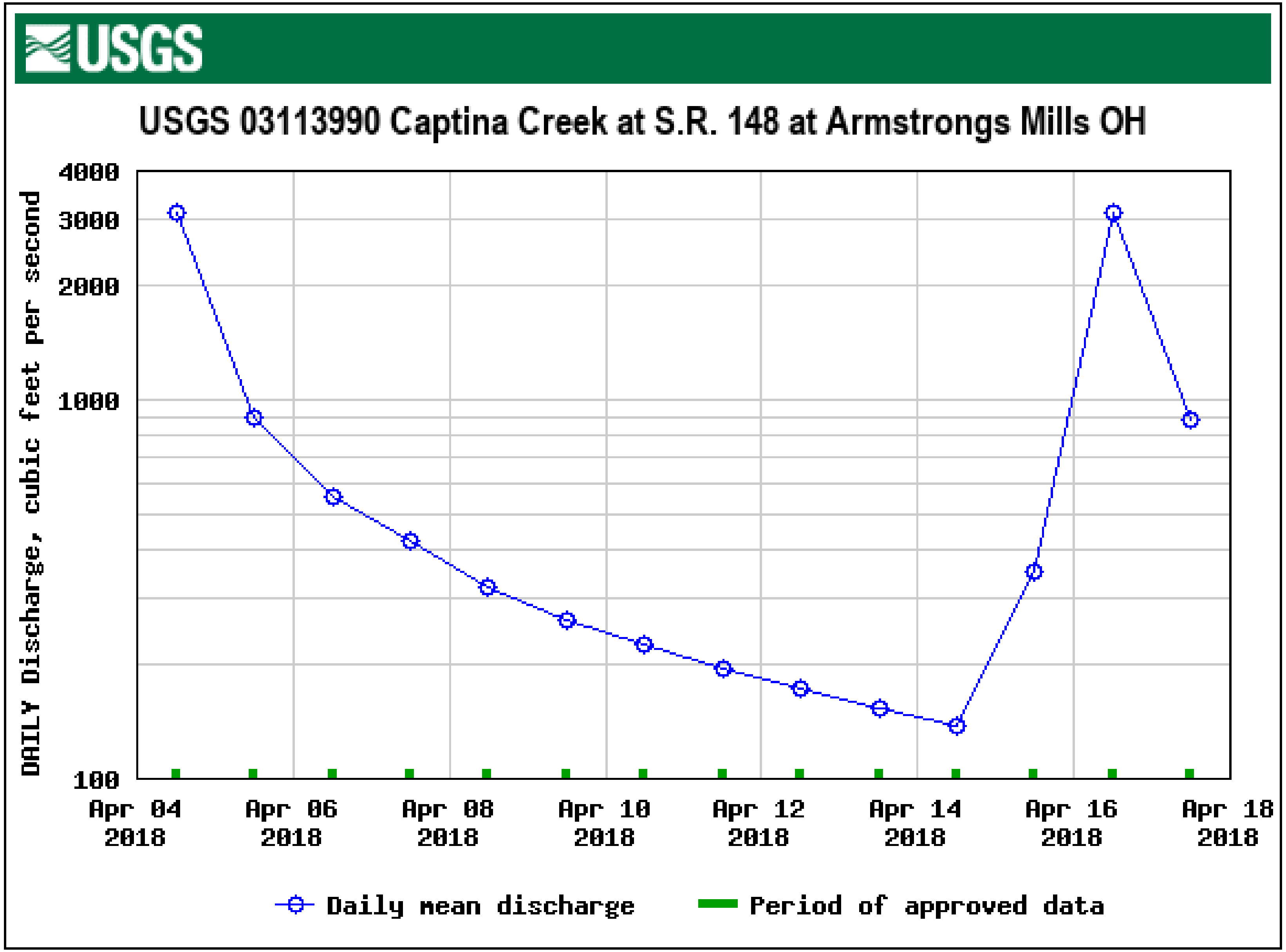
Hydrograph for Captina Creek January 2018



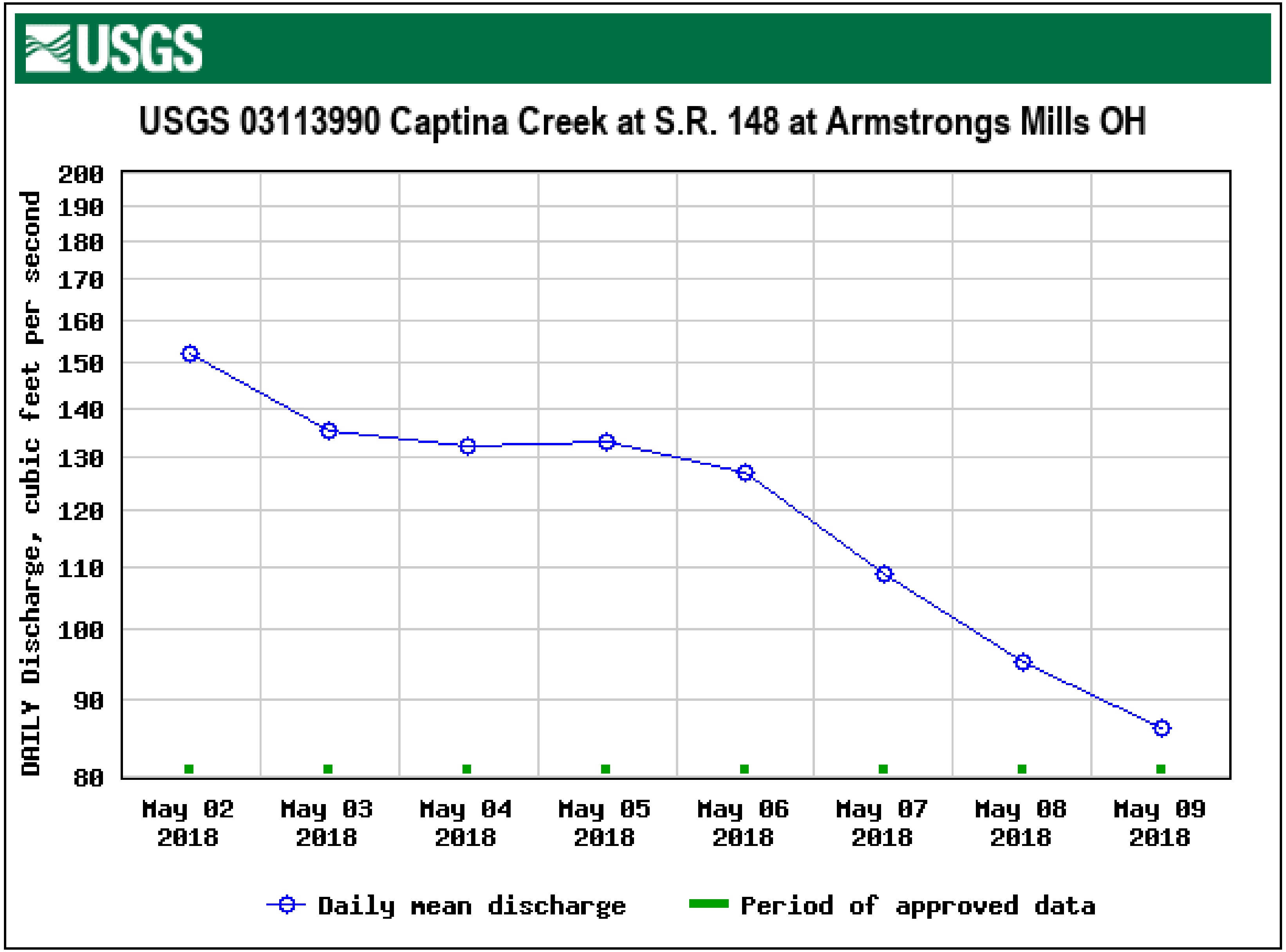
Hydrograph for Captina Creek February 2018



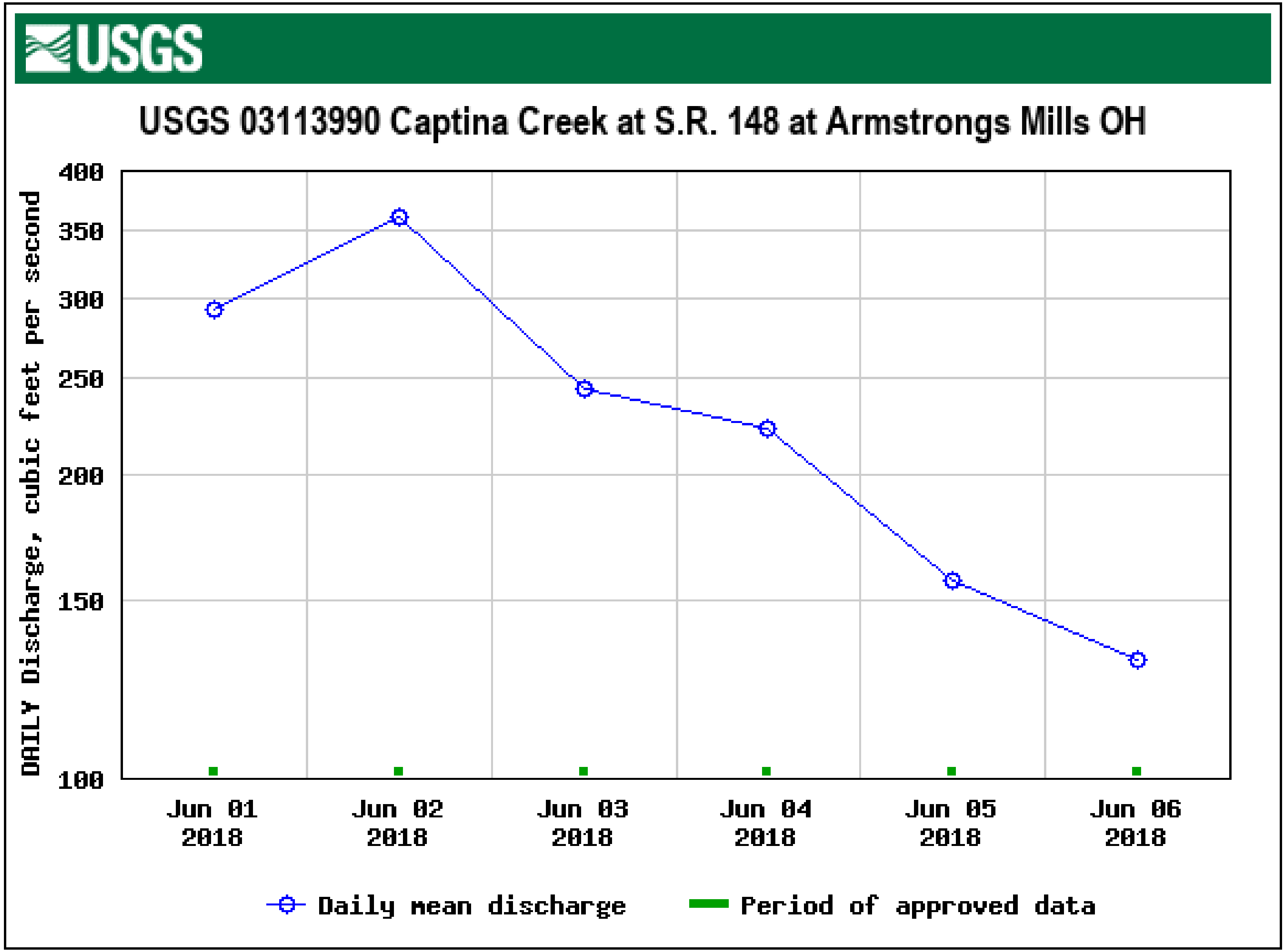
Hydrograph for Captina Creek March 2018



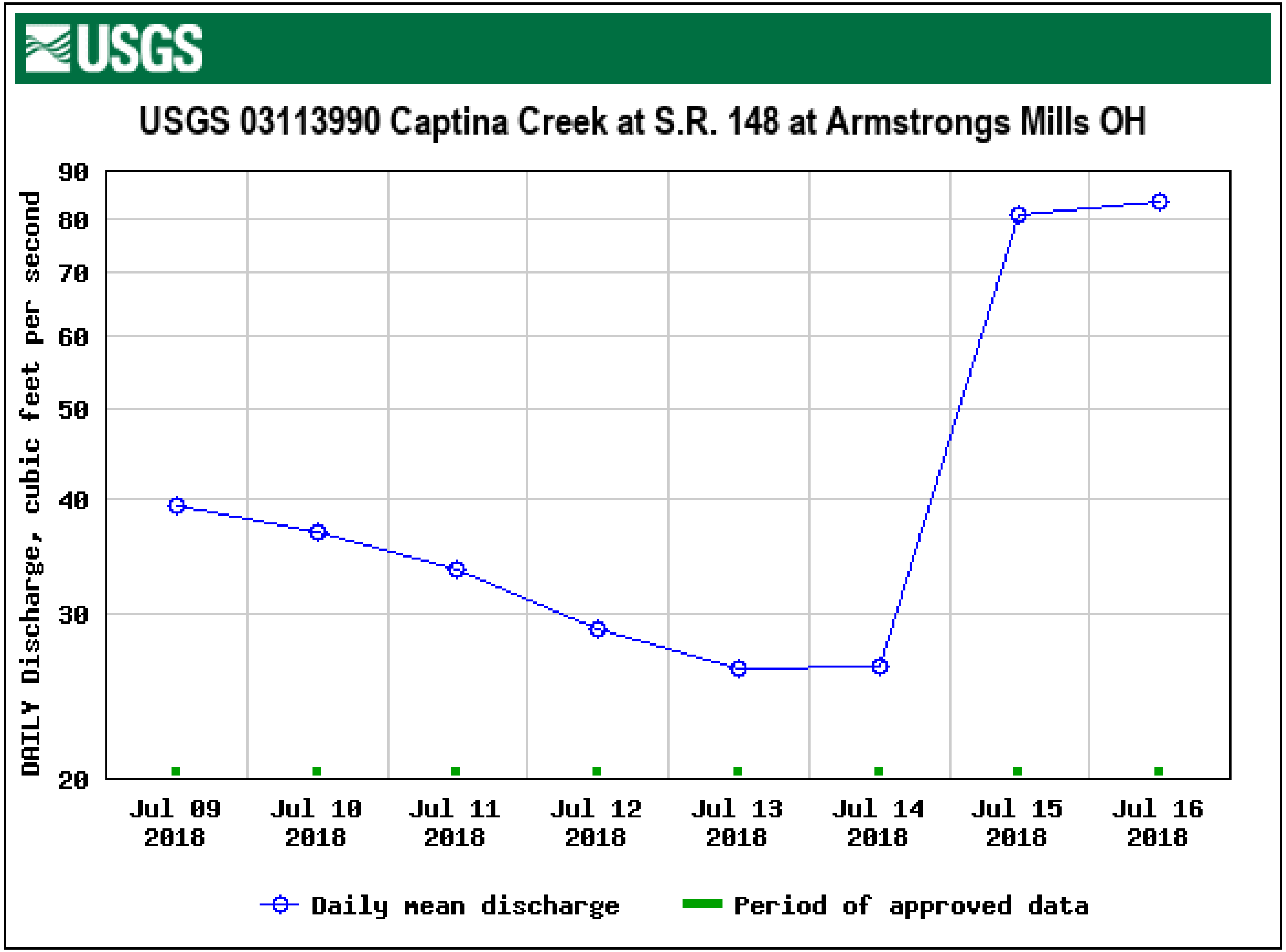
Hydrograph for Captina Creek April 2018



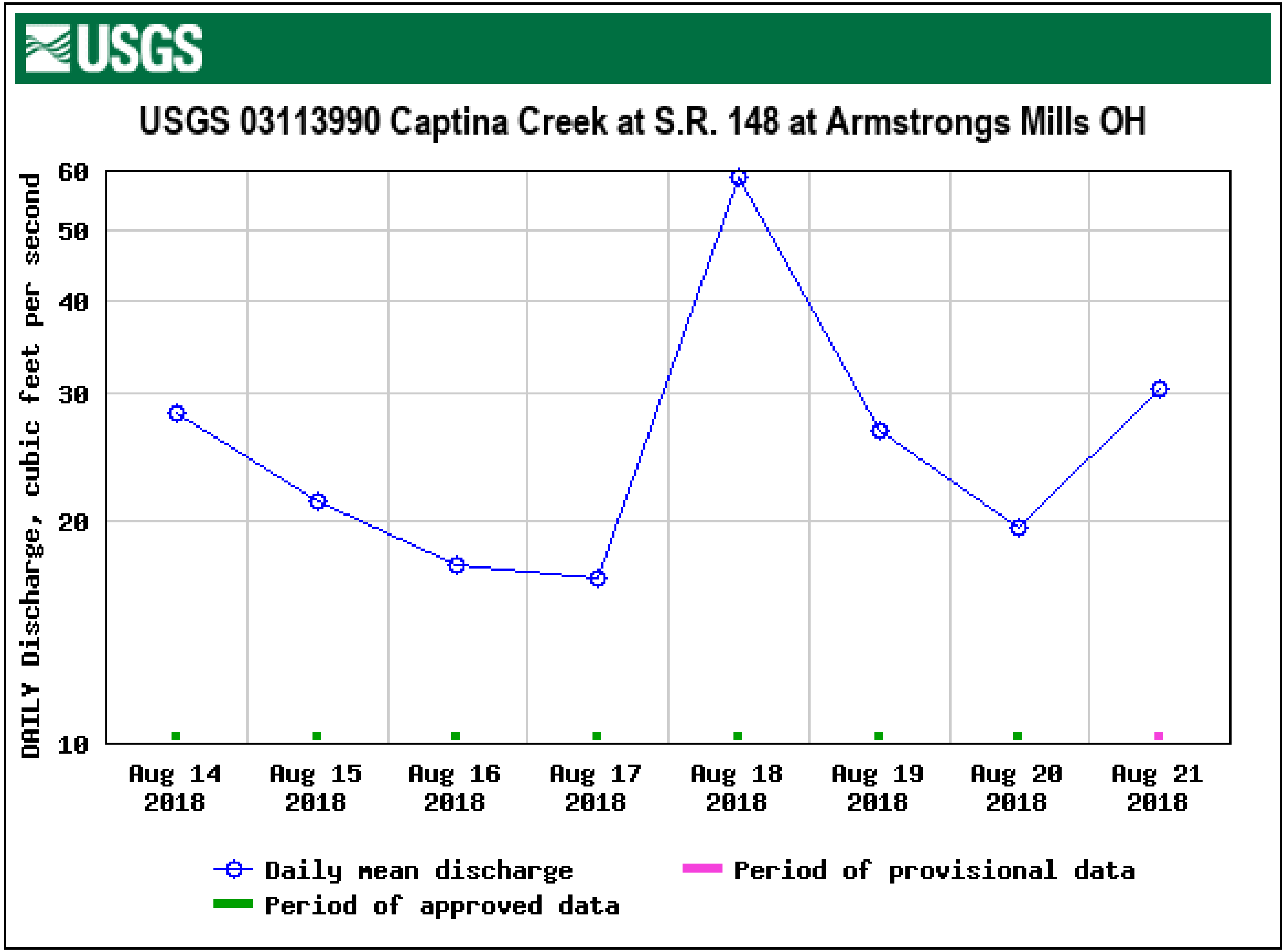
Hydrograph for Captina Creek May 2018



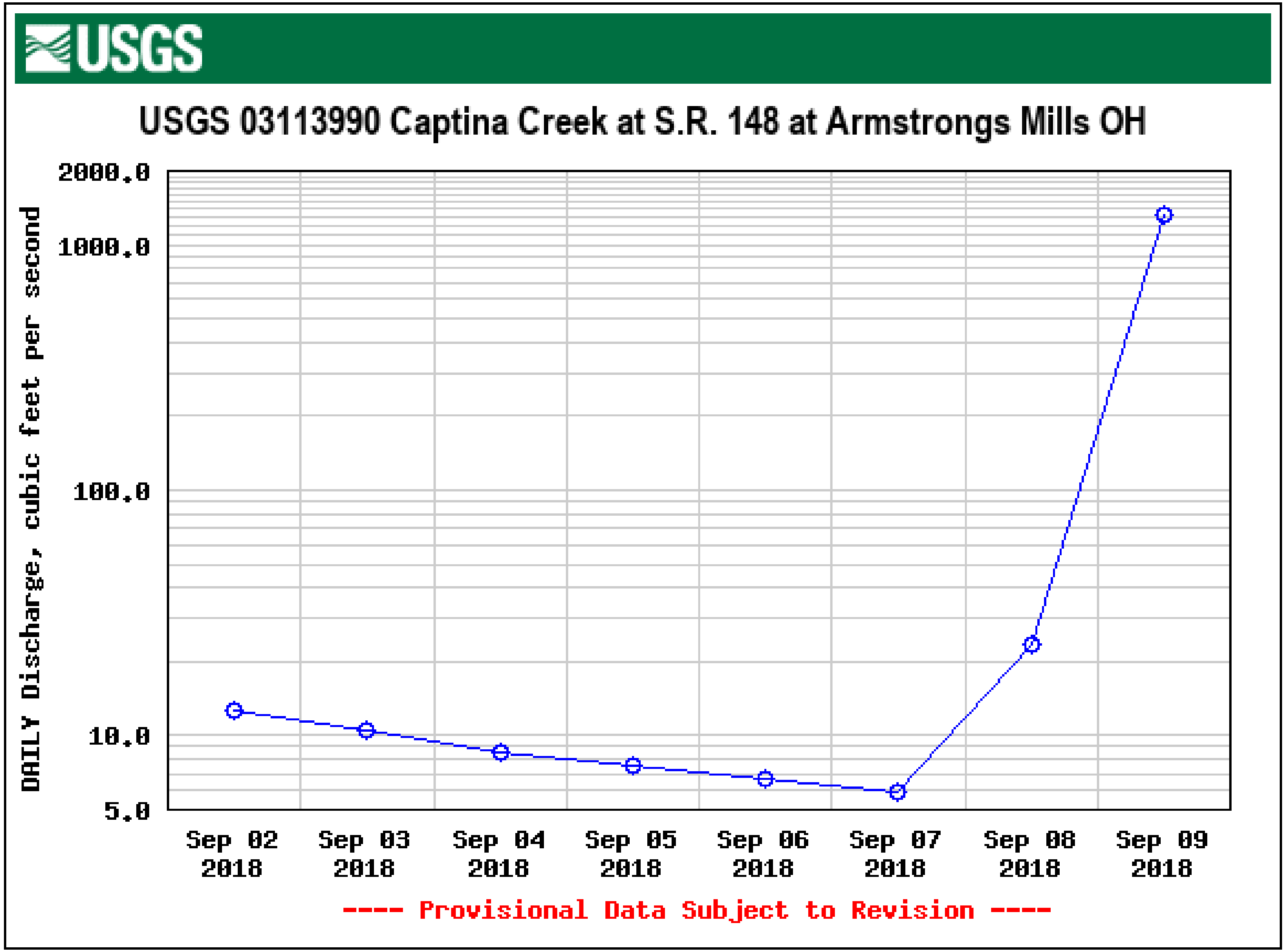
Hydrograph for Captina Creek June 2018



Hydrograph for Captina Creek July 2018



Hydrograph for Captina Creek August 2018



Hydrograph for Captina Creek September 2018