

INVESTIGATION OF AN INVASIVE CRAYFISH AND ITS RELATION TO TWO
IMPERILED NATIVE CRAYFISHES; ANTHROPOGENIC INFLUENCES, MULTI-
SCALE HABITAT ASSOCIATIONS, AND CONSERVATION OPTIONS

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by
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The undersigned, appointed by the Dean of the Graduate School, have examined the
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IMPERILED NATIVE CRAYFISHES; ANTHROPOGENIC INFLUENCES, MULTI-
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ABSTRACT

Introduced crayfishes often pose a threat to native aquatic biodiversity and the natural function of aquatic systems. In the St. Francis River drainage of Missouri the introduced Woodland Crayfish (*Orconectes hylas*) has become established and is spreading throughout the drainage. The native crayfish fauna is altered at locations where introduced populations of *O. hylas* become established and often the imperiled Big Creek Crayfish (*Orconectes peruncus*) and St. Francis River Crayfish (*Orconectes quadruncus*) are extirpated from those locations within a few years. The goal of this study was to look for evidence that may explain how *O. hylas* displaces either of the two natives and provide managers with potential conservation actions to ensure the persistence of native crayfishes. The study objectives were to 1) investigate the association of natural environmental factors and anthropogenic alterations at a coarse-spatial scale with the distributions of *O. hylas*, *O. peruncus*, and *O. quadruncus*, 2) investigate habitat use and selection at a fine-spatial scale for *O. hylas* and *O. quadruncus*, and 3) develop a monitoring framework to assess the range expansion of *O. hylas* and the range contractions of *O. peruncus* and *O. quadruncus*.

Native crayfishes are often extirpated from portions of their range due to interactions with invasive species, anthropogenic alterations to environmental conditions,

or a combination of these factors. Chapter 2 focused on the objective of identifying coarse-scale natural and anthropogenic factors associated with the current distributions of the invasive *O. hylas* and two endemic crayfishes (*O. peruncus* and *O. quadruncus*) in the St. Francis River drainage and provide insight about the potential role of anthropogenic factors in facilitating species displacement.

I used classification trees to model coarse-scale natural and anthropogenic environmental factors and their relation to the presence or absence of each species. Model results were further used to predict probability of presence for each species within each stream segment throughout the St. Francis River drainage. Factors related to geology and soils were the best predictors of species distributions and a dichotomy of these factors explained much of the discrete distributions of the two native species. Agricultural-related factors were identified as the most influential anthropogenic activity related to species distributions. All associations between the invasive species and anthropogenic factors were negative which suggested the invasive was not likely to occur in heavily impacted areas. Overall, models had high correct classification rates and reliably predicted the presence of the invasive in the invaded drainage. Given the negative associations of the invasive with anthropogenic alterations at a coarse spatial scale, I believe that other mechanisms are likely to be responsible for the widespread displacement of the two native species.

Chapter 2 focuses on how *O. hylas* and *O. quadruncus* use and select habitat at a fine-spatial scale. This approach provides two benefits. First, habitat associations of the two species can be compared to look for similarities or differences that suggest competitive exclusion has occurred. Second, learning about the habitat associations of

both species can improve our understanding of their ecology and may provide information that can be used to manage the species. These methods also allowed for investigation of the space-use of the two species, movement, growth, and select life-history characteristics. *Orconectes peruncus* was not included in this portion of the study because of logistical constraints.

To achieve the objectives of Chapter 3, individual adult crayfish were tagged with passive integrated transponder tags at locations where *O. hylas* and *O. quadruncus* are syntopic and where *O. quadruncus* are not exposed to *O. hylas* (allopatric). Crayfish were tracked using a portable antenna during summer, fall, and spring seasons along with diel tracking in the summer. Habitat use was examined by graphing used and available locations on bar graphs and habitat selection was examined by using discrete choice analysis to parameterize models and AIC to select the most supported model.

Habitat use results indicated that both species use a wide range of habitats in the streams where sampling occurred and can be considered generalist species. *Orconectes hylas* and *O. quadruncus* both selected habitats with coarse cover rock and slow current velocities. There was little difference between how the two species used or selected habitats indicating that the potential for competitive exclusion may be high, but that it has not yet occurred at the study sites. Other observations from crayfish tracking provided information on growth, movement, and life-history traits of both species. Crayfish were observed to grow more in the summer and spring than in the autumn or winter, but there was no difference in growth between species. *Orconectes hylas* was observed to move at 10.6 m/hr; a rate that if sustained for four days, would equal a kilometer. Perhaps the most important life-history trait observed was related to crayfish reproduction. An *O.*

hylas male was observed mating with an *O. quadruncus* female which suggests hybridization is a possible mechanism of species replacement, but viable hybrids have not been documented.

Chapter 4 focused on the design of a monitoring framework to assess the status of crayfishes in the St. Francis River drainage. A monitoring strategy using occupancy modeling to estimate the occupancy and detection probabilities of *O. hylas*, *O. peruncus*, and *O. quadruncus* was suggested. This approach uses periodic sampling of many streams throughout the drainage to observe increases in occupancy (spread) of *O. hylas* and decreases in occupancy (contraction) of the two natives. Sampling procedures, alternative methods, advice on data interpretation, and a budget were proposed.

The findings of this study can be used to assist in conservation activities such as creation of refugia for native species and direct future research to more specifically identify the mechanism(s) of species displacement. Several conservation actions are discussed in Chapter 5 both in the context of invasive crayfishes in general and in the context of *O. hylas*. Abundance reduction methods for *O. hylas*, biological control, and manipulation of habitat conditions are not likely viable management options in this situation. Until a more viable management strategy can be identified, the best option for protecting the native crayfishes in the St. Francis River drainage is to utilize existing barriers to the dispersal of *O. hylas* and protect native populations that are isolated from *O. hylas* by those barriers. Additional research and implementation of conservation actions are likely required to ensure the long-term persistence of *O. peruncus* and *O. quadruncus*.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Crayfish Imperilment

Crayfish play vital roles in aquatic ecosystems. They are known to consume benthic invertebrates, detritus, macrophytes, algae, and fish in streams (Huryn and Wallace 1987, Charlebois and Lamberti 1996, Whitley and Rabeni 1997, Taylor and Soucek 2010). Consumption of those resources contributes to high levels of energy processing and secondary production (Momot et al. 1978, Mitchell and Smock 1991, Roell and Orth 1992, Momot 1995). Crayfish are also known to be consumed by over 200 species of animals, including mammals, birds, reptiles, amphibians, fishes, and other invertebrates (DiStefano 2005). Crayfish are primary components of the diet of certain sport fishes (Rabeni 1992, DiStefano 2005). Crayfish can function as ecological engineers through bioturbation of fine and coarse sediments and by processing detritus (Creed and Reed 2004). They are also used commercially for fishing bait by humans (DiStefano et al. 2009) and certain species are raised for human consumption (Huner 1994). Crayfish contribute to overall biodiversity in terrestrial, lentic, and lotic environments (Taylor et al. 2007).

Despite their importance, they represent one of the least protected and most imperiled faunal groups in North America (Master 1990, Lodge et al. 2000, Taylor et al. 2007). Of the reported 363 taxa of crayfish in the United States and Canada, less than 1% receive federal protection under the U. S. endangered species act but almost half (47.9%) are listed by the American Fisheries Society (AFS) as endangered, threatened, or vulnerable (Taylor et al. 2007). For the 169 taxa listed as endangered, threatened, or

vulnerable by the AFS, the criteria cited for listing are restricted range (99% of listed species), habitat destruction or modification (33%), and a collection of natural or anthropogenic factors (4%) such as introduced species, hybridization, predation, and competition (Taylor et al. 2007). Other factors affecting crayfish populations are disease and overharvest, which are poorly understood in North America (Taylor et al. 2007). Many of these factors may have interactive effects on crayfish populations, thus complicating efforts to understand and protect populations. For instance, Lodge et al. (2000) suggest that unfavorable environmental changes for native crayfishes may promote the establishment of introduced species.

The leading criterion for listing crayfishes, restricted range, is unlike the other factors because it often results from a degree of endemism that occurs naturally. Restricted range does not threaten crayfish populations; rather it predisposes them to greater vulnerability to other (often anthropogenic mediated) factors. For example, the federally endangered *Orconectes shoupi* exists in only two counties in Tennessee and its persistence is hypothesized to be threatened by urbanization (Bizwell and Mattingly 2010). Other crayfishes are considered to have restricted ranges based on limited survey data and occasionally a species once thought to be rare or narrowly distributed is actually found to be abundant and undeserving of the “restricted range” label when more intensive sampling is conducted (Westhoff et al. 2006, Taylor et al. 2007). This is a symptom of a larger problem stemming from the fact that much uncertainty remains about crayfish distributions and basic ecology, areas of research that have been more thoroughly pursued for many other taxa.

The second leading criterion for listing crayfish, habitat alteration, results from various anthropogenic alterations to the landscape including agricultural practices, urbanization of the watershed, channelization, mineral mining, and in-stream gravel mining (Jones et al. 1999, Wang et al. 2001). Alterations such as these can increase sedimentation (Southerland et al. 2002), alter natural hydrologic and stream hydraulic conditions (Kondolf 1997), alter nutrient dynamics (Carpenter et al. 1998), alter the chemical properties of water (Rallo and García-Arberas 2002, Allert et al. 2008), and disrupt temperature regimes (Whitledge et al. 2006). Additionally, habitat alteration can occur at multiple spatial and temporal scales which may affect crayfish locally (e.g., gravel mining) or over larger portions of their ranges (e.g., global climate change).

A growing threat faced by many crayfish populations stems from the introduction of nonindigenous species, most notably, other species of crayfish (Lodge et al. 2000, Taylor et al. 2007, Larson and Olden 2010). These introductions occur due to live-bait release, legal and illegal stocking, discarded aquarium pets or classroom study animals, and aquaculture practices (Ludweig and Leitch 1996, Lodge et al. 2000, Larson and Olden 2008, DiStefano et al. 2009). Some introductions are relatively benign, whereas others may lead to the replacement of existing native species or cause drastic habitat alterations (Lodge et al. 2000, Olden et al. 2006). Demonstrating a causal relation between invasive species and declines in native species can be difficult because there are numerous ways in which invasive species can affect native species such as disease, hybridization, interspecific competition, and fish predator interactions (see Lodge et al. 2000 for review).

Some of the most notable invasive species problems associated with crayfish have occurred in Europe. Several species of North American crayfish were introduced for aquaculture purposes throughout Europe and have since become widespread and replaced native species. Many North American crayfishes are carriers of a fungal pathogen termed the crayfish plague (*Aphanomyces astaci*), which does not appear to affect North American species, but is often lethal to European species (Söderhäll and Cerenius 1999). Crayfish plague was first documented in Italy in 1859 and has spread throughout mainland Europe, Great Britain and Ireland (Söderhäll and Cerenius 1999). Crayfish plague has resulted in the local extinction and decline of many native crayfish stocks in Europe. This combination of the effects of introduced species and disease is another example of multiple threats that cause crayfish declines. In the United States, disease is rarely examined as a cause for declines in native crayfish populations (Lodge et al. 2000).

In North America, the most well known and understood invasive crayfish is the Rusty Crayfish, *Orconectes rusticus*. Native to parts of the Ohio River drainage, introduced populations of *O. rusticus* exist in at least 15 states, Ontario, and the Laurentian Great Lakes (Olden et al. 2006). This invasion has been associated with declines in other crayfishes (Lodge et al. 1986), negative effects on other invertebrates (Olsen et al. 1991, Wilson et al. 2004), algae and macrophytes (Charlebois and Lamberti 1996, Lodge and Lorman 1987), and disruption of fisheries (Wilson et al. 2004).

When a species is introduced to an area where a closely related species exists naturally, hybridization may occur, and can result in the permanent loss of unique genetic material and thus biodiversity (Perry et al. 2002). Hybridization between invasive and native crayfishes is often hypothesized but rarely tested or documented (Lodge et al.

2000, Perry et al. 2002). The only study using genetic markers in crayfish to examine hybridization was conducted in Wisconsin where the invasive *O. rusticus* was shown to hybridize and create viable offspring with *Orconectes propinquus* mostly through pairings of female *O. rusticus* and male *O. propinquus* (Perry et al. 2001). Butler and Stein (1985) showed that both the invasive *O. rusticus* and native *Orconectes sanborni* males preferentially mated with *O. rusticus* females. Other authors claim intermediate morphological characteristics as evidence of crayfish hybridization (Capelli and Capelli 1980, Smith 1981).

Competition is frequently hypothesized as a cause for crayfish species replacements and can occur in a variety of ways (Butler and Stein 1985, Light et al. 1995). For example, the invasive *Pacifastacus leniusculus* has been shown to outcompete the native *Astacus astacus* for shelter thereby exposing the native to greater fish predation (Söderbäck 1994). Hill and Lodge (1999) found the invasive *O. rusticus* had higher fitness than two natives under different combinations of competition and predation and negatively affected growth and mortality of the natives. In other instances competition is hypothesized, but not proven. Rabalais and Magoulick (2006) confined invasive *Orconectes neglectus* to cages with native *Orconectes eupunctus* in an Ozarks stream but found no differences in growth or survival when the native was exposed to the invasive.

Aggressive behavior and agonistic interactions between invasive and native crayfishes are often used to examine competition. Guiaşu and Dunham (1999) paired native crayfish with invasive crayfish in laboratory trials, monitored agonistic behavior, and determined the invasive species was dominant. Morphological features such as

larger body or chelae size of some invasive crayfish may result in greater success during agonistic interactions (Garvey and Stein 1993, Gherardi et al. 2000). Roth and Kitchell (2005) found that fish consumed lower than expected numbers of the invasive *O. rusticus*, based on body size and hypothesized that selective predation facilitated invasion success. Beyond aggression, aspects of crayfish life history (e.g., growth rate and timing of hatch) are hypothesized to give invasive species a competitive edge (DiStefano et al. 2002).

Overharvest is not currently a concern for most species of North American crayfish but may occur in some areas (Roell and Orth 1992, Taylor et al. 2007). Species most commonly harvested for bait or human consumption are widespread or locally abundant. Further, most lotic crayfishes have little realized commercial value and are rarely harvested.

The resultant complexity from factors of imperilment acting alone or in combination creates unique situations that require systematic study of imperiled crayfish populations. Some situations may require examining several factors that cause crayfish imperilment to discover which have the greatest probability of negatively impacting populations. Further complications arise when considering these problems at multiple spatial and temporal scales. Many causes of imperilment occur on different spatial scales which require investigations to use multi-scale approaches. Temporally, populations fluctuate between years adding variability to estimates and making broader generalizations difficult when studies are based on only a few seasons of data (DiStefano et al. 2003). Factors causing imperilment may also act differently on crayfish as they progress through life history stages.

Study Location

The St. Francis River begins in Southeast Missouri and flows south into Arkansas where it joins the Mississippi River and can be divided into upper and lower subbasins based on underlying geology. The Upper St. Francis River subbasin (Boone 2001) is defined as the portion of the drainage upstream of Wappapello Dam which is contained within the Ozark Highlands section (Nigh and Schroeder 2002). Within this region, the St. Francois knobs and basins section is dominant and consists of the oldest (Precambrian), highest (540 m above sea level), and the greatest relief (152-244 m) areas in Missouri (Nigh and Schroeder 2002, Boone 2001). Precambrian igneous rock (i.e., granite, rhyolite, felsites) dominates the Upper St. Francis River subbasin. As the river approaches Wappapello Reservoir the geology resembles the rest of the Ozark Uplands and consists of sandstone, dolomite, and cherty limestone (Boone 2001). The Lower St. Francis River subbasin (Boone 2001) includes everything downstream of Wappapello Dam and drains through the Mississippi River Alluvial basin section (Nigh and Schroeder 2002) which consists mostly of alluvial plains (unconsolidated gravel, sand, silt, and clay). This study will only deal with the Upper St. Francis River subbasin as the species of concern are not known to exist in the lower subbasin (Pflieger 1996).

River geomorphology in the Upper St. Francis River subbasin differs from other Ozarks streams because of the relative distinctiveness of the underlying igneous geology. Soils tend to be less fertile and more acidic due to the erosional products of the igneous rock (Boone 2001). Springs are rare in this region because impervious bedrock limits groundwater recharge. Permanent streams in this region generally require 26 to 51 km²

of watershed to sustain one kilometer of permanent stream (Boone 2001). The St. Francis River reaches a maximum of sixth order (Strahler 1957) where it becomes impounded by Wappapello Dam and is the largest stream in the subbasin.

Historical land use in the upper subbasin is similar to that of the other regions of the Ozarks (Jacobson and Primm 1994, Boone 2001). Historically, some of the major land use issues include fire suppression, decline of shortleaf pine stands, mineral deposit mining, large-scale timber operations, and livestock overgrazing (Boone 2001). Many of these activities still occur today, but on a reduced scale and under tighter regulation (Boone 2001). The subbasin is mostly (77%) forested (oak-hickory-shortleaf pine dominated) with some grassland (10%), cropland (7%), and developed (6%) lands (Boone 2001). Farmington (population 13,924), Fredricktown (population 3,928), and Ironton (population 1,471) are the largest human communities in the subbasin which is mostly rural and sparsely populated (U.S. Census Bureau 2000, Boone 2001).

Species Background

Six species of crayfish are thought to occur naturally in the Upper St. Francis River subbasin of Missouri (defined below): *Cambarus diogenes*, *Cambarus hubbsi*, *Orconectes luteus*, *Orconectes peruncus*, *Orconectes punctimanus*, and *Orconectes quadruncus* (Riggert et al. 1999). Of these, *O. peruncus* and *O. quadruncus* are endemic only to the Upper St. Francis River subbasin and both are listed as threatened by the AFS, imperiled by the state of Missouri, and Vulnerable on the IUCN Red List (Taylor et al 2007, DiStefano 2008a, DiStefano 2008b). Within the St. Francis River drainage there is little overlap between the ranges of *O. peruncus* and *O. quadruncus* (Figure 1). This

discrete distributional pattern is further evidenced by the co-occurrence of the species at only seven locations (5%) of the 128 locations sampled for crayfish in the St. Francis River drainage (J. Westhoff, unpublished data).

Procambarus acutus, *Orconectes harrisoni* and *Orconectes hylas* have been documented in this subbasin and are considered introduced species (Riggert et al. 1999, Boersig and DiStefano 2007). *Procambarus acutus* is known from one location (Boersig and DiStefano 2007) and *O. harrisoni* is known from three locations in the drainage (J. Westhoff, unpublished data). Neither of these introduced species has been studied in this drainage so the effects of their presence are unknown. *Orconectes hylas* is widespread in several streams and appears to be the dominant species in some reaches (Riggert et al. 1999). Widespread expansion and numerical dominance (Stage V introduction *sensu* Colautti & MacIsaac 2004) of *O. hylas* warrants the labeling of this species as invasive. This study focused on the two endemics, *O. peruncus* and *O. quadruncus*, and the invasive *O. hylas* (referred to as the three species of interest throughout this document).

Orconectes hylas is believed to have been introduced into the subbasin from the neighboring Black River and Big River drainages (Figure 2) where it is native (Fetzner and DiStefano 2008). The exact methods, dates, and locations of the introductions are not known, but are believed to have occurred within the past 35 years as it was first detected in 1984 in Stouts Creek (DiStefano 2002). *Orconectes hylas* has since spread throughout portions of Stouts Creek and appeared in Big Creek, Carver Creek, Marble Creek, Orr Hollow Creek, and Big Lake Creek (Figure 3) and is estimated to occupy between 5 and 20% of the total stream km of the St. Francis River drainage (DiStefano and Westhoff, in press).

These introductions are of concern because *O. peruncus* and *O. quadruncus* often disappear from stream reaches where *O. hylas* is found (DiStefano 2008a, DiStefano 2008b). Two or more species “coexist” if they are able to persist indefinitely when syntopic (Chesson 2000, Siepielski and McPeck 2010). Species “co-occur” when niche differentiation is not achieved between competing species and although they appear to coexist, this is only because of slow extirpation rates, neutral dynamics, or a source population is supplying the system (Siepielski and McPeck 2010). Although *O. hylas* co-occurs with the natives at some locations, the species may not be able to coexist over time. Other alterations to the structure and processes of the aquatic community at locations invaded by *O. hylas* may exist, but have not been investigated.

Life history characteristics have been recorded for each of these three species. All three species reach similar size at maturity (average 17.6 – 24.5 mm carapace length; CL), but *O. hylas* are known to grow to a larger maximum CL (36.8 mm) than either *O. peruncus* (28.0 mm) or *O. quadruncus* (33.2 mm) (DiStefano et al. 2002). Juvenile *O. hylas* sampled from introduced populations also grew faster and larger than *O. hylas* from native populations (in Black River drainage) and *O. peruncus* juveniles (DiStefano et al. 2002). Female *O. hylas* released young earlier than the natives, were more fecund than *O. peruncus*, and are known to become ovigerous up to two months before either native (DiStefano et al 2002). Sex ratios vary somewhat seasonally, but are approximately 1:1 for all species (Riggert et al. 1999, DiStefano et al. 2002).

Orconectes hylas is similar in size and appearance to the two endemics but methods have been established to distinguish them (Appendix A). The most identifiable morphological characteristic to distinguish the species is the shape of the male gonopods.

The following descriptions are taken from Pflieger (1996). Male *O. hylas* have long and slender gonopods that have primary and secondary processes of approximately equal thickness that touch along their length. The gonopods of *O. peruncus* are similar to *O. hylas*, but the processes tend to be more separated and the secondary process is thicker and curves away from the primary process. The processes of *O. quadruncus* gonopods are of equal length and much shorter than those of the other two species.

Female crayfish are less easily identified to species, but slight morphological differences do occur in the shape of the annulus ventralis among the three species. *Orconectes quadruncus* is the most distinct in that the fossa is shallow and the sinus has three, gently curving directional changes. The fossa of *O. peruncus* and *O. hylas* are both relatively deeper than *O. quadruncus* and the sinus has five directional changes along its length. In *O. peruncus*, the directional changes appear more angular than in *O. hylas*, which sometimes emulates a question mark. Further, the annulus ventralis is relatively compressed along the rostral-caudal axis in *O. hylas* as compared to the slightly elongated form in *O. peruncus*.

The phylogenetic relation between *O. hylas*, *O. peruncus*, and *O. quadruncus* is of particular interest in this invasion situation. The three species of interest were found to be more phylogenetically similar to each other than any other species based on an assessment of the mitochondrial genomes of 20 native Ozark crayfish species (Crandall 1998). Fetzner and DiStefano (2008) reported haplotype sequence divergence ranged from 2.4 – 3.7% among the three species and only 0.2 – 0.6% within a given species, indicating the species are closely related but have little genetic variation within species. Unique haplotypes do exist for *O. peruncus* at Twelvemile and Dry Creek, as well as

several other small streams in the drainage (Fetzner and DiStefano 2008). The molecular diversity of *O. quadruncus* was half that of *O. peruncus*, and the most unique population was located in Upper Rock Creek (Fetzner and DiStefano 2008). The genetic similarity among species supports statements by Creaser (1934) that hypothesize a population of *O. hylas* was naturally introduced into the St. Francis River basin by headwater capture at some point in geologic history and subsequently evolved into *O. peruncus*. Creaser (1934) further speculates that populations of *O. peruncus* became isolated by the deepening of the St. Francis River and gave rise to *O. quadruncus*.

Study Framework

This project is part of a larger, ongoing study initiated by the Missouri Department of Conservation. Several published studies exist involving these three species of interest and address topics including life history, distribution, genetics, and competition (Table 1). These studies provide the groundwork for formulating hypotheses about the decline of *O. peruncus* and *O. quadruncus* and the spread of *O. hylas*. Interspecific competition was identified as a potential mechanism of species displacement based on comparisons of life history characteristics of the species (DiStefano et al. 2002) and has been experimentally investigated. Rahm et al. (2005) examined agonistic interactions (in the absence of food or shelter) of adults and juveniles between the three species of interest as a potential driver of interspecific competition and thus a mechanism of species displacement. Their experiments did not show that the invasive *O. hylas* did not dominate the two endemics in size-matched interspecific interaction trials. The same result was described when adult *O. hylas* were given a slight size advantage. Westhoff et

al. (in review) evaluated the effect of *O. hylas* on the growth and survival of *O. peruncus* at two locations in the former range of *O. peruncus*. The interspecific effects of *O. hylas* on *O. peruncus* were no different than the intraspecific effects of *O. peruncus* on themselves, providing no evidence for interspecific competition between juveniles of these species.

Information gained from previous research efforts has addressed some information gaps and hypotheses about species displacement, but are not conclusive. Many avenues of research remain that may reduce uncertainty about the mechanism or mechanisms responsible for the decline of the two endemic species of interest and provide information that can be used to ensure their persistence. Incomplete information or invalid assumptions about system functioning could result in well-intentioned, yet ineffective conservation actions. These objectives were presented in the dissertation by covering habitat associations (Chapter 2 and 3), importance of anthropogenic alterations (Chapter 2) and the use of monitoring (Chapter 4).

Habitat Associations

Environmental variables affect aquatic communities at differing levels and at different spatial scales (Rabeni and Sowa 1996). This relation creates a hierarchical filter whereby species distributions and abundances are shaped by environmental and anthropogenic factors from the watershed to the microhabitat scale (Frissell et al. 1986, Brewer et al. 2007). Each smaller scale is nested in the larger scale and thus potentially impacted by changes at those larger scales (Rabeni and Sowa 1996, Frissell et al. 1986). A species must first be able to exist based on features of the larger scale before features

of the smaller scale become limiting. This relation emphasizes the need to examine habitat at multiple spatial scales whenever possible.

Habitat partitioning has been demonstrated by sympatric crayfish species and is believed to allow for the coexistence of multiple species on small spatial scales (Rabeni 1985, DiStefano et al. 2003, Flinders and Magoulick 2007). Some species exhibited ontogenetic shifts in habitat association (DiStefano et al. 2003) and shifts during times of predator exposure (Mather and Stein 1993). This variation in habitat use can be difficult to explain, especially with studies limited by spatial and temporal constraints.

Quantitative habitat association information is known for only a few species of crayfish and is lacking for the three species of interest. It is important to design field habitat studies that incorporate as much of this variation as possible to most accurately describe habitat associations.

A better understanding of the habitat associations for these species at multiple spatial scales can have several benefits. Observed patterns of habitat use and niche requirements can be used to look for evidence of competitive exclusion between crayfish species (Bovbjerg 1970, Flynn and Hobbs III 1984). The most important factors related to habitat use can be identified which provide managers information on how to prioritize habitat protection and enhancement efforts. Understanding habitat relations between aquatic organisms and environmental variables can also allow for predictive modeling that provide specific levels of habitat characteristics that are most suitable for a given species.

Anthropogenic Alterations

Many anthropogenic activities are hypothesized to, or have been shown to, negatively affect stream biota (Allan 2004). These activities most likely affect different species and different streams in varying ways, especially in heterogeneous landscapes (Wang et al. 1997). Some activities may not affect one species, but may cause physiological stress on individuals of another species (Allan 2004). Any alteration in stream community structure has the potential to stress members of that community and it has been suggested that stressed communities are more likely to be successfully invaded (Lodge et al. 2000, Sakai et al. 2001). Thus, anthropogenic alterations to the landscape or stream may enhance the ability of an invasive species to become established and spread if that species is more tolerant of the stressors than the native species are. This idea of habitat alteration and disturbance favoring introduced species establishment is found throughout the literature (Jones et al. 1999, Byers 2002, Meador et al. 2003, Marvier et al. 2004, Moyle and Marchetti 2006) and is a potential mechanism by which *O. hylas* has expanded and *O. peruncus* and *O. quadruncus* have declined. Collection of evidence that identifies certain anthropogenic alterations to the landscape as facilitating species displacement would help managers target those land-use practices that could be modified to mitigate their adverse effects.

Monitoring

Managers require accurate information on distribution and population status of imperiled organisms to guide management and conservation efforts (Gibbs et al. 1998, Block et al. 2001). Monitoring is important when known threats such as invasive species

are thought to be adversely affecting native populations. Changes in distribution or population abundance can signal success or failure of current management strategies and can aid identification of proper management approaches. Many types of monitoring metrics exist, but one approach that has become more popular recently is to use occupancy modeling which can estimate the proportion of sites occupied by an organism of interest and accounts for incomplete detection of the species (MacKenzie et al. 2006). Occupancy and detection probability are informative metrics for monitoring changes in species distributions at both fine and coarse scales. Monitoring, in conjunction with information on species ecology and mechanisms of species displacement, promises to provide the most holistic approach to effectively preserving *O. peruncus* and *O. quadruncus*.

Research Objectives

The purpose of this study was to gather information that facilitates efforts to ensure the persistence of *O. peruncus* and *O. quadruncus* and reduce the uncertainty related to observed species displacement. This was accomplished by 1) examining associations of crayfish distributions to coarse-scale natural and anthropogenic factors that may be influencing those distributions, 2) examining fine-scale habitat selection of *O. hylas* and *O. quadruncus*, and 3) designing a monitoring plan as a tool to inform decision makers about the expansion of the invasive *O. hylas*, the decline of *O. peruncus* and *O. quadruncus*, and the conservation status of these two imperiled endemics. Information gained was used in conjunction with existing information on this study

system to form a better understanding of crayfish imperilment issues and to recommend specific conservation actions.

Table and Figures

Table 1. Scientific studies related to various topics involving *Orconectes hylas*, *Orconectes peruncus*, and *Orconectes quadruncus*.

Topic of Investigation	Species	Study
Species description	<i>O. hylas</i>	Faxon 1890
Species description	<i>O. peruncus</i>	Creaser 1931
Species description	<i>O. quadruncus</i>	Creaser 1933
General Background	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	Pflieger 1996
Distribution	<i>O. peruncus</i> , <i>O. quadruncus</i>	Riggert et al. 1999
Life History	<i>O. peruncus</i> , <i>O. quadruncus</i>	Riggert et al. 1999
Life History	<i>O. hylas</i>	DiStefano et al. 2002
Genetics	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	Crandall 1998
Genetics	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	Fetzner and DiStefano 2008
Bioenergetics	<i>O. hylas</i>	Whitledge and Rabeni 2002
Competition	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	Rahm et al. 2005
Competition	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	Westhoff et al. in review
Conservation	<i>O. peruncus</i> , <i>O. quadruncus</i>	DiStefano 2008a, DiStefano 2008b
Rate of Invasion	<i>O. hylas</i> , <i>O. peruncus</i> , <i>O. quadruncus</i>	DiStefano and Westhoff in press
Water Quality	<i>O. hylas</i>	Allert et al. 2008
Water Quality	<i>O. hylas</i>	Allert et al. 2009

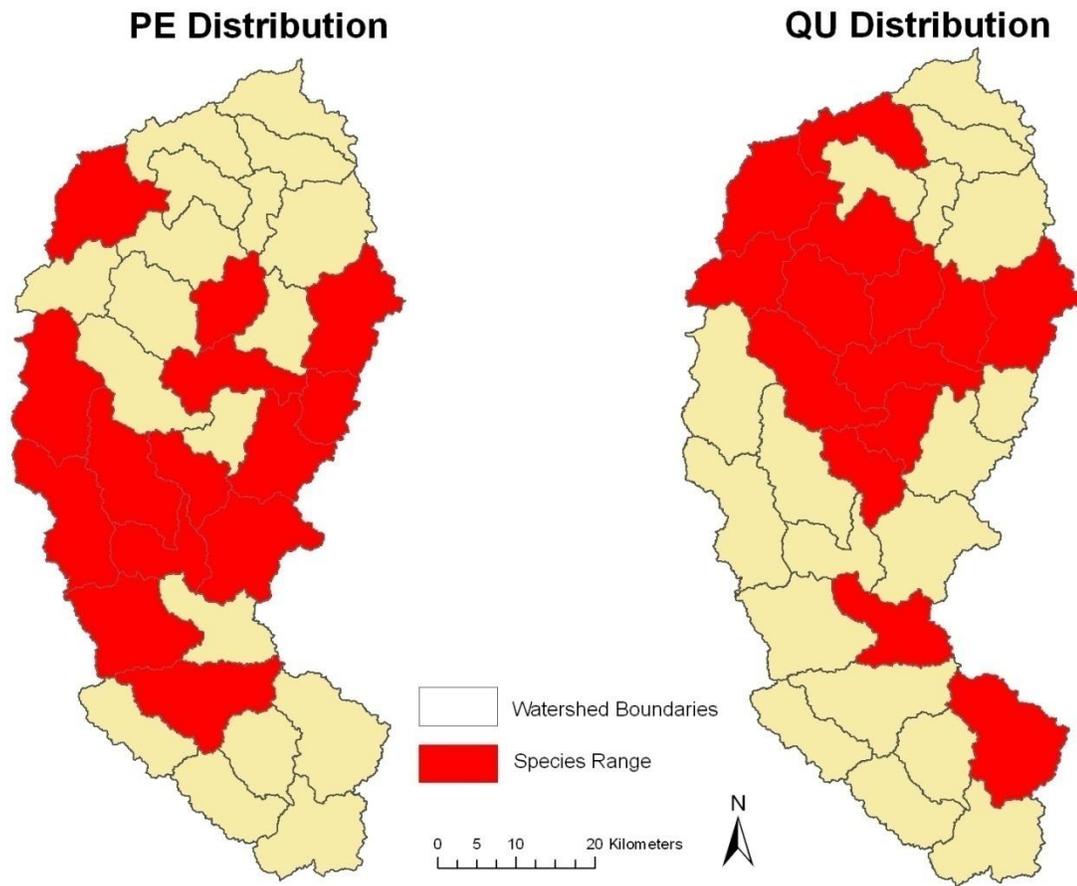


Figure 1. Distribution of *Orconectes peruncus* (PE) and *Orconectes quadruncus* (QU) in the St. Francis River Drainage of Missouri. Watershed boundaries represent 12-digit Hydrologic Units (HU) and a given species was considered present in the HU if it was known to occur within at least one stream with that HU.

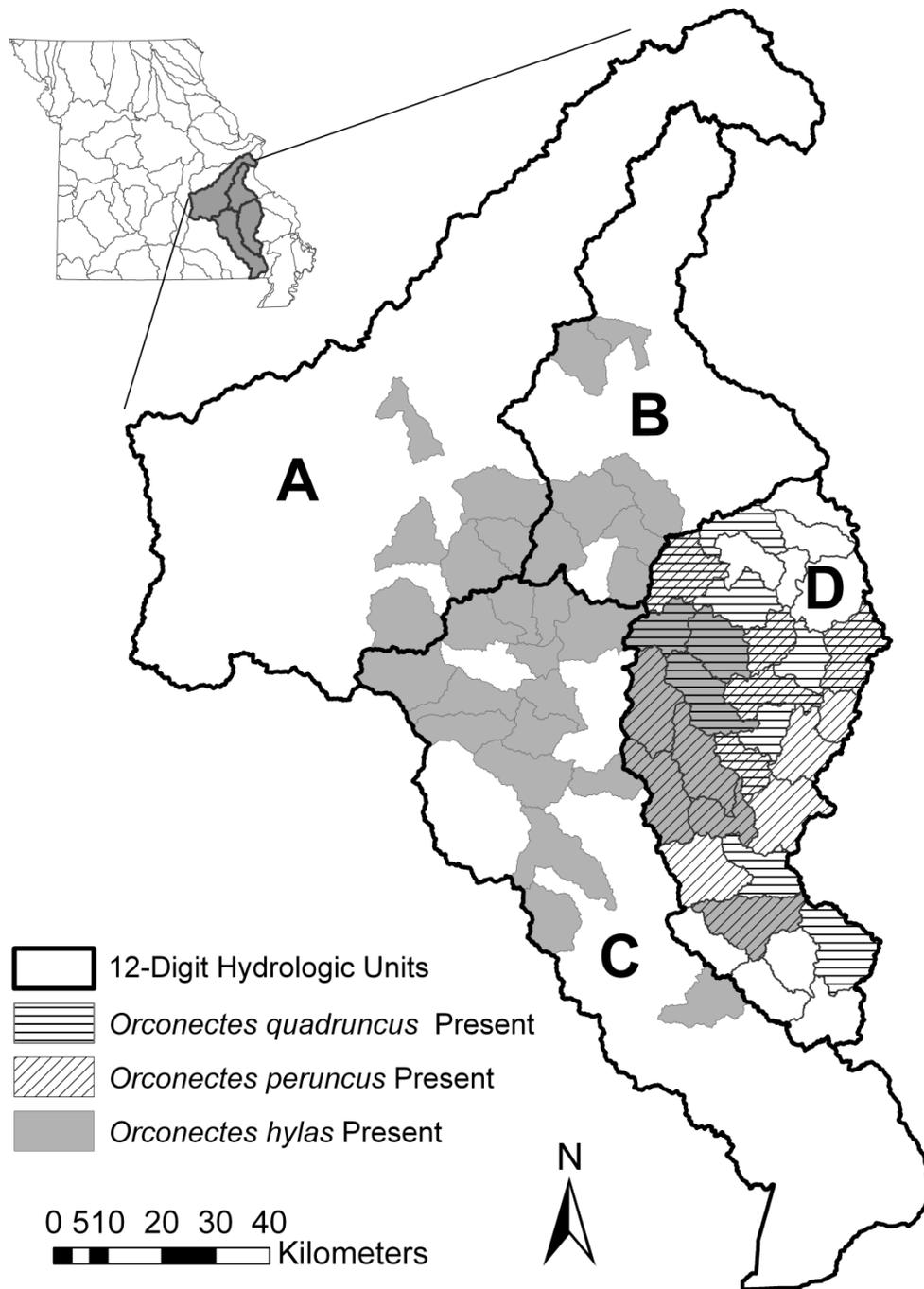


Figure 2. Sub-drainages known to harbor *Orconectes quadruncus* (horizontal lines) *O. peruncus* (diagonal lines), and *O. hylas* (gray areas) in the Meramec River (A), Big River (B), Black River (C), and St. Francis River (D) drainages in the state of Missouri, USA. Gray areas in the St. Francis River drainage represent introduced populations.

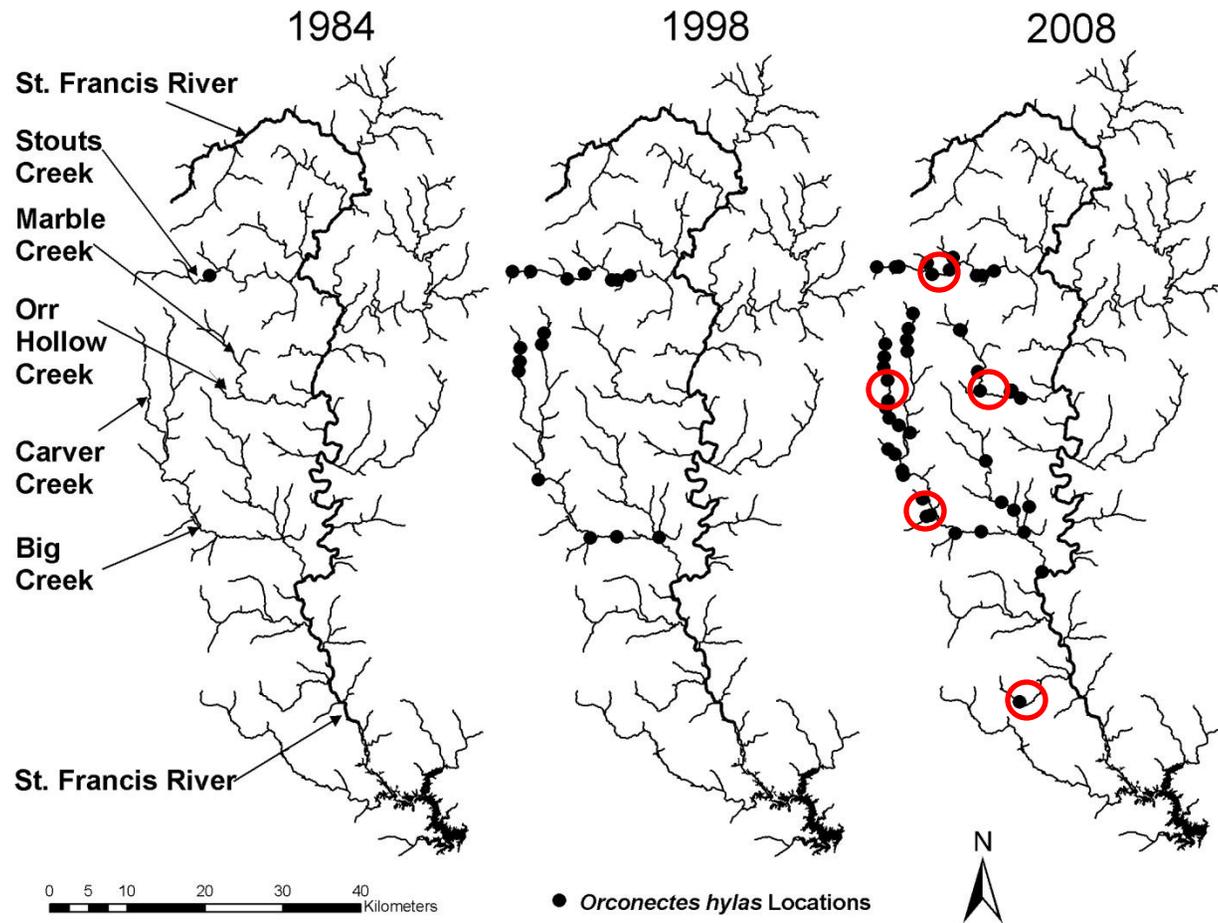


Figure 3. Progression of *Orconectes hylas* invasion through the St. Francis River drainage in Missouri, USA over 24 years. Red circles represent hypothesized approximate locations of separate introduction events based on sampling.

CHAPTER 2

COARSE-SCALE ANTHROPOGENIC ALTERATIONS, ENVIRONMENTAL
CONDITIONS, AND SPECIES DISTRIBUTION

INTRODUCTION

Habitat alteration and invasive crayfish are frequently implicated as causes of native crayfish imperilment in North America (Lodge et al. 2000, Taylor et al. 2007). These threats can operate independently of one another, or interact, whereby habitat alteration impairs native population persistence and enhances invasive species establishment or persistence (Ross et al. 2001, Rahel 2002, Meador et al. 2003, Marvier et al. 2004, Didham et al. 2007). Anthropogenic actions that stress aquatic organisms can reduce native population size or eliminate native species, thus creating a situation of altered competitive ability or empty niche space in which invaders can establish (Moyle and Light 1996, Byers 2002). In this scenario, native species may persist in unaltered areas despite the presence of an invasive species, but if the environmental conditions are altered by humans, the invasive species may gain a competitive edge and eliminate the native species. This argument addresses the ecological question of why invasive species are able to replace native species despite the fact the native species has evolved in the system and is theoretically better adapted (Byers 2002).

Uncertainty about the mechanism(s) responsible for local extirpations of native crayfishes results in difficulty for management (Larson and Magoulick 2009). Understanding the natural and anthropogenic factors that shape species' distributions at broad spatial scales can reduce this uncertainty (Hansen & Richardson 2002, Meador et al. 2003, Steen et al. 2008). By identifying conditions associated with species distributions, managers can better understand if the native species are declining due to

anthropogenic habitat alterations alone, or a combination of habitat alteration and interactions with the invasive.

Natural and anthropogenically altered conditions that best predicted species distributions throughout the drainages of interest were identified using classification tree models. Positive association between anthropogenic variables and stream reaches occupied by the invasive *O. hylas* suggests the invasive may have an advantage in those streams. Additionally, if anthropogenic variables better predict species' distributions than do natural variables, it may indicate the propensity of anthropogenic activities to override the influence of natural variables. The results of this analysis can focus future efforts on either species displacement mechanisms not related to anthropogenic alterations or alternatively provide a refined list of anthropogenic land-use activities to likely have the greatest effect on the crayfishes studied. The results of my study are intended to complement existing field studies aimed at identifying a mechanism of species displacement by providing a collective context for the interpretation of the studies at multiple spatial scales (*sensu* Rabeni and Sowa 1996). Interpolation of model results is used to predict the potential distribution of the invasive species in the St. Francis River drainage and discuss potential implications of future conservation actions.

METHODS

Species distribution models relate occurrence data to environmental conditions to predict species distributions (Peterson 2003, Usio et al. 2006, Elith and Leathwick 2009). Two models were created for each of the three species of interest to predict each species' potential distribution. Each model for a given species included natural variables, but one model also included a suite of anthropogenic variables. This was done to assess the relative influence of natural and anthropogenic variables on the distribution of each species. The two models created to predict the distribution of *O. hylas* used only samples and variables from that species' native range. The use of samples from the St. Francis River drainage was avoided because it would have included landscape features in the analysis that might not influence *O. hylas* distribution and would have been confounded by samples from areas where *O. hylas* had not yet had the opportunity to disperse. All models for *O. peruncus* and *O. quadruncus* were based on samples collected in the St. Francis River drainage where the species are endemic. Because neither species is distributed throughout the entire system, I recognized that the models could provide insight into why they are restricted to their respective distributions within the catchment.

Crayfish Data

Crayfish samples from the Big (N = 31), Black (N= 32), Meramec (N= 45), and St. Francis (N= 128) river drainages were used for model development. These samples were collected by numerous individuals from 1976 – 2008. Most St. Francis River drainage samples were from a distributional survey conducted in the late 1990s (Riggert et al. 1999) or collected during 2007-2008 crayfish sampling for other objectives. Most

samples from the other three catchments were collected in the early 1980s by various collectors. Because these data were collected by many different biologists using different methods (i.e., seining, trapping, hand collecting, and quadrat samples), only species presence or absence was used for modeling. When multiple collections occurred on the same stream segment (section of stream between two tributary junctions), the most recent collection was used. This was done to reduce the influence of stream segments that were repeatedly sampled on the probability of presence values and ensure sample sizes were not artificially inflated.

Natural Factors

Fifty natural environmental variables characterizing geology (n=8), soils (n = 22), natural land cover (n = 6), stream size/location (n=5), and various other conditions (n = 9) were included as predictors in the models (Table 2). Originally collected as part of a GAP analysis project, these data were obtained from the Missouri Resource Assessment Partnership (MoRap) as a 1:100,000 digital network of stream segments linked to natural environmental attributes (Sowa et al. 2005). The spatial scale to which each variable applies is either the stream segment scale (immediate catchment of a stream segment not including upstream influences) or the catchment scale (entire catchment above the given stream segment). Many catchment-scale variables represent the percentage of the total catchment in a given condition (e.g., soil type or land cover), whereas those at the stream segment scale relate to local variables that vary somewhat independently from catchment level processes (e.g., gradient or stream size). Brewer et al. (2007) discussed how many of these natural variables relate to the distribution and ecology of aquatic biota.

Some variables were correlated, but all variables were included in the model to retain the unique information associated with each. An example of this would be variables related to stream size or location, which comprise several variables that assign some metric or category based on different criteria. Some variables, such as Strahler stream order, are well recognized metrics (Strahler 1957); however, this metric is often criticized and is a coarser metric than one such as Shreve link (Shreve 1966). Each variable describes something slightly different than a related variable and may influence how model results are interpreted.

Anthropogenic Factors

Anthropogenic predictor variables for each stream segment in the catchments of interest were obtained from MoRAP and varied in spatial resolution (Annis et al. 2010). Some variables were excluded from the analysis because they lacked appropriate spatial resolution, leaving 25 anthropogenic variables as possible predictors for the model (Table 2). These variables were grouped into five broad categories: Agriculture, Hydrology, Mining, Urbanization, and Water Quality (Table 2). Variables took the form of either the percentage of that factor in the catchment (e.g., percentage of row crop land use), the number of occurrences of the factor in the catchment (e.g., the number of headwater impoundments in the catchment), the minimum distance to an upstream occurrence of that factor (e.g., the distance to the closest upstream lead mine), the average distance to all upstream occurrences of that factor in the catchment (the average distance to all upstream lead mines), or a combination of two or more of these formats (Table 2). Annis

et al. (2010) provides detailed information about each variable and a literature review of their effects on aquatic biota.

For some variables, such as lead mines, it is informative to examine both the total number of occurrences in the catchment above the stream segment as well as the distance to individual lead mines. This variable is based on the spatial location of active and abandoned lead mines recorded in the EPA's Better Assessment Science Integrating Point and Non-point Sources 2001 database. Because the anthropogenic variables only had spatial location data associated with them, the analysis assumed that each mine has a similar influence on the system. Uncertainty exists regarding the magnitude of the disturbance, the amount of heavy metals or other contaminants in the system, the mitigation practices in place, and any other information that may help us understand which individual occurrences are more harmful relative to other occurrences. It is known *a priori* that this assumption is not valid for many of the anthropogenic variables, but it is unavoidable given the available information. Including both the number of mines in the catchment and minimum distance to the closest mine (or average distance to all of mines in the catchment) enabled examination of these individual anthropogenic variables locally (based on distance) and collectively (based on number or percent coverage).

The use of individual anthropogenic variables allowed for modeling of individual effects, but did not address the cumulative effect of multiple anthropogenic variables on species distributions; the later could theoretically have a more pronounced influence on distributions. To address cumulative effects, individual anthropogenic variables under each of the five categories listed above were grouped into five threat indices based on their general source or impact on the natural environment and added to the model as

predictors (Table 2). These indices were related to agriculture (threats related to livestock or crop production), hydrology (threats that alter the natural flow regime), mining (threats related to point-source mining activities), urbanization (threats related to high density human populations), and water quality (threats related to point-source water quality impairment). Several anthropogenic variables could be placed in multiple indices (e.g., impervious surface coverage could be placed in the urban or hydrologic indices); however, each anthropogenic factor was only used in a single index as shown in Table 2. A sixth index, the grand index, included all anthropogenic variables and gave the most complete view of potential threat to aquatic organisms. This approach allowed for examination of anthropogenic variables from different conceptual angles, both individually and in aggregation.

Only variables representing the percentage of catchment or number in catchment were used to create threat indices. The values for each variable were ranked from lowest expected threat (1) to highest expected threat (n). Segments with the same value were given the same rank. Each variable was then scaled from zero (lowest threat value) to 100 (highest threat value). Variables in a given index category were then summed and rescaled from zero to 100 to create the final index. The grand index resulted from summing the other indices categories and rescaling.

Analyses

Predicted distributions of each species were determined using decision tree models (Olden and Jackson 2002). Classification trees (a type of decision tree model) were used because the response (crayfish presence or absence) was categorical. Natural

and anthropogenic variables discussed above were the predictor variables. Decision tree analyses are used to predict species distributions because they are a robust analysis technique capable of examining non-linear data, high-order interactions, and other complex relations that are frequent in distributional ecology (De'ath and Fabricius 2000, Usio et al. 2006, Brewer et al. 2007). They also allow for visualization of patterns (maps) by applying predictive model results in geographic information systems.

All models were created using the Decision Tree Extension of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL). The exhaustive CHAID algorithm was selected over the other possible algorithms for these models because it thoroughly examines all possible splits and allows for nodes to be broken into more than two categories. This algorithm uses adjusted p-values created with chi-square tests to find the strongest interaction between the predictor and explanatory variables.

Classification tree models sometimes split predictor variables at a range of values that is not informative. For example, one node may contain samples with 1% or less of a land use type and the other node contains samples with 2% or greater of the land use type. This split likely has little biological significance because the difference between a site with 1% or 2% of a land use type is probably not detectable by an organism. To avoid this, many predictor variables were divided into ten ordinal categories of equal bin size so each category represented 10% of the values for that variable (Table 2). This method reduces some resolution in the models, but questions at this coarse-spatial scale are better understood by identifying more generalized associations when data lack high resolution.

Additional model parameters were set to control the tree growth and reduce model overfitting. Trees were allowed to grow up to five levels because interpretation of the

model requires that each split considers the relations in the preceding splits and can become complicated if tree depth is not limited. Parent node (a node that is subsequently divided into child nodes) size was set at 21, which was 16%-19% of the total samples used depending on the model. Child node size was set at 7. Node sizes indicate the minimum number of sites that fit the node criteria. These node sizes differ from those used by others (Brewer et al. 2007), but were necessary because smaller node sizes result in overfit models when using small sample sizes. Significance level (α) used to determine a node split (are the sites within a node significantly different than the other node given the conditions of the split) was set at 0.05 and was corrected using Bonferroni adjustment to account for the increased probability of Type 1 error.

Probability of species presence (POP) values are located in each node of a classification tree and represent the percentage of samples where the species was present out of the total number of samples meeting the node criteria. Model outputs were saved in a syntax format that could be used in ArcView 9.3 (Esri, Redlands, CA) to create maps using POP values. Model syntax was used to query the stream network file to select stream segments based on attributes defined in the model and assign the appropriate POP value to the given segment. Each stream segment in the catchments of interest was assigned a POP for each species.

Grand index values for stream segments on sampled locations within the St. Francis River drainage occupied by *O. hylas* (n = 32) were compared to sampled segments where *O. hylas* was not detected (n = 96) using a t-test with unequal variance. This allowed me to determine if those sites where *O. hylas* has established outside of its

native range have differing overall threat index values than other sampled sites in the drainage.

Model Validation and Performance

All models were validated using cross validation by dividing the data into ten folds (subsamples) used to create separate tree models and holding out one fold each iteration. Misclassification costs were averaged across all iterations to estimate the final model cross-validated risk estimate. Results from the *O. hylas* models were applied to the introduced range to validate model performance and predict areas the invasive species may be likely to occupy. Independent model validation data on the presence of *O. hylas* was obtained from crayfish samples taken in the St. Francis River drainage. The presence of *O. hylas* in a given segment was determined using the relative 50 rule developed by Sowa et al. (2005), which provides a quantitative method for deciding which POP values actually represent sites where the species can be considered present. This method considers a species to be present in a stream segment only if the POP for that segment is at least 50% of maximum observed POP in the catchment. For example, in a model where the highest POP value was 80%, a species was considered present in a stream segment if the POP value was 40% or greater and considered absent if the POP value was 39% or lower.

RESULTS

Of the 128 samples taken in the St. Francis River drainage, 50 (39%) contained *O. peruncus* and 36 (28%) contained *O. quadruncus*. The two natives were only collected in the same stream segment in seven of 128 samples. *Orconectes hylas* was present in 51 of 108 (47%) samples from its native range and in 39 (30%) samples taken in the St. Francis River drainage.

Natural Factors

The first level of model splits (the split that explains the most variation) for the two native species was related mostly to soil texture and geology in the catchment. Stream segments with high amounts (> 60%) of very cherty silty loam textured soils (common in limestone and dolomite dominated areas) in the catchment had the greatest probability of harboring *O. peruncus* (Figures 4 and 5). Stream segments with igneous or sandstone geology dominant in the catchment were more likely to harbor *O. quadruncus* (50%) than those with catchments dominated by limestone or dolomite (21%) (Figure 6 and 7). The distribution of the invasive *O. hylas* was best explained by the presence (POP 64%) or absence (POP 16%) of glade complex cover (Figures 8 and 9), which are areas of exposed bedrock on the terrestrial landscape that are relatively void of woody vegetation and have high runoff potential (Baskin & Baskin, 2000).

Soil characteristics and geology were also common predictors in the second level of model splits. These splits specify conditions nested within the higher level (parent node) and provide new POP values for sites meeting all the node criteria. For example, in the *O. quadruncus* model, stream segments with limestone or dolomite as the dominant

geology in the catchment had a POP of 21%, but if one of those sites also had 0% very cherty silty loam textured soil in the catchment, the POP would increase to 63% (Figure 6). Of interest regarding the distributions of the two natives was the relation between very cherty silty loam textured soils and the presence of *O. quadruncus* (negative relation; Figure 6) and *O. peruncus* (positive relation; Figure 4). In the second level of the *O. hylas* model, higher POP values were obtained when igneous geology was present in the catchment or cherty loam textured soils (generally a coarse soil type, but not relative to the other soil types in the region) constituted greater than 10% of the catchment (Figure 8). Stream size, stream segment gradient, and amount of deciduous forest land cover were also identified as natural predictors of the presence for the native species (Figures 4 and 6).

Anthropogenic Factors

Variables related to agriculture were the most influential anthropogenic variables in all three all-variable models (Figures 10-15). Native species responded in differing ways to the agricultural index (Figures 10 and 12). *Orconectes peruncus* was more likely to be present when the agricultural index value for a stream segment was low (<34 = 58% POP; >34 = 20% POP) whereas *O. quadruncus* was more likely to be present when the value was higher (<34 = 16% POP; >34 = 41% POP).

For *O. hylas*, the positive relation with glade complex land cover observed in the natural factor model remained the first split; however, the presence of confined animal feedlot operations (CAFOs) entered the model and was followed by splits representing the effects of coal mining and hydrologic threats (Figure 14). For each of these

anthropogenic variables, *O. hylas* exhibited a negative relation to the presence of the perceived threat. Other model components remained similar to those observed in the natural factor models in all three of the all-variable models.

Stream reaches occupied by *O. hylas* in the St. Francis River drainage were not more threatened by anthropogenic alterations relative to the surrounding regions (Figure 16). Grand Index scores were significantly lower ($p = 0.02$) for sample locations with *O. hylas* (mean = 28.4) than locations without *O. hylas* (mean = 37.3) in the St. Francis drainage.

Model Performance and Validation

Correct classification rates varied from 33.3 to 97.8% among the models (Table 3). The models generally predicted absence better than presence. Total risk (the percent chance the model will misclassify a given sample) was consistent across models and species, with *O. hylas* models performing the best. Cross-validation risk estimates were close (difference less than 7.5%) to the final model risk estimates in the *O. hylas* and *O. quadruncus* all variable models, but were greater in the remaining models (difference of 14 - 22.7%) (Table 3). Greater differences between risk estimates indicate the importance of individual data folds and, thus, weaker models.

Probability of presence values from the *O. hylas* models were applied to stream segments in the St. Francis River drainage (Figure 9). Of the 39 stream segments known to harbor introduced populations of *O. hylas*, 34 (87.2) were correctly predicted by both models. Of the five remaining sites, two had a zero POP and three had a 7% POP.

DISCUSSION

Natural Factors and Crayfish Distributions

Broad catchment-scale variables (i.e., geology and soil texture) were the most highly associated with species distribution. Geology and soils often play a major role in the distribution of aquatic taxa as they constrain the processes and conditions occurring at finer spatial scales within streams (Frissell et al. 1986, Cannan and Armitage 1999, McRae et al. 2004). The geology and soils of a region influence physiochemical characteristics of water (Hynes 1975), affect runoff and discharge rates by influencing water infiltration (Smakhtin 2001), and can determine channel-bed characteristics (Stock and Montgomery 1999). Geology, along with latitude and climate, are considered major factors in the distribution and range sizes of crayfishes (France 1992). However, little is known about the relation of geology and soils to lotic crayfish distributions as most crayfish habitat association studies examine relations at finer spatial scales (Garvey et al. 2003, Flinders and Magoulick 2007). Catchment geology was an important predictor for fish and crayfish distributions in New Zealand (Joy and Death 2004). Geology may not be an important factor for all crayfishes as it was not an important predictor in a multi-scale habitat association analysis for *Orconectes williamsi* (Westhoff et al. 2006).

Orconectes quadruncus was positively associated with igneous geology, whereas *O. peruncus* was positively associated with the coarse textured very cherty silty loam soils common in the limestone and dolomite dominated areas. Although no strong correlation existed between soil texture type and geology, visual inspection of patterns indicated that igneous geology was commonly associated with very stony silty loam soil

textures whereas limestone and dolomite dominated areas have high levels of very cherty silty loam soil textures. The modifier of “very” for a soil texture type indicates that 35 – 60% of that soil is composed of the texture that the word is modifying (U.S. Department of Agriculture, National Soil Survey Handbook). Stony designation is given to coarse rock fragment greater than 25 cm and cherty refers to diameters of 2 – 35 cm (U.S. Department of Agriculture, National Soil Survey Handbook). Hydrology of igneous areas that contain little or no very cherty silty loam may be characterized by faster runoff rates through shallow cobbly or stoney loam soils in some soil classifications (U.S. Department of Agriculture, Soil Survey of Iron County, MO). Excessive rock fragments in the soil horizon can lower the water capacity of the soil (NRCS 2006) which may lead to faster runoff and less available water to the stream in drought conditions. The associative nature of the relation does not allow us to fully understand the mechanism, but suggests it is possible that *O. quadrunclus* is found in flashier streams that perhaps have less through-soil water delivery during dry times than streams harboring *O. peruncus*. Soil conditions related to runoff potential have been related to the distribution and abundance of smallmouth bass *Micropterus dolomieu* in Missouri streams (Brewer et al. 2007). Light (2003) found a negative relation between the invasive crayfish *Pacifastacus leniusculus* and high peak discharge, suggesting that runoff may differentially affect crayfishes.

Another possible explanation for the discrete distributional pattern of the natives may relate to in-stream chemical conditions based on geology. Limestone and dolomite dominated areas have higher amounts of CaCO_3 than do igneous dominated catchments from the high solubility of carbonate rocks in limestone. The St. Francois Mountains

area (igneous) has lower dissolved solids and alkalinity than streams on the Salem Plateau (limestone and dolomite) (Adamski et al. 1995). This may be important as calcium salts are required for crayfish growth and molting (Wheatley and Ayers 1995); however, it is unknown if required calcium levels differ among the species of interest. *Orconectes rusticus* is known to be limited by pH levels below 5.5 and Ca^{2+} levels below 2.5 mg/l (Olden et al. 2006). Higher acidity levels (lower pH) are often associated with igneous areas and may affect the species physiology differently or alter chemical reactions in the lotic environment (McMahon and Morgan 1983).

Geology and soil texture were the driving factors in the models for the two native species, but another catchment-scale variable, glade complex land cover, was the overriding factor in the *O. hylas* models. Glades are commonly associated with areas where erosion has removed loose soil and vegetation is sparse, so these areas may have high runoff potential as little water is held by the shallow, rocky soils and few plants exist to trap water for transpiration (NRCS 2006). If glades are absent, igneous geology in the catchment may replicate the hydrologic effects of the glades (faster runoff and less water storage capacity in the soil) and improve the probability of *O. hylas* presence. When glades are present, the presence of cherty loam soil texture (likely greater soil water capacity than very cherty silty loam) lowers the probability of *O. hylas* presence. When this relation is considered within the context of hydrology, it appears *O. hylas* may use those areas where runoff may be flashy, similar to *O. quadruncus*.

Coarse-scale associations with geology and soil texture were modified in the *O. quadruncus* models by variables related to stream size. Previous distributional work by Riggert et al. (1999) in the St. Francis Drainage indicated both native crayfishes were

most abundant in third order or smaller streams. This study allowed for expansion on these findings and put them in a slightly different context. For *O. quadruncus*, distribution in small or large streams was dependent on geology. When igneous geology was present, they were more likely to occur in creeks, small rivers, and larger rivers than in headwaters (Stream size categories were a general classification based on fish community data from Missouri and delineated based on Shreve link values by Sowa et al. (2005)). This may relate to hydrology again in that igneous areas may have less water between rain events and thus may be more susceptible to stream desiccation. By occupying larger stream systems, the natural disturbance of stream desiccation may be offset somewhat. Stream drying has been hypothesized as a factor that may negatively affect crayfish populations (Flinders and Magoulick 2003). When in areas dominated by limestone and dolomite with high levels of very cherty silty loam soil textures, *O. quadruncus* are more common in order 1 and smaller streams, which may have more sustained soil through flow of water. However, the order 1 streams identified in this model split are those streams that occur in the periphery of larger stream systems that are at the edge of the igneous dominated area. The immediate downstream segments are in areas dominated by igneous geology and lack very cherty silty loam soils. *Orconectes quadruncus* occurs throughout the remainder of the small catchment and may be able to exist in these isolated stream segments dominated by very cherty silty loam because *O. peruncus* was unable to access them. It is also possible that the smaller streams identified in this split may have characteristics that allow *O. quadruncus* to persist such as high allochthonous energy inputs, shallower depths, slower velocities, higher gradients, and different communities of organisms than larger streams (Vannote et al. 1980).

Anthropogenic Factors and Species Displacement

The idea of unnatural disturbances promoting invasive species is well cited in the terrestrial (Stapanian et al. 1998, Byers 2002, Marvier et al. 2004) and aquatic literature (Meador et al. 2003, Marchetti et al. 2004, Riley et al. 2005, Strayer 2010). The primary goal of my study was to determine anthropogenic activities that may be negatively related to the native imperiled crayfishes and/or are creating an environment conducive to the establishment or spread of *O. hylas*. The relation of anthropogenic variables to species distribution did not implicate those variables in the distribution pattern of *O. hylas* in the St. Francis River drainage. A potential influence from agricultural practices was observed for all three species, but the pattern suggested that *O. peruncus* and *O. hylas* respond negatively to agricultural related activities, whereas *O. quadruncus* responds positively.

The agricultural index equally weighted data on the several agricultural activities including percent pasture, percent row crop, number of CAFOs, and pesticide application rates. Of these, pasture is the most common agricultural land use throughout the region and is used for cattle grazing and to a lesser extent to grow hay for livestock consumption. Approximately 60% of the stream segments in the St. Francis River drainage had greater than 10% pasture land use in their catchments. Most pasture in the region is in gently sloping valleys bordering streams, which often give livestock access to the riparian area and stream channel. The riparian area is essential to proper stream function (Hynes 1975, Naiman and Décamps 1997) and the negative effects of livestock grazing on it include increased bank erosion, addition of fine sediment to the stream, and

altered nutrient levels (Kauffman and Krueger 1984, Lyons et al., 2000, Jansen and Robertson 2001, Allen 2004). These effects impact stream biota by changing energy flow, species composition, and species richness (Fitch and Adams 1998, Petersen 1998, Allan 2004). Chandler Schmutzer et al. (2008) showed streams with livestock access had altered water quality, reduced detrital biomass, and lower amphibian abundance and species richness. Alternatively, Rinne (1999) discussed the many potential impacts of grazing on riparian condition, but found that very few authors had effectively shown evidence of negative impacts on fish communities.

Other components of the agricultural index may be important as examples exist relating each of them to negative impacts on aquatic biota. Richter et al. (1996) identified control of nonpoint source pollution from agricultural activities as a high priority for protecting freshwater fauna. Steep slopes, highly erodible soils, and tilling can all lead to high sedimentation rates in streams which are known to fill interstitial spaces in the substrate and disrupt native aquatic communities (Berkman and Rabeni 1987, Petts 1988, Wood and Armitage 1997). Elevated levels of nutrients from fertilizer application can increase primary productivity, which in turn alters community structure (Carpenter et al. 1998). Chemicals used to control crop pests are known to affect macroinvertebrates by altering community structure, reducing densities, and increasing drift mortality (Schulz and Liess 1999). CAFOs were negatively related to the distribution of *O. hylas* in its native range. They are not common in the study area, but do occur in the northern sections of the Big and Meramec River drainages, where *O. hylas* has never been collected. It is possible that the higher nutrient loads and chemical

inputs associated with CAFOs are driving the model, but it is more likely an artifact of CAFO spatial distribution.

Two other anthropogenic variables were useful for modeling the POP for *O. hylas*. When glades and CAFOs were present in the catchment, the presence of coal mining in the catchment resulted in a zero percent POP for *O. hylas*. Drainage from coal mines was shown to decrease both the density and richness of benthic macroinvertebrates by altering pH and heavy metals (Chadwick and Canton 1983). When coupled with the negative effects from CAFOs, *O. hylas* may be precluded due to poor water quality. However, the occurrences of coal mining in our data were restricted to the Meramec River drainage in an area where *O. hylas* has never been collected. So, this relation was likely a result of the spatial distribution of coal mines more so than a true negative association with *O. hylas*. Hydrologic index was also a predictor of *O. hylas* presence in the all variable model, but the difference between a high and low index value decreased the POP from 100% to 85%, given the constraints of the previous model splits. Despite the importance of hydrology to aquatic organisms (Poff and Allen 1995), its effects are relatively weak in our model.

Other commonly identified threats to aquatic systems are not prevalent in the St. Francis River drainage. Levels of urbanization above 8 – 10% in catchment are known to have negative impacts on native aquatic fauna (Booth and Jackson 1997, Wang et al. 2001, Riley et al 2005); however, less than one percent of stream segments in the St. Francis River drainage had 10% or greater urban land use in their catchment. Thus, the threats closely tied with urban areas such as waste-water treatment facilities and impervious surface coverage were not likely to have an identifiable effect on crayfish

distribution given this approach. Other stressors may affect crayfishes, such as environmental estrogens (Mills and Chichester 2005), but were not included in the models because there was not sufficient information on their possible presence in the drainage. Any number of these anthropogenic alterations, or combinations of them, may have substantial impacts on crayfish populations.

The idea that one native species is negatively impacted by agriculture fits with the original hypotheses; however, it was not predicted that the invasive species would have a negative association with the presence of CAFOs, hydrological threats, or coal mines. The negative relation between the invader and anthropogenic alteration may implicate alternative mechanisms of species displacement not related to its ability to tolerate degraded abiotic conditions. This evidence does not support the general hypothesis that anthropogenic activities have enhanced invasive species establishment or spread in this situation. Without controlled, mechanistic studies one cannot conclude that anthropogenic alterations to the system have no effect on species distributions. However, there is little evidence to suggest that anthropogenic factors significantly affect invasive *O. hylas* presence or native *O. peruncus* or *O. quadruncus* decline at a coarse spatial scale in the St. Francis River watershed.

Model Performance and Validation

Models that incorporate coarse-resolution data are more reliable if validation procedures confirm model accuracy with independent data (Olden et al. 2002). Models predicted POP well (classification rates of 78.1% to 85.2%), indicating that there was sufficient information in the predictor variables to estimate species presence. A

distributional study of smallmouth bass *Micropterus dolomieu* in Missouri that used decision tree analysis reported a similar classification rate of 75% for their model (Brewer et al. 2007). Low correct classification for both *O. quadruncus* models was likely influenced by the small number of known present locations for the species. The positive association of *O. quadruncus* to agriculture may be explained by examining model performance. Despite predicting absence well (98% correct classification rate) the all-variable *O. quadruncus* model did not predict presence well (33% correct classification rate). Thus, the agriculture index explains where *O. quadruncus* is not likely to be found, but is not reliable when predicting where it should be found. These results agree with the known distribution of *O. quadruncus*, which is absent in much of the northern section of the drainage and in larger streams, the areas where the agricultural index was highest (Figure 16).

None of the all variable models predicted high POP for any species in the northern part of the St. Francis drainage. This area was the most altered by anthropogenic activities (Figure 16). Crayfish samples from this area indicate low densities of *O. peruncus* and *O. quadruncus* relative to samples taken in other parts of the drainage (Riggert et al. 1999, unpublished data). It is possible that these anthropogenic factors are negatively impacting native crayfish populations throughout this portion of their distributions and thus should be monitored for invasive species other than *O. hylas*, which appears to be similarly negatively impacted.

Interpolation of model predictions outside of the range where the model was developed can be risky (Thuiller 2004, Pearson et al. 2006). However, this is a common option when the goal is to predict the occurrence of a species outside of its known range

or in un-sampled systems (Peterson 2003, Morrison et al. 2004). Comparison of the predicted distribution of *O. hylas* in the St. Francis River drainage and the independent occurrence data showed high model validity (87% correctly classified as present) indicating that these results can be used to inform monitoring efforts and predict future spread of *O. hylas*.

Modeling Constraints

Many possible explanations exist as to why *O. hylas* has become established outside of its native range. One explanation was examined, anthropogenic disturbance, at a coarse spatial scale using generally low-resolution data. The associative nature of this approach did not directly address potential effects of anthropogenic actions on crayfish abundance, behavior, physiology, or individual fitness in a mechanistic way. However, this approach identified natural and anthropogenic variables (i.e., influence of geology and soil texture on hydrology and water chemistry or the influence of agricultural practices on physical processes) that may affect crayfish distribution and warrant continued investigation using controlled studies in a hypothesis testing framework.

Studies using finer resolution data or that investigate processes that occur at finer spatial scales (i.e., biotic interactions) may provide additional insight on the mechanistic effects of anthropogenic actions on these crayfishes. Portions of Big Creek in the St. Francis River drainage were affected by lead mining activities and no crayfish were found in those stream segments despite nearby populations of *O. peruncus* and *O. hylas* that should be capable of dispersing to those locations (J. Westhoff, unpublished data). Allert et al. (2008) documented the negative effects of heavy metals released in lead

mining on *O. hylas* abundance in the Black River drainage. Their study was conducted at a finer spatial scale than the present study and used more detailed information about lead mines (e.g., heavy metal concentrations), which may explain why the models did not include this relation. Sargent et al. (2011) linked coarse-scale associations and finer-scale mechanistic explanations for crayfish invasion patterns. They investigated differences between water temperature preference of and invasive and native crayfish and associated those findings with crayfish abundance patterns in the Flint River, Georgia. They concluded that alteration of groundwater inputs and riparian zone condition may affect invasion success (Sargent et al. 2011).

Inference was limited because abundance data was not used in these models. Presence and absence information tells less about the quality of a site than abundance information, which can indicate relative site quality. Brewer et al. (2007) reviewed issues with the data structure and variable creation for a similar dataset and identified problems such as condensing categorical data into fewer classes and poor spatial accuracy of different data types. Modeling could also be improved by collecting and using information on the magnitude of certain anthropogenic activities then weighing certain threat occurrences based on their suspected impact on the biota. These issues present a constant challenge for coarse-scale modeling and can hopefully be addressed as more data become available and at finer spatial resolutions.

Data on introduction vectors (mode of introduction) and propagule pressure (number of individuals introduced) are important to understanding where introductions may occur and are often included in predictive modeling for introduced species (Puth and Allen 2004, Colautti et al. 2006). Information on these variables was not included in the

POP model for *O. hylas* that was applied to the St. Francis River drainage. Information about fishing pressure, or other vector related data, could refine these predictions to also identify areas where *O. hylas* is likely to be introduced. Hybrid models that combine phenomenological approaches (e.g., habitat suitability) and species demography or mechanistic relations represent a more holistic approach to invasive species forecasting (Gallien et al. 2010).

Conclusions and Conservation Actions

The objectives were to identify coarse-scale factors, either natural or anthropogenic, related to the current distribution of three species of crayfish. Future work to relate species distributions should further investigate geology and soil related factors. Finer scale data (e.g., specific soil series, soil depth data, physiochemical parameters related to geology type) may further inform the models and allow for more robust inference about species relations to natural factors. Anthropogenic activities may influence the distributions of these species to a degree, but are not positively associated at a coarse scale with the current range of the invasive *O. hylas* in the St. Francis River drainage. Strong evidence does not exist for any mechanism of species displacement, so managers may be best served to investigate other causal mechanisms responsible for the apparent displacement of *O. peruncus* and *O. quadruncus* such as hybridization, reproductive interference, or disease (Perry et al. 2002, Edgerton et al. 2004).

This approach predicted the POP for unsampled stream segments which enhances the ability of decision makers to prioritize locations for the implementation of conservation action alternatives. Stream segments with the least anthropogenic influence

and the highest POP for the natives may provide ideal sites for creating refugia from the invasive or focusing conservation efforts; however, the positive relation of *O. quadruncus* and agricultural practices should be investigated further and considered in those decisions. Alternatively, stream segments with high anthropogenic influence may be targeted for mitigation efforts or avoided as sites for possible reintroductions of native crayfishes if finer scale impacts are noted in other studies. Finally, the models can be applied in a strategic framework to identify locations where *O. hylas* is likely to spread based on POP values. These sites can be monitored to more efficiently track the spread of the invasive or the decline of the natives. Further range contraction of either native species could result in an increase in their protection status and more effort focused on preserving existing populations.

Table 2. Natural and anthropogenic variables included as predictors in species distribution models. Asterisk (*) indicates that variables were included in the threat index, but not as an individual predictors in the model. Variable names with superscripts represent variables used in the models in nominal format (¹) or scale format (²). Codes in the original number format column (number of occurrences in catchment = **1**, percent of catchment = **2**, categorical = **3**, minimum distance to closest upstream occurrence = **4**, average distance to all upstream occurrences = **5**) indicate the format of the data when it was collected and prior to dividing them into 10 equal categories (no superscript).

Variable Type	Variable Name	Original Data Format
Natural Variables		
Geology	Dominant geology in catchment ¹	3
	Igneous in stream segment catchment ¹	3
	Alluvium geology	2
	Clay geology	2
	Limestone geology	2
	Sandstone geology	2
	Dolomite geology	2
	Igneous geology	2
Soils - runoff potential	Hydrologic soil group A (HGA)	2
	Hydrologic soil group B (HGB)	2
	Hydrologic soil group C (HGC)	2
	Hydrologic soil group D (HGD)	2
Soils - surface texture	Loam soil	2
	Loamy fine sandy soil	2
	Loamy sandy soil	2
	Sandy loam soil	2
	Silty loam soil	2
	Silty clay soil	2
	Silt/clay/loam	2
	Clay	2
	Stoney silt loam	2
	Very cherty silty loam	2
	Very cherty loam	2
	Stoney loam	2
	Cherty loam	2
Very stony silty loam	2	

	Very cherty loam	2
	Gravelly/sandy loam	2
	Rocky soil	2
	Clayey soil	2
Land cover	Glade complex	2
	Eastern red cedar forest	2
	Bottomland hardwood forest	2
	Deciduous forest	2
	Pine forest	2
	Open water	2
Stream size	Stream size classification	3
	Strahler stream order	3
	Shreve link ²	1
	Size discrepancy 1 category	3
	Size discrepancy 2 category ¹	3
Flow	Flow permanence ¹	3
	Losing reach ¹	3
Elevation	Minimum elevation	1
Gradient (m/km)	Relative gradient	3
	Reach gradient	1
	Segment gradient	1
Springs	Number of springs	1
	Total spring flow	1
Temperature	Temperature code ¹	3
Anthropogenic Variables		
Agriculture	Confined animal feeding operation (CAFO)	1,4,5
	Cropland cover	2
	Crop pesticides	1
	Pasture/grassland cover	2
Hydrology	Certified wells	1
	Dams	1,4,5
	Headwater impoundments	1
	Major impoundments	1
	Road/stream crossings	1
Mining	Coal mines	1,4,5

	Lead mine	1,4,5
	Other mines	1,4,5
Urbanization	Impervious surface cover	2
	Population in year 2000*	1
	Roads	1
Water quality	Comprehensive Environmental Response Compensation, and Liability Information systems sites (CERCLIS)	1
	Landfills	1,4,5
	Leaking underground storage tanks*	1,4,5
	National Pollution Discharge Elimination System sites (NPDES)	1,4,5
	Resource Conservation Recovery Information (RCRIS) site	1,4,5
	Toxic release Inventory (TRI) sites	1,4,5
	Waste water treatment facility	1,4,5
Indices	Agricultural index ²	1
	Hydrologic index ²	1
	Mining index ²	1
	Urbanization index ²	1
	Water quality index ²	1
	Grand index ²	1

Table 3. Summary of model performance and validation results. True negative and true positive columns represent the percentage of samples correctly classified by individual models. Risk estimates for the final model (% samples misclassified in final model) and for the cross-validation (% samples misclassified in iteration runs) are displayed.

Model	True negative (%)	True positive (%)	Risk estimate model	Risk estimate cross-validation
<i>Orconectes peruncus</i> – Natural	78.2	82.0	20.3	43.0
<i>O. peruncus</i> - All	88.5	62.0	21.9	35.9
<i>Orconectes quadruncus</i> – Natural	90.2	50.0	21.1	34.4
<i>O. quadruncus</i> - All	97.8	33.3	20.3	22.7
<i>Orconectes hylas</i> - Natural	78.9	88.2	16.7	19.4
<i>O. hylas</i> – All	96.5	72.5	14.8	22.2

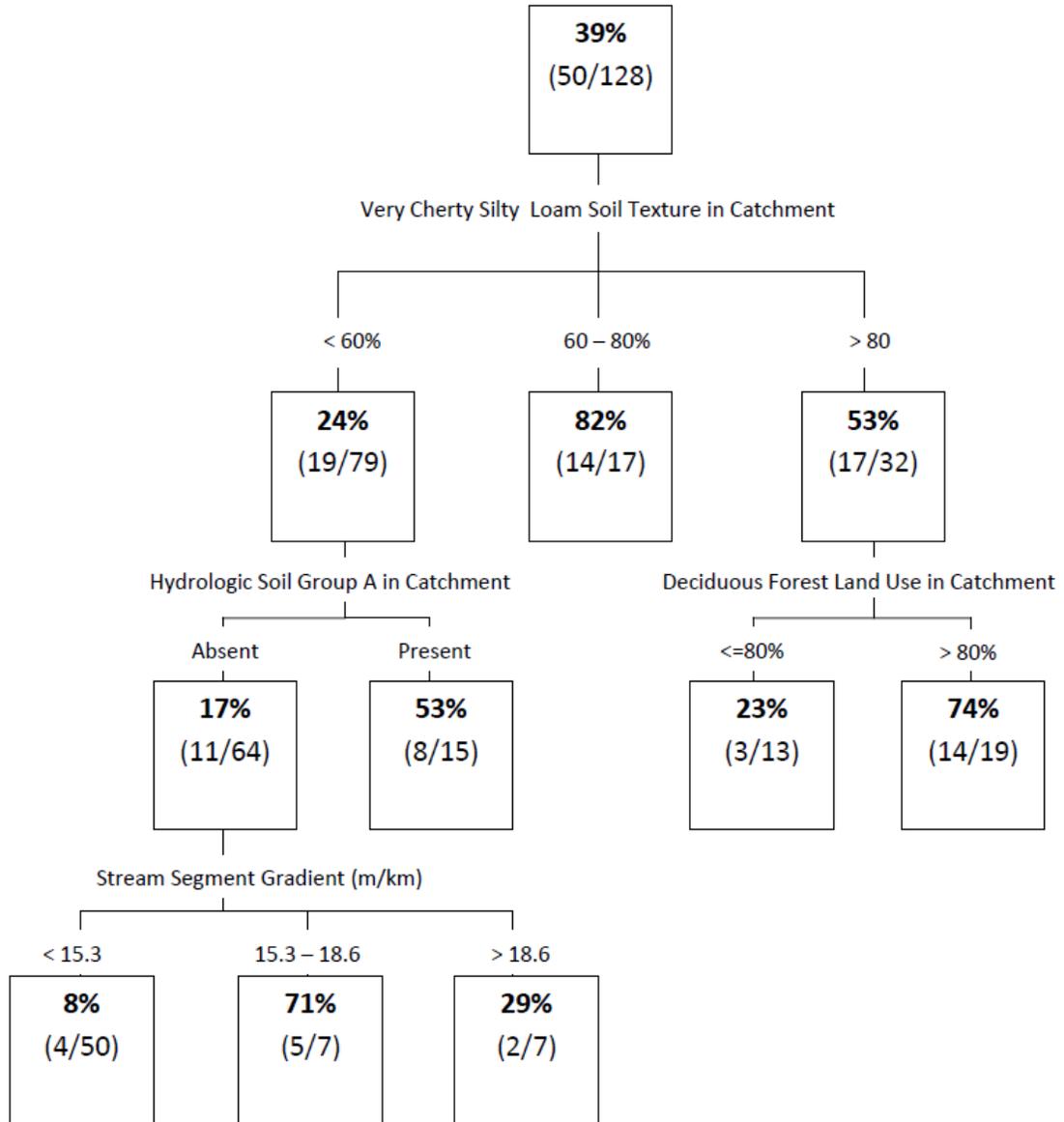


Figure 4. Predicted probability of presence estimates (bold numbers) for *Orconectes peruncus* from classification tree models using natural factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

**PE Probability
of Presence
Natural Variables**

Probability of Presence

- 0 - 25%
- 26 - 50%
- 51 - 75%
- 76 - 100%

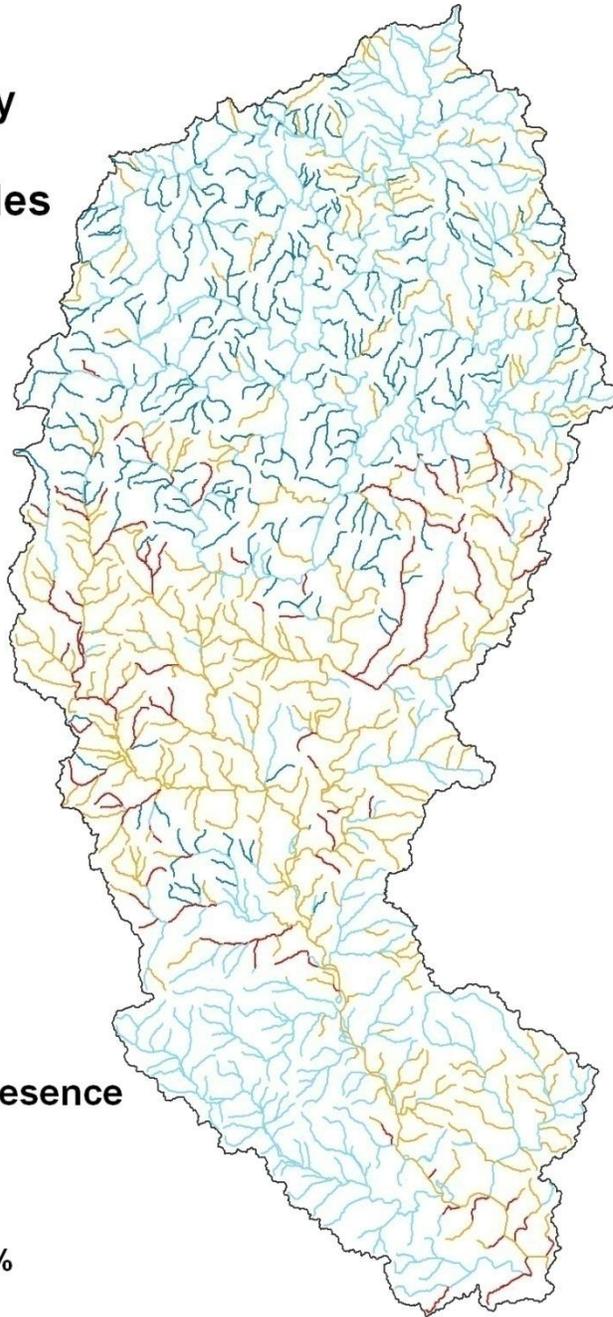


Figure 5. Probability of presence (POP) map for *O. peruncus* in the St. Francis River drainage based on the natural variable only classification tree model. The probability of *O. peruncus* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

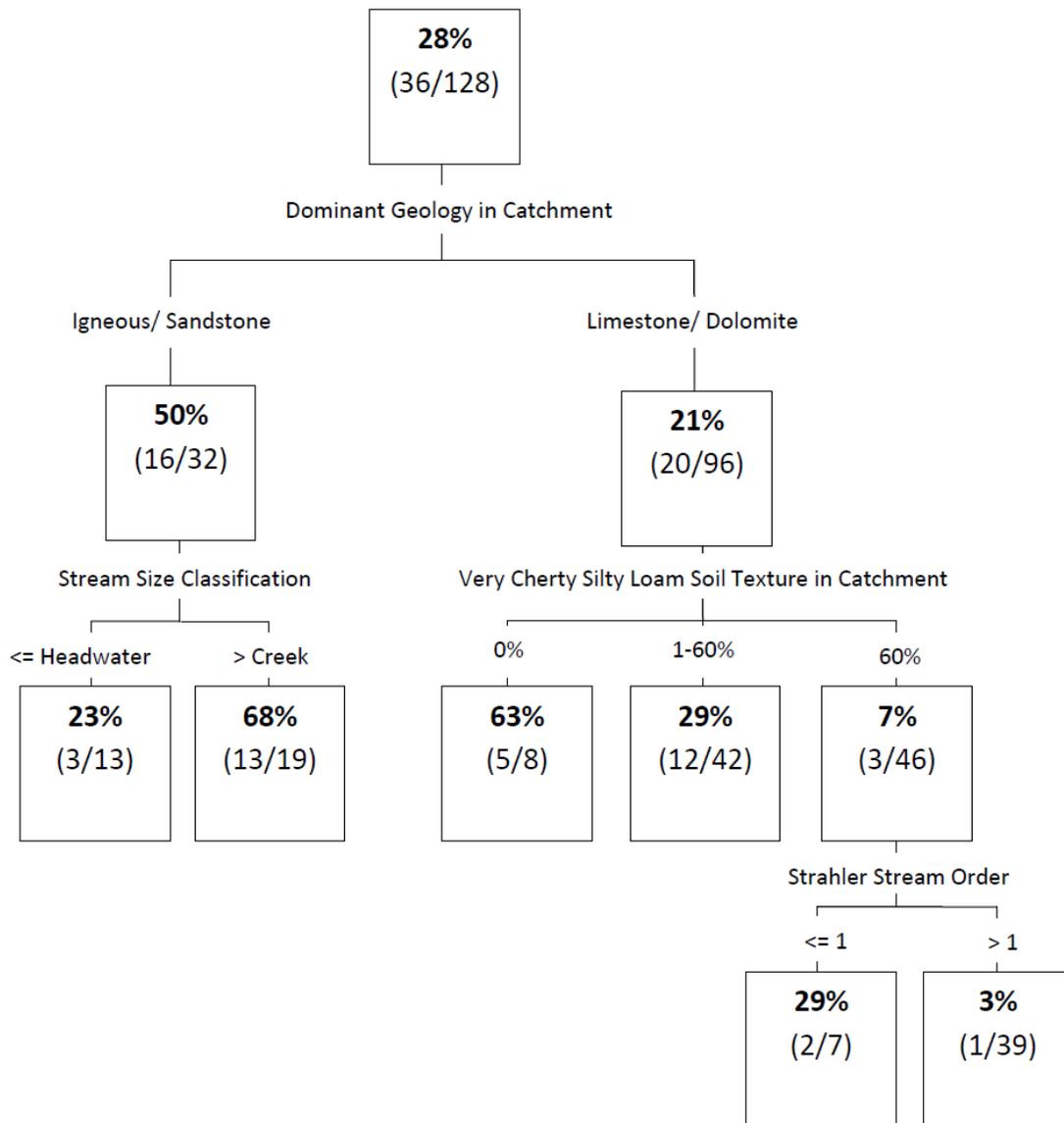


Figure 6. Predicted probability of presence estimates (bold numbers) for *Orconectes quadruncus* from classification tree models using natural factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

**QU Probability
of Presence
Natural Variables**

Probability of Presence

- 0 - 25%
- 26 - 50%
- 51 - 75%
- 76 - 100%

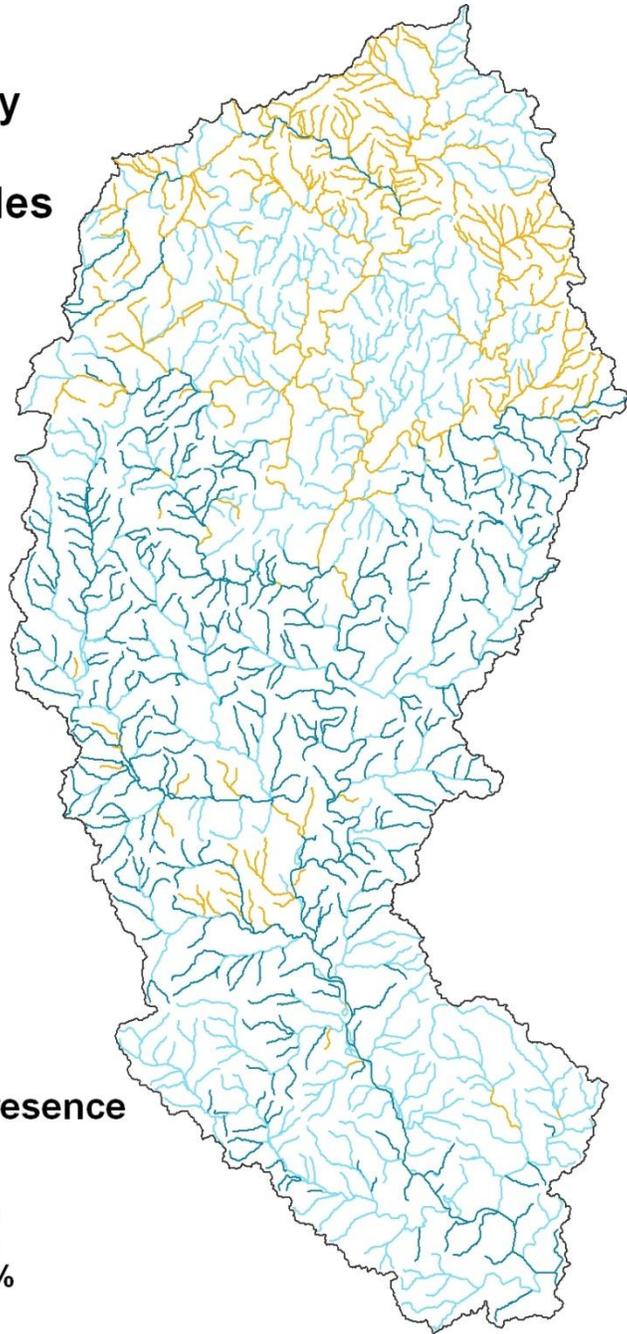


Figure 7. Probability of presence (POP) map for *O. quadruncus* in the St. Francis River drainage based on the natural variable only classification tree model. The probability of *O. quadruncus* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

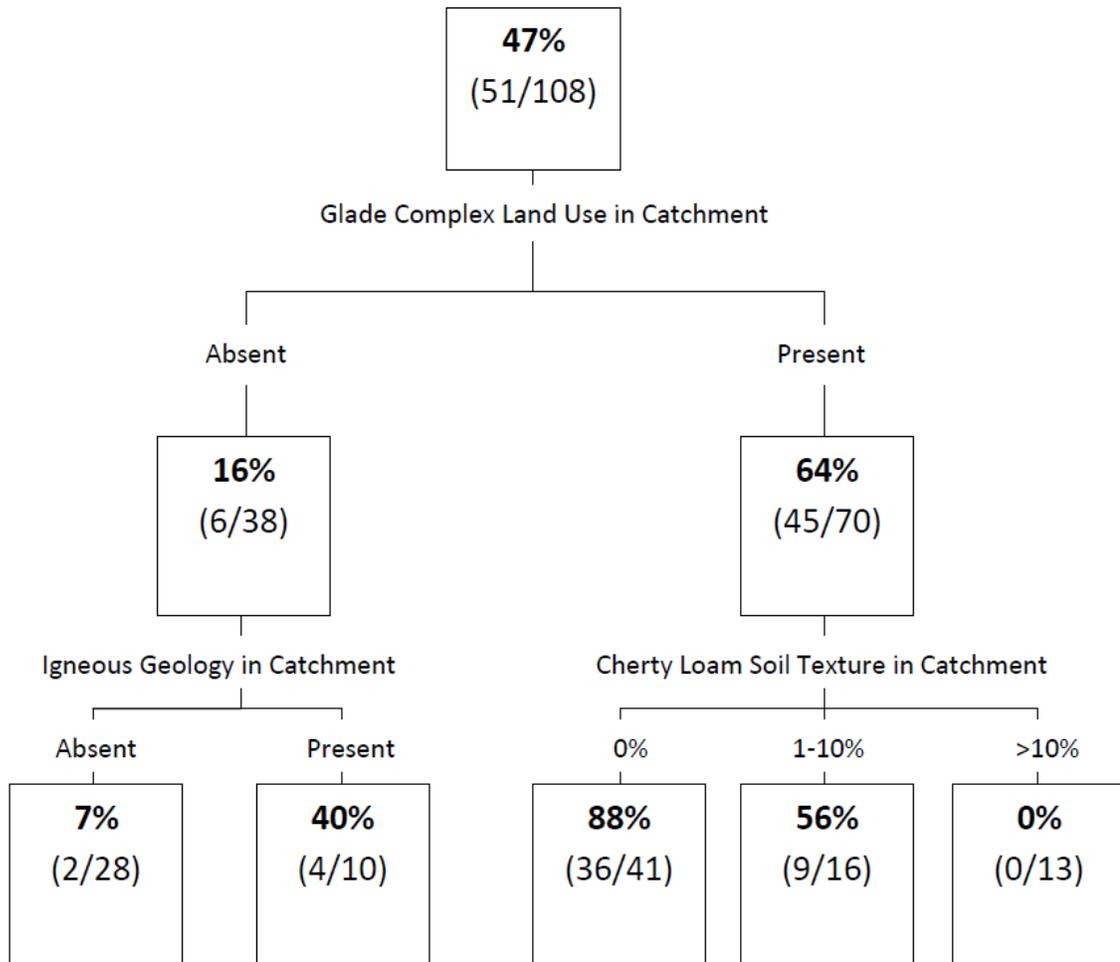


Figure 8. Predicted probability of presence estimates (bold numbers) for *Orconectes hylas* from classification tree models using natural factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

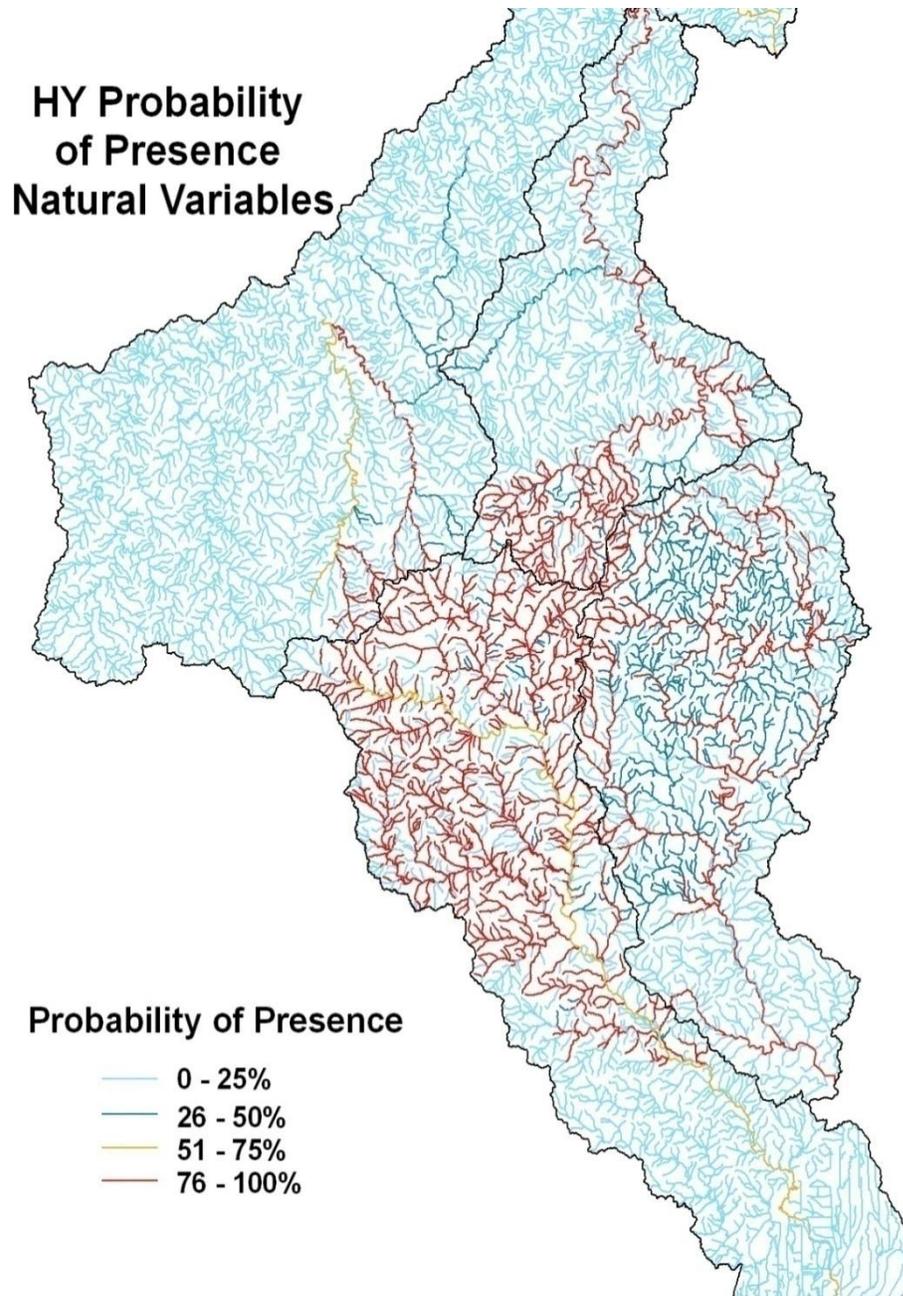


Figure 9. Probability of presence (POP) map for *O. hylas* in the St. Francis River drainage based on the natural variable only classification tree model. The probability of *O. hylas* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

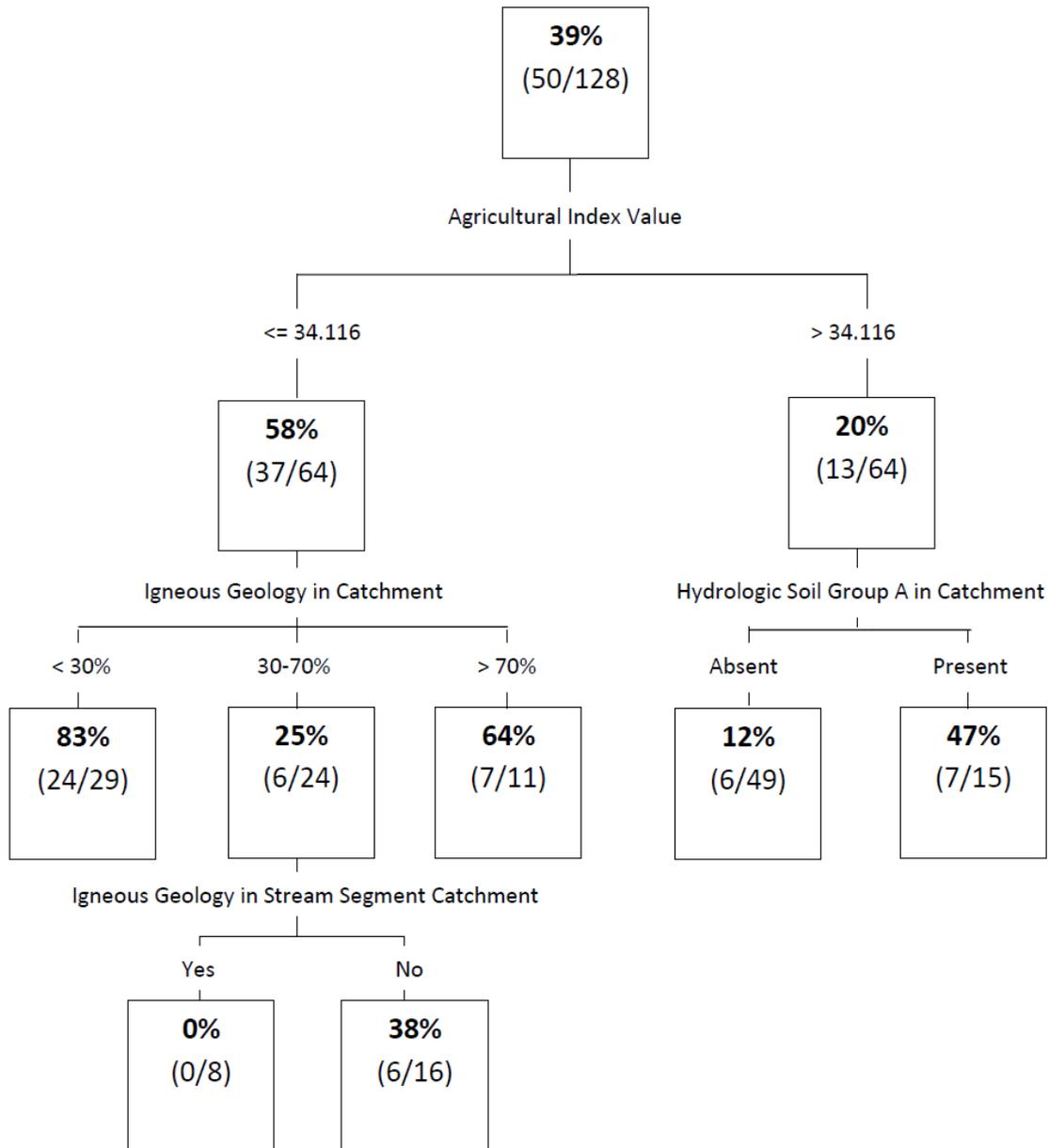


Figure 10. Predicted probability of presence estimates (bold numbers) for *Orconectes peruncus* from classification tree models using natural factors and anthropogenic factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

**PE Probability
of Presence
All Variables**

Probability of Presence

- 0 - 25%
- 26 - 50%
- 51 - 75%
- 76 - 100%

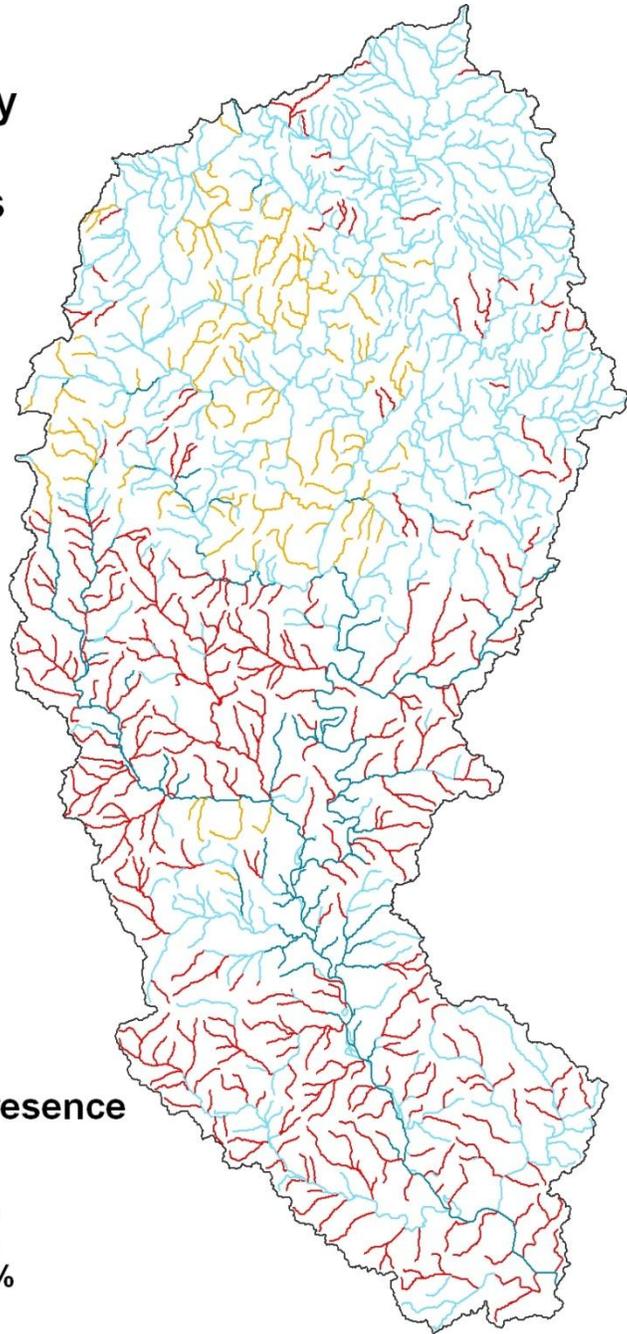


Figure 11. Probability of presence (POP) map for *O. peruncus* in the St. Francis River drainage based on the all variable classification tree model. The probability of *O. peruncus* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

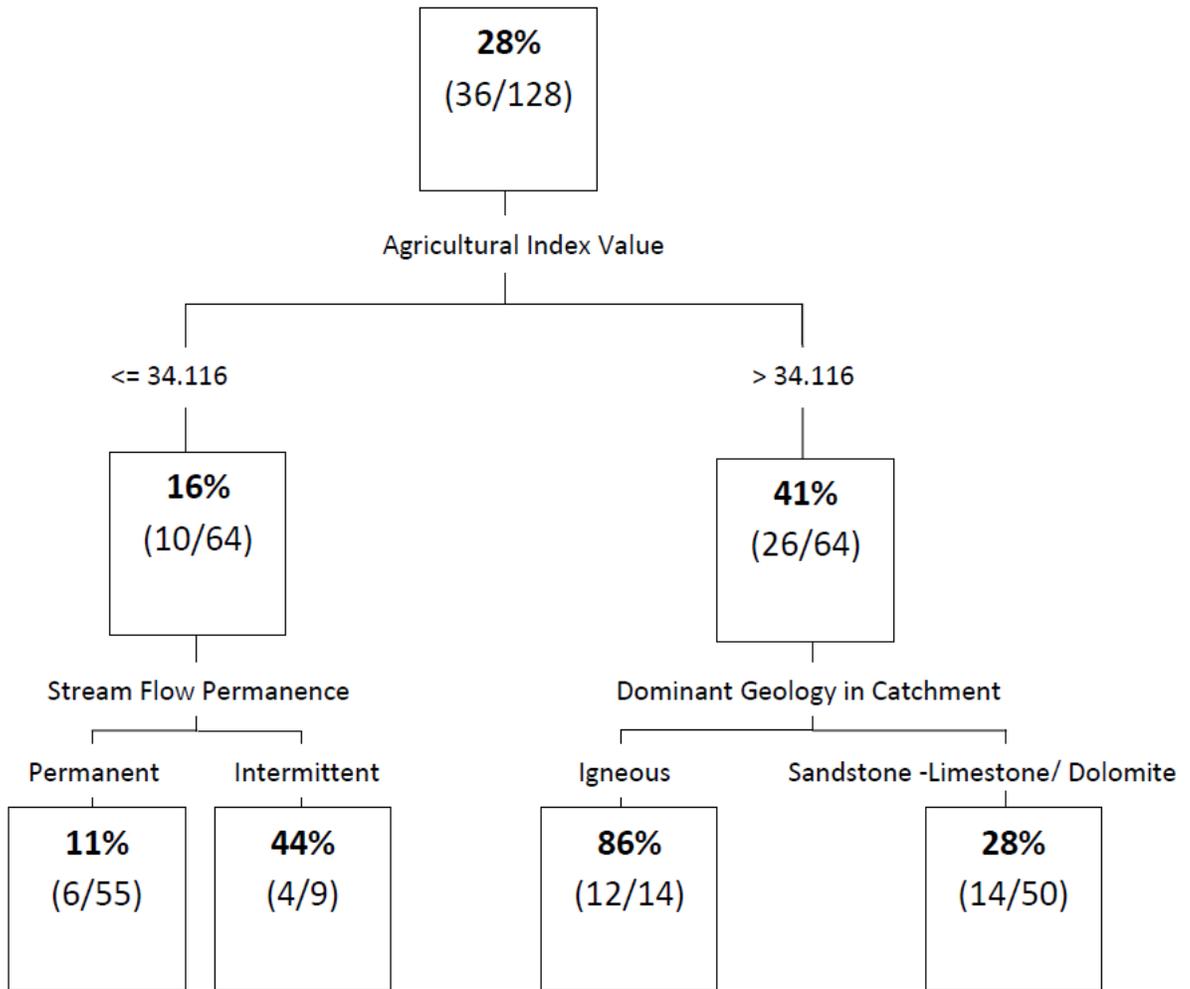


Figure 12. Predicted probability of presence estimates (bold numbers) for *Orconectes quadruncus* from classification tree models using natural factors and anthropogenic factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

**QU Probability
of Presence
All Variables**

Probability of Presence

- 0 - 25%
- 26 - 50%
- 51 - 75%
- 76 - 100%

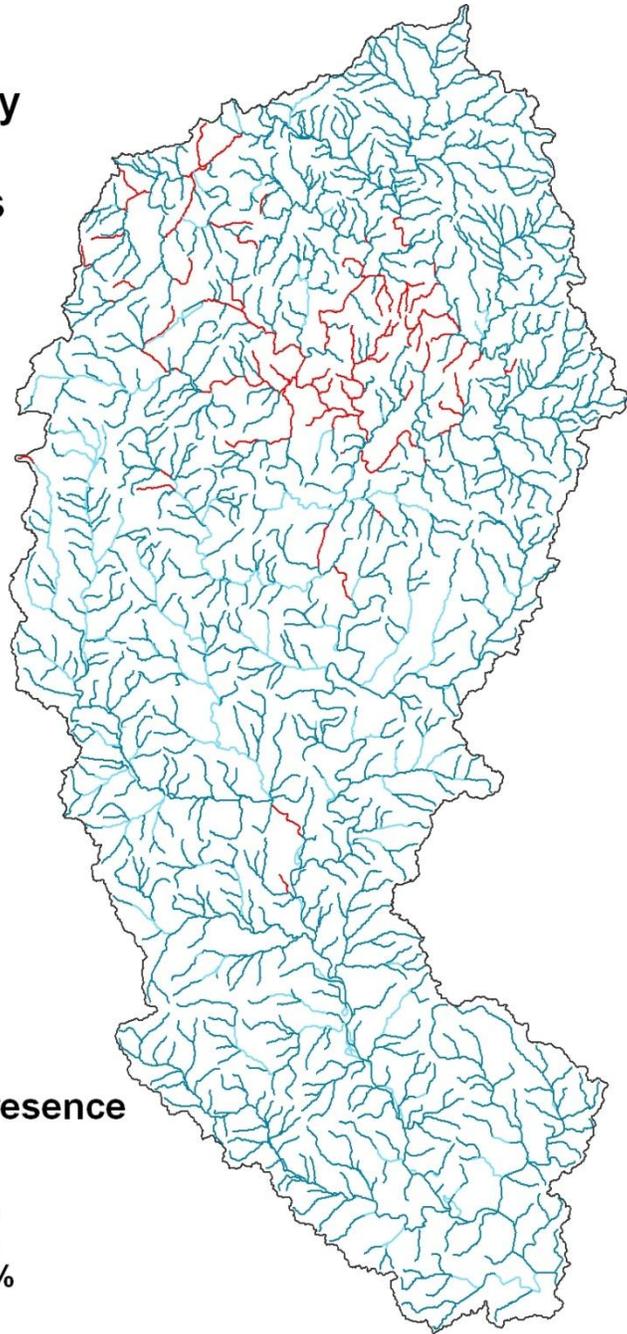


Figure 13. Probability of presence (POP) map for *O. quadruncus* in the St. Francis River drainage based on the all variable classification tree model. The probability of *O. quadruncus* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

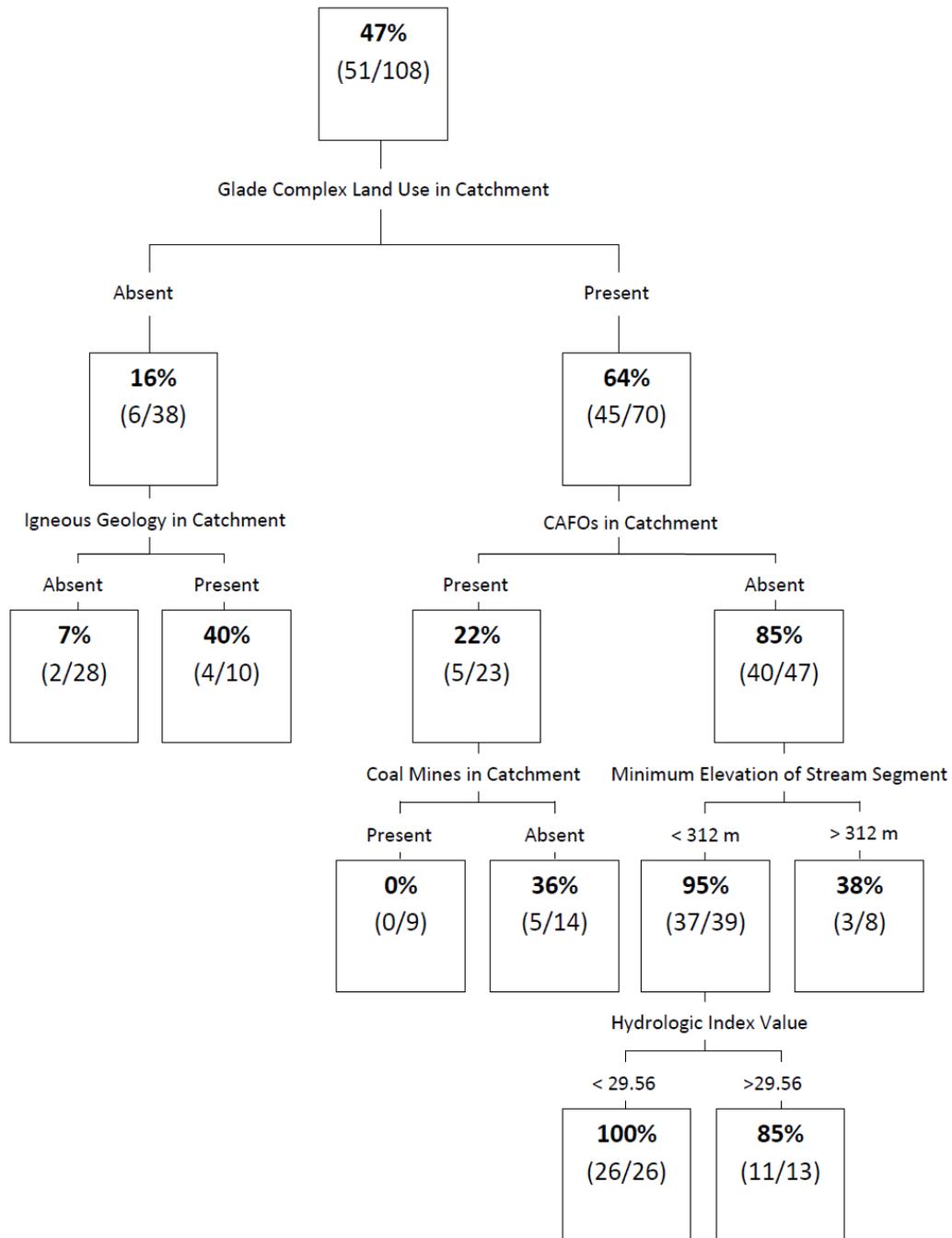


Figure 14. Predicted probability of presence estimates (bold numbers) for *Orconectes hylas* from classification tree models using natural factors and anthropogenic factors. Numbers in parentheses indicate sample size (the number of presence samples over the total number of samples in the node).

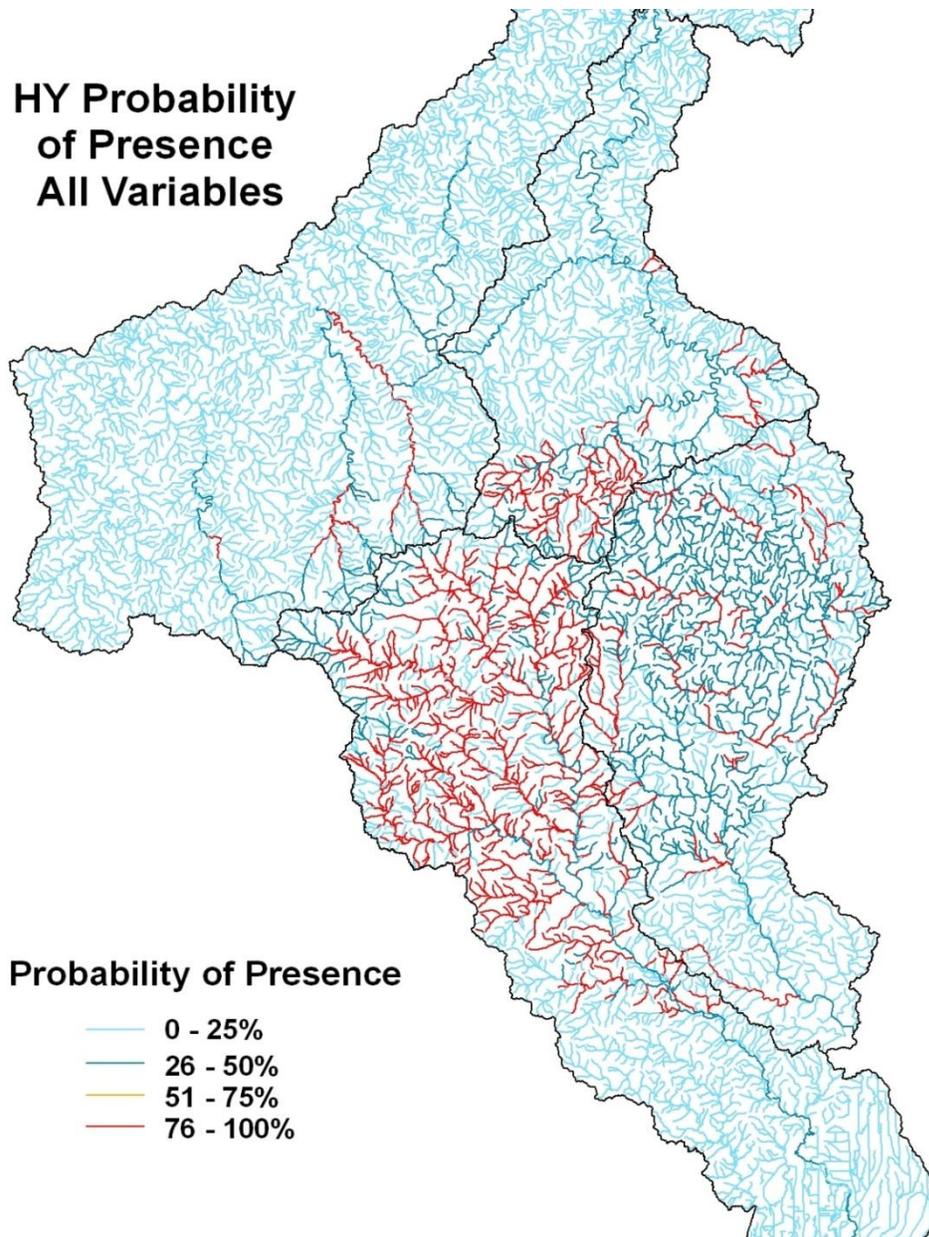


Figure 15. Probability of presence (POP) map for *O. hylas* in the St. Francis River drainage based on the all variable classification tree model. The probability of *O. hylas* occupying a given stream segment (section of stream between tributary junctions) is noted by its color.

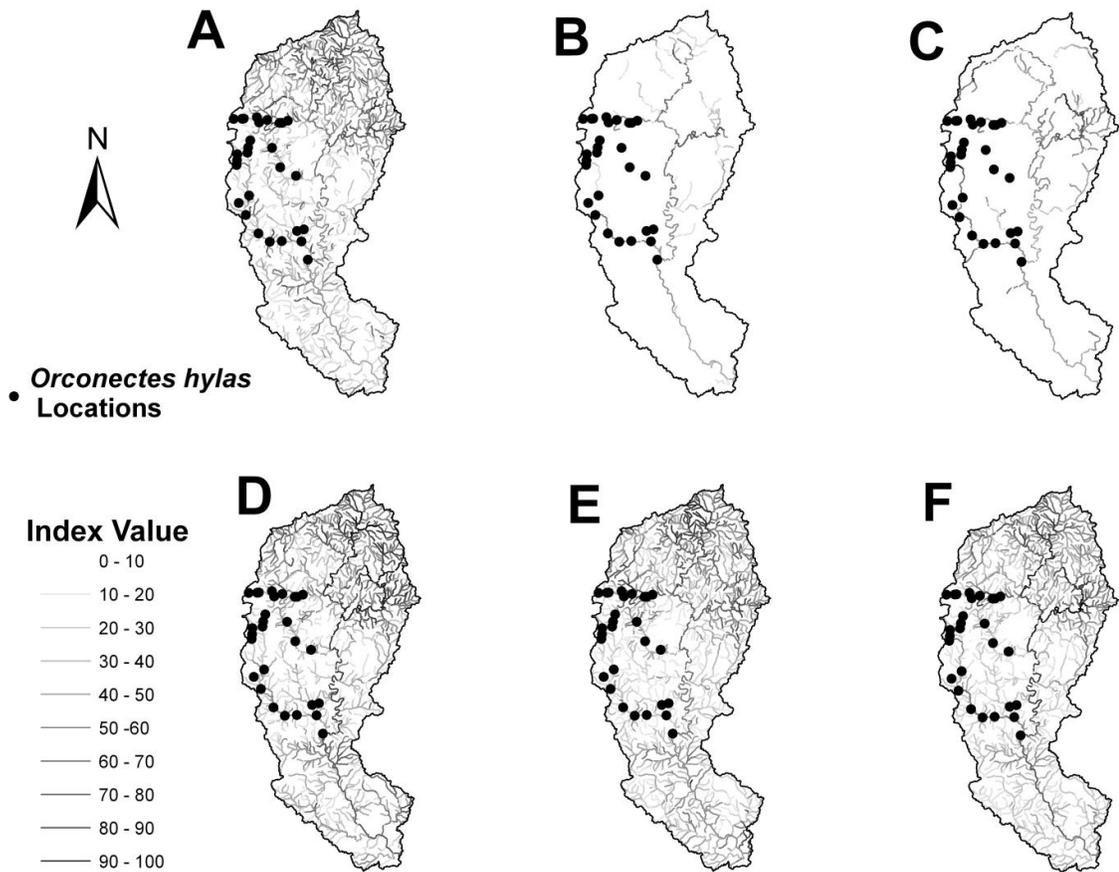


Figure 16. Stream segments in the St. Francis River drainage of Missouri, USA, with the collection locations for the invasive Woodland Crayfish, *Orconectes hylas*, indicated by black circles. Threat index values for the hydrologic index (A), urbanization index (B), agricultural index (C), water quality index (D), mining index (E), and grand index (F) for each stream segment. Darker (higher) values indicate greater predicted stress to aquatic organisms.

CHAPTER 3
FINE-SCALE HABITAT USE AND ASSOCIATION

INTRODUCTION

The term ecology was defined by Begon et al. (2006) as “the scientific study of the distribution and abundance of organisms and the interactions that determine distribution and abundance”. Understanding how a species interacts with characteristics of its physical environment is paramount to fully understanding its ecology (Rosenfeld 2003). Information about a species habitat use can be used to protect it from threats (e.g., designation of critical habitat under the U.S. Endangered Species Act), enhance its populations to increase utility to humans (e.g., Wildlife Habitat Incentive Program), explore theoretical relations (e.g., competitive exclusion principle), or simply to gain knowledge of our natural environment. Proper management of a species often relies on researchers quantitatively describing the species’ habitat requirements (Langton et al. 1996). Quantitative habitat relations exist for few crayfishes despite the prevalence of habitat alteration as a listing criterion for imperiled crayfishes in North America (Taylor et al. 2007).

Organisms can be thought of as occupying an n-dimensional hypervolume where the dimensions represent environmental conditions in which an organism can exist (Hutchinson 1957). This set of conditions represents a fundamental niche, but biological interactions can result in a species only occupying a portion of its niche, which Hutchinson termed the realized niche. When two species compete for similar resources, the more competitive species can force the other to use less desirable conditions, or eliminate it altogether, which is termed competitive exclusion (Hardin 1960). In order for competing species to coexist, they must have sufficient separation of their niche

requirements. Overlap in niche requirements (especially habitat requirements) and the resulting interspecific competition is frequently identified as a potential mechanism of species displacement when invasive species are present (Bovbjerg 1970, Flynn and Hobbs 1984, Pintor and Sih 2009, Hoagstrom et al. 2010).

Orconectes hylas possesses life-history traits that may enable it to be a superior competitor to *Orconectes quadruncus* (DiStefano et al. 2002). Local declines and extirpations of *O. quadruncus* populations have been observed at locations where *O. hylas* has become established (DiStefano and Westhoff, in press). This information, combined with the morphological and genetic similarity between these species, suggests the competitive exclusion of *O. quadruncus* by *O. hylas* may be occurring and is the mechanism of species displacement. One way to implicate competitive exclusion is to determine if both species require similar habitat conditions at a fine spatial scale. Niche overlap may or may not be occurring in other important dimensions (i.e., food resources or physiochemical attributes), but understanding habitat requirements is an important component in understanding this relation. Interspecific competition has not been demonstrated in experimental settings for these species (Rahm et al. 2005, Westhoff et al. in review), but evidence of competition may only be observable *in situ*.

Few studies have used habitat selection information collected from uniquely marked crayfish as size limitations of tags used for telemetry are often prohibitive and recapture rates for other marking methods are often low (Bubb et al. 2002a, Bubb et al. 2002b). Therefore, much of what we know about crayfish habitat associations *in situ* is derived from studies that use gear types that sample populations such as traps, seines, or quadrat samplers (DiStefano et al. 2003, Westhoff et al. 2006, Jones and Bergey 2007).

These approaches provide valid information, but habitat conditions are determined at a spatial scale that can include areas of non-use (e.g., if you catch one crayfish in a 1-m² quadrat sample, you don't know if the crayfish was using the cobble sized substrate within that 1-m² or the sand that may also exist in the sampled area). Habitat selection based on observations of individuals located to within a few centimeters removes much of the spatial variation associated with those techniques.

Space-use overlap is a frequently used approach to investigating how species or individuals use physical space in relation to one another (Kernohan et al. 2001, Millspaugh et al. 2004, Vokoun and Rabeni 2005). One method to compare space use is to compare home ranges, but this does not take into account the level of use within the home range (Kernohan et al. 2001, Millspaugh et al. 2004). The volume of intersection technique allows for assessing space use based on the level of use within the home range or larger area of interest (Millspaugh et al. 2000). This comparison can use static or dynamic analyses, where static comparisons are done without accounting for exact location of an individual or species during a specified time period and dynamic comparisons use simultaneous data to assess animal interaction (Millspaugh et al. 2004). In streams, the volume of intersection analysis has been adapted to consider only two dimensions, resulting in an area of intersection metric (Vokoun and Rabeni 2005). The degree to which overlap occurs can indicate the potential for competition or competitive exclusion.

Life-history studies exist for less than 16% of the crayfish species in the United States and Canada (R.J. DiStefano, personal communication). Although some life-history information exists for the three species of interest in my study (Riggert et al.

1999, DiStefano et al. 2002), it represents limited portions of the overall population. Information about these species that can be obtained from uniquely marked individuals represents an opportunity to complement existing knowledge. This baseline information on species ecology provides a more complete knowledge base on which to base management decisions.

Knowledge of movement patterns and capabilities provide additional context for understanding the ecology of crayfishes (Hazlett et al. 1979). Crayfish are able to make active movements against flows enabling them to disperse both in upstream as well as downstream directions (Robison et al. 2000, Bubb et al. 2004). Differences in movement and dispersal capabilities between native and introduced species may affect the success of a given invasion and subsequent impacts on native crayfishes (Bubb et al. 2006a). Movement patterns and capabilities for crayfishes have been investigated for some introduced species of crayfish (Byron and Wilson 2001, Barbaresi et al. 2004, Bubb et al. 2004, Aquiloni et al. 2005, Bubb et al. 2006b), but nothing was known about movement for the three species of interest.

Objectives

There are five objectives of Chapter 3. The main objective is to assess the fine-scale habitat selection of *O. quadruncus* and *O. hylas* at locations where the species are syntopic (invaded stream reaches) and where *O. quadruncus* is allopatric (un-invaded stream reaches) to provide managers information about the habitat associations of these species and to gain insight about the potential for competitive exclusion of *O. quadruncus* by *O. hylas*. This objective is supported by a secondary objective to determine the

amount of space-use overlap between *O. hylas* and *O. quadruncus* at sites where they are syntopic. The remaining objectives sought to capitalize on the use of individually marked crayfish to enhance the overall knowledge of the ecology of *O. hylas* and *O. quadruncus*. The third objective was to determine if differences existed in crayfish growth between species, seasons, or sex. The fourth objective was to examine movement data from individual crayfish to look for differences in crayfish movement between sex, species, and time of day, determine maximum movement rates, relate movement distance to crayfish size, and note dispersal and migration movements. The final objective was to note life-history characteristics observed throughout the study. Logistical constraints did not allow for similar investigation for the other crayfish species of interest in the St. Francis River drainage, *Orconectes peruncus*.

METHODS

Study Sites

Orconectes hylas and *O. quadruncus* were captured, uniquely tagged with passive integrated transponder (PIT) tags, and subsequently searched for using a portable PIT antenna at two streams in the Upper St. Francis River drainage in Missouri (Figure 17) during several temporal periods. These sites were selected to meet criteria for species presence (i.e., proper species was present and in substantial numbers), habitat conditions (i.e., range of substrates, current velocities, etc.), stream size (i.e., streams larger than 15 m wetted width or with pools deeper than 2 m were excluded), and access (landowner consent required). Streams that did not meet these criteria represented locations where sampling would not be logistically feasible given the sampling design. Orr Hollow Creek

is an eastern-flowing, first order tributary of Marble Creek with average wetted width of 4.7 m and no pools deeper than 1.2 m. The stream's catchment is composed of mostly mixed deciduous forest with some pasture, and the riparian zone is intact through the length of the stream. The stream substrate is dominated by gravel, pebble, and cobble with only minor occurrences of exposed bedrock. Shallow stream depth likely precludes adult smallmouth bass (*Micropterus dolomieu*) and other large predatory fishes (J. Westhoff, personal observation). Snapping turtles, snakes, and herons appear to be among the most prevalent potential predators of adult crayfish in the stream (J. Westhoff, personal observation).

The crayfish community of Orr Hollow Creek comprises five native species: *O. quadruncus*, *Orconectes luteus*, *Orconectes punctimanus*, *Cambarus hubbsi*, and *Cambarus diogenes* (Riggert et al. 1999). A sixth species, *O. hylas*, has invaded the lower section of Orr Hollow Creek at the stream's confluence with Marble Creek and is spreading upstream (DiStefano and Westhoff, in press). The invasion of Orr Hollow Creek by *O. hylas* likely began in the around the year 2000 and as of 2009, encompassed the lower 1,207 m of the stream (DiStefano and Westhoff, in press). Unlike other streams occupied by *O. quadruncus* and invaded by *O. hylas*, *O. quadruncus* has not been locally extirpated and has co-occurred with *O. hylas* for nearly ten years.

Three, 200-m long sampling zones were delineated along the length of Orr Hollow Creek (Figure 18). Site 1 began 380 m upstream of the confluence and was bordered on its upstream edge by Site 2. Both Site 1 and 2 harbored syntopic populations of *O. quadruncus* and *O. hylas*. Site 3 was 600 meters upstream of the upstream border of Site 2 and was upstream of the stream reach known to be occupied by *O. hylas*, but did

harbor *O. quadruncus*. Previous sampling (J. Westhoff, unpublished data) yielded mean estimates of adult crayfish densities (number/m²) at the lower sites for *O. hylas* (0.8), *O. quadruncus* (0.7), *C. hubbsi* (0.6), *O. luteus* (0.6), and *O. punctimanus* (0.05) and at Site 3 for *O. hylas* (0.0), *O. quadruncus* (1.8), *C. hubbsi* (0.5), *O. luteus* (0.8), and *O. punctimanus* (0.05).

Captain's Creek is a second order tributary of the St. Francis River and flows south through mixed deciduous woodlands and active pasture land. A forested riparian zone is present for much of the length of the stream, but is less intact than in Orr Hollow Creek. The substrate is also dominated by pebble and cobble sized rocks, but has a greater influence from exposed bedrock. Captain's Creek is wider (5.9 m average wetted width) and has deeper pools than Orr Hollow Creek (max pool depth 1.6 m). Captain's Creek likely has low numbers of fishes capable of consuming adult crayfish, but other predators similar to Orr Hollow Creek may be present (J. Westhoff, personal observation). Captain's Creek is not known to harbor any introduced crayfishes, but does harbor two native species, *O. quadruncus* (most numerically dominant species) and *O. punctimanus*. There does not appear to be any immediate threat of invasion by *O. hylas* into Captain's Creek based on the locations of current established invasive populations. Crayfish densities in Captain's Creek are not known, but appear to be greater than in Orr Hollow Creek (J. Westhoff, personal observation). One, 200-m reach was delineated in Captain's Creek approximately 2.1 km upstream of the confluence with the St. Francis River and was called Site 4 (Figure 19). This site served as a replicate of the *O. quadruncus* only site used in Captain's Creek.

These four sample sites were given alternate names to enhance clarity for the reader. Sites 1 and 2 on lower Orr Hollow Creek where *O. hylas* and *O. quadruncus* are syntopic were given the abbreviation “SYN”. Although data collection at these two sites was done as though they were independent, they were combined for data analysis purposes and are referred to as “1SYN”. Site 3 on upper Orr Hollow Creek did not harbor *O. hylas* and was given the abbreviation of “2ALLO” to indicate that the species of interest are allopatric at that site. Similarly, Site 4 on Captain’s Creek was given the abbreviation of “3ALLO” to represent the lack of *O. hylas*.

Crayfish Collection and Marking

Crayfish were collected using kick seines, baited wire-mesh minnow traps, and hand collecting. Each collection method introduced various advantages and biases. For example, kick seining does not produce size bias, but does alter the habitat and can be biased based on habitat type (i.e., collecting crayfish from deep pools is often not effective with a seine compared to collecting crayfish from shallow riffles). Trapping selects for larger and more active individuals, but is less affected by habitat type and does not alter habitat conditions (J. Westhoff, personal observation). Hand collecting was rarely used, but does introduce a habitat bias as deep pools and fast flowing water are difficult to sample. Crayfish that received PIT tags were collected from within the site in which they were released or within 100 m of that site. Effort was made to limit the number of crayfish stocked in a site that were not caught there to avoid inflating crayfish density, which may alter behavior (Fero and Moore 2008). Attempts were made to limit

disturbance to the stream bottom from crayfish capture methods to avoid disrupting existing home-ranges, densities, behaviors, and habitat conditions.

No crayfish smaller than 17 mm carapace length (CL; distance from tip of rostrum to anterior end of cephalothorax) or larger than 32 mm CL was tagged. Black et al. (2010) suggested only internally tagging crayfish greater than 22 mm CL with 8.5 mm PIT tags; however, a pilot study demonstrated the ability of internally tagged 17 mm crayfish to survive and grow (J. Westhoff, unpublished data). Additionally, sample sizes would have been reduced if these smaller individuals had not been used. A total of 19.4% of tagged crayfish were less than 22 mm CL. Approximately equal numbers of males and females were tagged.

Prior to tagging, CL was recorded with veneer calipers to the nearest 0.1 mm along with species, sex, and reproductive form (I or II). Crayfish were internally tagged with 8.5 mm PIT tags (Biomark, Inc.) by holding the individual upside down and making a 3-mm incision on the ventral surface of the abdomen using the tip of a 12-gauge hypodermic needle (Black et al. 2010). The incision was located on the left lateral side between the base of pleopod I and II except during the pilot season, in which the incision location was between pleopod I and periopod V (Figure 20). The pilot season incision location was abandoned because it was difficult to tag small male crayfish between pleopod I and periopod V given the limited space available due to the presence of the male gonopod. Once the incision was made, the tag was pushed anteriorly into the cephalothorax with either the tip of a pair of forceps or the tagger's finger until it could no longer be seen protruding from the crayfish. Crayfish were weighed with an electronic scale to the nearest 0.01 g after air drying on a paper towel for approximately

two minutes. Each crayfish was placed in its own 0.24 l plastic holding chamber filled with water and checked one hour after tagging to determine if it was alive and responsive. Crayfish were not returned to the exact location from which they came, but the location of their initial capture and their release location was recorded. They were not returned to the exact location because some crayfish were brought in from outside of study reach boundaries.

Scanning for Tags and Collection of Environmental Data

Each site was scanned for tagged crayfish during three seasons (summer, autumn, and spring) and for two, 72-hr periods (diel sampling) to assess the habitat use of crayfishes both seasonally and over a diel period (Table 4). Winter sampling was not done because it was not logistically feasible. Date ranges for the sampling seasons corresponded with major life-history events for both *O. hylas* and *O. quadruncus* as summer is the time when growth is maximized, autumn is when the crayfish mate, and spring is when females are ovigerous and have hatchlings (Riggert et al. 1999, DiStefano et al. 2002). Each site was scanned 13 times during the summer 2009 season, except the Captain's Creek site which lost almost all surface water due to drying. All four sites were scanned 10 times each during the autumn 2009 and spring 2010 seasons. Only the upper 200 m of 1SYN (Site 2) and 2ALLO were used during the diel sampling because increased travel time from visiting other sites would have reduced the temporal resolution of the data. One site was scanned (approximately 3 hr) and then the other site was scanned immediately. This was repeated continuously for 72 hr by using headlamps

during the nighttime samples. Each scanning event during the diel period was classified as day or night depending on its relation to sunset and sunrise.

Crayfish were tagged and released prior to each season (except the summer season) to supplement the sample population (Table 4). Once crayfish were released they were given a minimum of 16 d to acclimate prior to the initiation of scanning. At the start of each season, a random scanning order was determined for the four sites and kept throughout the season. No more than three sites could be scanned in one day, so each day a different site than the previous day would be scanned in the morning. This was intended to address the influence of diel activity trends on crayfish location. Not every site was scanned on successive days, no site was scanned more than four times over seven days, and scanning for the seasonal sampling occurred during daylight hours.

Generally, a crew of two people scanned and recorded habitat conditions. One person scanned the wetted stream channel in an upstream direction using a portable PIT antenna (FS2001F-ISO base unit, Biomark, Inc.). Scanning was done slowly passing the antenna as close to the substrate surface as possible and covering all wetted areas in the sample reach. When a tag was encountered the unique tag code was recorded and a numbered marker was placed at the location within the stream. Markers were left in the stream between scanning events. It was noted if a tag code was identified at the location of an existing marker, indicating the tag was encountered in the same location in the previous scanning event. If a tag was encountered within 1 m of an existing marker the marker was moved to the new location and the distance was estimated from the previous marker location. Time spent scanning a 200 m site varied, but generally took from 2 to 4 h. Antenna power was monitored during each scanning event and scanning was stopped

if power fell below 3.2 amps, but normally was at 3.8 or 4.0 amps. Antenna power levels below 3.2 amps lowered the antenna read range below acceptable levels. Read range of the portable antenna varies based on tag orientation and other factors (O'Donnell et al. 2010), but was typically 12 cm.

Once a marker was placed on the stream bottom, the second person collected a series of environmental data from the “used” location. Water depth and stream bottom current velocity were measured at the exact location of the marker. The type of macrohabitat (riffle, run, pool, backwater) was recorded. Surface substrate composition was determined using a cross shaped 25 x 25 cm grid placed directly over the marker (Litvan et al. 2010). Substrate particle size based on a modified Wentworth scale (Bovee and Milhous 1978) was recorded at the terminal ends of the grid (12.5 cm upstream of the marker, 12.5 cm right and left laterally of the marker, 12.5 cm downstream of the marker) and directly at the marker. Particle-size classes included silt and sand (< 2 mm), gravel (2-16 mm), pebble (17-64 mm), cobble (65-256 mm), boulder (> 256 mm) and bedrock or clay hardpan (0 mm; effectively no interstitial space). The presence of organic detritus, woody debris, vegetation, or fresh leaves within the substrate grid was recorded. The longitudinal distance along the site where the marker was located was estimated from flagging placed every 10 m along the length of the site. Markers were left in the stream at the location of tag detections and on the next visit each was left in place if a tag was still present at that location. The marker was removed if no tag was detected at the marker, unless a tag was encountered within two meters of the previous marker, in which case the marker was moved and the distance between the locations was estimated to the nearest 10 cm. This was done collect fine resolution data on movement of individuals.

Once all data for a marker were collected from the used location, a random number generator was used to determine the location of two “available” habitat points (Figure 21). One available habitat point was taken with a 360 degree radius centered on the marker at a distance between 0.5 and 3 m from the marker and was referred to as the proximate-level available habitat point. This location represented a potential location within the median movement range of a crayfish in 36 hr as determined during pilot sampling. A second available location, reach-level available habitat, was selected between 3 and 20 m upstream or downstream from the marker. This distance range represented the 95% percentile of distance moved in 36 hr by crayfish during the pilot study. Movement distances in 36 hr were used to define available habitat based on the estimated time between successive scanning events at a site. Direction, longitudinal distance along the stream, and distance from the right descending bank were randomly selected for each available habitat location. Environmental data at the proximate-level and reach-level available habitat points were collected in the same way as the used locations. Upon completion of scanning and environmental data collection, stream discharge was determined at an established location for the site.

During the last scanning event at each site during each season (excluding diel sampling) an attempt was made to physically recapture marked crayfish after the environmental data had been collected for the encounter. The substrate was carefully removed while looking for the marked individual. Live individuals were captured by hand or by using a seine if in swift or deep water. If no crayfish was found, the substrate was excavated using sturdy plastic cups until the tag was recovered. Marked crayfish that were recaptured were weighed and measured (CL) using the same technique as

outlined above and notes were made on the physical condition of the crayfish (e.g., missing appendages) and on the life-history events (e.g., ovigerous, reproductive form, presence of glair). Crayfish were then returned to the same location where they were most recently encountered. Tags that were recovered were cleaned using isopropyl alcohol and re-used in later tagging events. The process of physically recapturing marked individuals was only done at the end of the season to avoid biasing crayfish movement and disturbing habitat during the season.

Data Validation and Corrections

All data were entered in MS Excel twice and double-pass verified to eliminate data entry errors. Encountering a tag in the stream did not ensure the individual corresponding to the tag was alive. To avoid using data from tags that had been expelled or from dead crayfish, a data validity rule-set was developed that identified each encounter as valid or invalid (Appendix B). Valid encounters were from crayfish that were confirmed to be alive after the encounter or had met movement criteria outlined in the data validity rule set. Invalid encounters included tags that were recovered without crayfish or from tags that did not meet the movement criteria. Uncertainty about the validity of some encounters remained, but was reduced substantially given the information (see Appendix B for further explanation).

Longitudinal distance data were corrected for each encounter to reduce variation incorporated from observer error. Distances along the reach were marked every 10 m, which resulted in the estimation of the exact distance for encounters that occurred between distance markers. If a tag was encountered only one time, the recorded distance

was not corrected. If the tag was encountered consecutive times, then the numbered marker placed in the stream associated with that code could be tracked through time. Additionally, it was noted if the numbered marker had unmatched distance records given the tag was encountered at the same location. For instance, if a tag was encountered five times with the associated distance records of 145 m, 145 m, 146 m, 145 m, and 146 m, the values were averaged rounded to the nearest integer (145 m in this example). If only two locations were recorded and they differed by a value of one, the lower number was used. Corrected distance data were used in place of the recorded distance data for all analyses and to determine valid encounter locations.

Fine-Scale Habitat Selection Analysis

Collection and analysis of habitat data for organisms can be accomplished many ways, each of which has advantages and disadvantages (Johnson 1980, Jones 2001, Rosenfeld 2003). Data can represent habitat use whereby an organism is found in a habitat, regardless of how much of that habitat is available. Habitat selection occurs when an organism occupies a habitat either more (positive selection) or less (negative selection) than the habitat is proportionally available. Habitat preference is sometimes confused with selection, but is best determined using experiments where outside factors like predation risk and competition are controlled and habitat types are presented in equal amounts for organisms to occupy (Johnson 1980, Rosenfeld 2003). Therefore, habitat selection represents habitat preference when outside factors are not accounted for (similar to a realized niche), whereas habitat preference may better represent a fundamental niche (Rosenfeld 2003). Habitat use and selection are commonly estimated from field studies

where the location or density of individuals is observed and associated with habitat features at the same location. Association does not explain causation so results of associative studies must be carefully interpreted. It is possible that although an organism is highly associated with a certain habitat, use of that habitat can result in lower fitness than a habitat that was not used or selected because a biotic interaction forced the organism to select the less profitable habitat (Rosenfeld 2003). Perhaps the most informative habitat studies examine the fitness consequences of habitat use by organisms and determine what habitat conditions are required by an individual to grow and reproduce. Although this is the most desirable level of investigation, it is difficult to achieve in the field or reproduce in a laboratory at a meaningful scale.

Habitat use and selection were examined for *O. hylas* and *O. quadruncus* at each sampled site and during each season. Bar graphs were created for each categorical factor (described below) to examine used and available resources by plotting the percent of the total observations of each level of a factor for each site, season, and species combination. Means and 95% confidence intervals were calculated to display the observed patterns in continuous variables. These figures show not only what habitat conditions were selected, but also those conditions that were used substantially but perhaps not selected more than they were available and thus, are not identified in modeling procedures.

Discrete choice models (Cooper and Millspaugh 1999) were created to examine habitat selection of *O. hylas* and *O. quadruncus*. Discrete choice models use maximum likelihood to estimate parameter values for variables in the model using a multinomial logit form. If only two choices are available and individuals make similar choices, then the multinomial logit form reduces to a logistic form. In essence, discrete choice analysis

is a logistic regression modeling approach that incorporates individual variation into the model. Thus, discrete choice models determine the probability of resource selection as a function of resource attributes given data on used and available locations of individuals (Cooper and Millspaugh 1999). This approach assumes that individuals experience utility (e.g., increased growth or fitness, decreased probability of predation) from certain habitat conditions and choose conditions that maximize utility over less desirable conditions (Cooper and Millspaugh 1999).

Habitat selection of organisms can be modeled using many approaches, most of which are valid given proper application and consideration of biological criteria (Manley et al. 2010). Discrete choice analysis is an approach that has several benefits. For instance, it is able to account for among-individual variation by including an error term for each individual (Cooper and Millspaugh 1999). When used locations are treated as independent observations some individuals may account for many observations and others account for few observations (e.g., snorkel surveys when individuals are not uniquely identified and the same reach is sampled repeatedly), differences in selection among individuals may bias results.

Habitat selection studies using standard logistic regression methods sometimes use data on available habitat conditions collected only once during the sampling timeframe and may be compared to used data that were collected over a period of weeks to years (Davis and Cook 2011). This approach is appropriate given habitat conditions do not change over the course of the study; however, in small streams habitat conditions can change. For instance, an individual may occur at a depth of five cm during baseflow conditions, but after a rain event the depth at the same location may be 15 cm. Depth at

available locations measured at the same time as the used location would reflect the 10 cm stream rise for both the used and available locations. Had available data been collected only at baseflow conditions, the depth at the used location recorded after the rain event would not be directly comparable to the depth at the available locations. The use of temperature by organisms as a resource may also be explored effectively with discrete choice models as it is known to fluctuate temporally in many systems (Bajer et al. 2007).

Finally, discrete choice models are able to match used locations with available locations based on biological knowledge of animal movement capabilities (Bodinof 2010). This is possible using other methods as well (Chisholm et al. 1987), but is not always done. For instance, some used-available studies define an arbitrary reach length at a study site and assume each individual has an equal opportunity to use the entire area within that reach despite possible movement limitations (Davis and Cook 2011). It could be argued that a location at the top of a 200 m stream reach is not truly available to a crayfish located at the bottom of that 200 m reach. Definition of available habitat based on individual location addresses this problem. The present study is the first known to use discrete choice analysis to assess habitat selection in crayfishes.

Data on environmental variables used as predictors in the model are listed in Table 5 and were plotted to look for non-linear relations and to assist in grouping of certain categorical data. Values of zero for continuous variables (i.e., depth and velocity) can be problematic in analysis so 0.01 was added to all values of these two variables. Exploratory analysis of velocity (V) values indicated a negative exponential relation and all values were converted using the negative exponential transformation. The relation

with depth also appeared to have a weak negative exponential relation, but it was not strong enough to warrant a conversion. There were few records of backwater macrohabitat so those records were combined with pools to create three macrohabitat levels (riffles, runs, and pools). Although several different types of cover (plant material capable of concealing a crayfish) were encountered (i.e., detritus, woody debris, vegetation, fresh leaves, and combinations thereof) they were rare and subsequently combined into one category of either present or absent.

Data for substrate particle size were condensed into four variable types. The substrate metric (S) is the geometric mean of substrate particle sizes determined using all five data points from the substrate grid. The mid-point diameter of each category of substrate size was used to calculate the geometric mean (See Appendix C). This method of converting categorical data into a continuous metric was not as desirable as using actual measurements of substrate diameter from the field, but does allow for examination of the overall substrate condition at each location using only one metric (i.e., one degree of freedom in the model) and does not require the increased field time needed to physically measure substrate particles. This metric appeared to have a quadratic relation based on used locations only, but the quadratic form did not fit well during modeling and S was only considered as a linear predictor.

For the second substrate variable, the eight substrate categories were combined into four categories based on occurrence of each in the data set and biological criteria (how the size of interstitial spaces created by a given substrate type related to the hypothesized size needed to conceal an adult crayfish). This was only done for the particle centered over the suspected crayfish location (cover rock) which was located at

the middle of the substrate grid. Silt, sand, and gravel were combined into the S_{FINE} category and represent substrate classes that create interstitial spaces smaller than the body size of adult crayfish. Pebble, which was commonly encountered, creates interstitial spaces approximately the size of adult crayfish and was retained as its own category (S_{PEB}). Cobble and boulder size classes were combined because boulder was not common and when it was, it was not much larger than the upper range of cobble. This size class (S_{COARSE}) represents substrates that create interstitial spaces larger than adult body size. The final category (S_{HARD}) comprised records of bedrock and clay hardpan, neither of which was common and have few interstices for adult crayfish to use. Because categorical data are modeled differently than continuous data, each of the four substrate categories were dummy coded (i.e., each had a value of 1 if that substrate type was present or 0 if absent) and the category S_{FINE} was withheld from the model and served as the baseline to which the other substrate category parameters must be interpreted.

The third substrate variable (U_{LARGE}) represented the upstream rock (grid point 1) and was coded as “1” if the record was for cobble or boulder and “0” if it was any other substrate type. This variable was considered in relation to water velocity as a factor that affected hydraulic conditions at the location where the crayfish was located. The final substrate metric (N_{SUB}) represented the number of unique substrate types recorded using the grid and represented substrate heterogeneity.

An *a priori* set of 20 candidate models was created representing various hypotheses about the relation of habitat variables to crayfish selection (Table 6). The models included individual metrics (models 1, 2, 4, 5, 6, and 8) along with additive

effects of multiple variables (models 7, 11, 12, 16, 17, 19, and 20) and select interactions (models 3, 9, 10, 14, 15, and 18). When categorical variables were used in a model, all levels of the categorical variable must be included, minus the dummy category (e.g., to examine macrohabitat, riffle was the dummy variable and was left out, but pool (present or absent) and run (present or absent) must both be in the model). The most complex model (model 20; the most parameterized) included each predictor variable, but did not include interactions. All models included a parameter (I) to account for variation among unique individuals. Discrete choice modeling was done in SAS (SAS Institute, Cary, NC) using the MDC procedure. Model selection was done for each unique dataset representing a combination of species (2), site (3), diel condition (2) and season (3) for a total of 17 models at each the reach and proximate level of availability. Individual models for each season were not run at the proximate level as selecting and parameterizing an additional 17 models would not likely yield much information beyond that provided from the other models and would have added complexity to interpretation. The data were also consolidated by combining all seasons into one dataset per site for each species that encompassed all seasonal sampling, excluding diel sampling. Diel samples were consolidated into one dataset (combined day and night observations) for each site where the diel sampling occurred. Data consolidation resulted in seven models at each selection level for a total of 14 models. This consolidation was done to simplify model interpretation given substantial differences were not observed in habitat selection among seasons.

Discrete choice analysis was used to fit each of the 20 candidate models for the given datasets, and then Akaike's information criteria corrected for small sample size

(AIC_c) was used to rank the candidate models and select those with the most support based on model weight, where (*w*) is the probability of that model being the best approximating model (Burnham and Anderson 2003). The highest ranking models from each dataset were selected by including any model whose weight was within 1/8 of the weight of the most supported model (Burnham and Anderson 2002). If more than one model was supported, the coefficient estimates from that model were averaged across models using

$$\hat{\beta} = \sum_{i=1}^R w_i \tilde{\beta}_i$$

where $\hat{\beta}$ is the model averaged estimate of the coefficient, w_i is the Akaike weight from the AIC_c values from the R supported models containing the given parameter, and $\tilde{\beta}_i$ is the estimated coefficient from model *i* (Burnham and Anderson 2002). Unconditional standard errors (Burnham and Anderson 2002) were calculated for model-averaged coefficients using

$$SE(\hat{\beta}) = \sqrt{\widehat{var}(\hat{\beta})},$$

where

$$\widehat{var}(\hat{\beta}) = \left[\sum_{i=1}^R w_i \sqrt{\widehat{var}(\tilde{\beta}_i) + (\tilde{\beta}_i - \hat{\beta})^2} \right]^2.$$

Model selection results indicate which environmental variables were the best predictors of habitat selection and parameter estimates show the direction and magnitude of the habitat selection for those variables. Parameter estimates are interpreted as odds ratios, which are calculated by raising the natural log (*e*) to the power of the parameter

estimate (Keating and Cherry 2004). Odds ratios are the probability of the individual selecting one level of the resource over another level (e.g., 10% more likely to select coarse than fine substrate).

Model Validation

Model selection in the AIC framework selects the most supported models from a candidate set but does not assess the predictive ability of the selected models (Burnham and Anderson, 2002). I used k-fold cross validation to assess the accuracy of each of the 14 models from the consolidated datasets at each spatial scale (Boyce et al. 2002). Eighty percent of the data in each dataset were randomly selected (while maintaining the 1:1 ratio of used to available locations) as training data for model fitting and the remaining 20% were used as test data. Five replicate datasets were created for each of the 14 models (70 test sets) and refitted using the top ranked models. Parameter estimates were averaged for datasets with multiple supported models (as above) and used to estimate the utility of individual observations in their companion test datasets. Relative probability of use was calculated for each used and available point using

$$P_j(A) = \left(\frac{\exp(U_{Aj})}{\sum_{A-i} \exp(U_{ij})} \right),$$

where A is one of the total I resources available and j is an individual (Cooper and Millsbaugh 1999). Probability of use for a given set of recorded conditions was compared between the used and available locations in each test dataset and the number of occasions when the used conditions had a higher probability of use than the available

conditions represented the percentage of correctly classified observations. Model validation was not done for the individual seasonal models.

Space Use Analysis

Joint space use was assessed for crayfish tracked using PIT telemetry using univariate utilization distributions (Millspaugh et al. 2000, Vokoun and Rabeni 2005). Each crayfish encounter was associated with a distance (m) along the sample reach and all valid encounters for a given species at a site during a selected season were analyzed as a group. This differs from other static joint space use approaches where telemetry locations were compared between two individual animals (Vokoun and Rabeni 2005). A false encounter location was added to each dataset at both 0 m and 200 m to ensure that each dataset was scaled the same and could be compared. Fixed univariate kernel density estimation was used to calculate a pseudo linear home range for the species. This is not a true home range estimate because it combines individuals across the site and sampling season and creates one estimate for the entire sampled population. I used the KDE procedure in SAS (SAS Institute, Cary, NC) and bandwidth selection using the Sheather-Jones plug-in method (Jones et al. 1996). The number of grid points was set at 201 (corresponding to the number of meters in each reach) and the bandwidth multiplier was left at the default value of one. Because the univariate kernel integrates to 1, the resulting output can be interpreted as a utilization distribution which indicates use intensity levels at corresponding linear stream locations. The utilization distributions for *O. hylas* and *O. quadruncus* can then be compared at a given site during a given season by the area of intersection (AI) index (Vokoun and Rabeni 2005) which was calculated by

$$\int_{-\infty}^{\infty} \min[\hat{f}_1(x), \hat{f}_2(x)] dx,$$

where \hat{f}_1 = the utilization distribution for species 1, \hat{f}_2 = the utilization distribution for species 2, and d = the density of the distribution. In simple terms, this equation sums the area under the utilization distribution curves for each species. AI values range from 0 to 1, where a zero value indicates no space-use overlap and a value of 1 indicates complete space-use overlap. Utilization distributions were compared within sites between species and within a species between day and night. Utilization distributions were also graphed within a site by season, but AI values were not calculated because the temporal difference between seasons may cause misleading results. Qualitative comparisons of the location of macrohabitat type and the utilization distributions were made as visual indicators of habitat use.

Crayfish Growth Analysis

Differences in growth between species, sites, and seasons were assessed for crayfish that were physically captured after the initial tagging. Instantaneous growth rate (IGR) was calculated for each individual using

$$IGR = \frac{\log_e wt_2 - \log_e wt_1}{t_2 - t_1},$$

where wt_2 = weight (g) at time of recapture, wt_1 = weight at time of initial capture, t_2 = day of recapture, and t_1 = day of initial capture (Guy and Brown 2007). The season of growth was determined by assigning the value of summer, autumn, winter, or spring, to each record based on the time between initial marking and recapture events. If more than one season occurred between capture events, the season with the most days of growth

was used which resulted in some overlap between date ranges used to determine seasons. Dates of crayfish growth for each season were summer, June 2, 2009 – September 30, 2009; autumn, September 30, 2009 – November 11, 2009; winter, September 30, 2009 – April 30, 2010; and spring, March 17, 2010 – April 30, 2010. The winter season overlapped with the autumn and spring seasons because no crayfish were tagged until the following spring and the next recapture event was not until the end of April. Crayfish with multiple seasons of growth (during summer, autumn, and spring) were not used in the analysis. Sample sizes were limited in many categories when the data were divided up by site, season, and species and did not allow for statistical testing using standard ANOVA methods to examine differences in those factors. Data were combined across sites and species to look for differences between seasons using a one-way ANOVA in SAS. Other comparisons were not done statistically, but means and 95% confidence intervals were calculated for each combination of species, sex, site, and season.

Movement Analysis

This study was not explicitly designed to examine movement, dispersal, or migration patterns of crayfishes. However, data were collected on location of tagged individuals throughout the study and provide information related to crayfish movement. Movement rates (m/hr) were calculated by determining the distance moved between successive encounters and the time in hr elapsed between encounters. Total movement distances were plotted to determine if they were related to crayfish size (CL). Using data collected during the diel sampling efforts, the absolute average movement rate (m/hr) of individual crayfish was compared between day and night samples, sex, and species using

repeated measures ANOVA in SAS (Cary, NC). Average movement rate values were square-root transformed prior to analysis after adding 0.01 to each value to account for heterogeneous variances resulting from zero-heavy data (Zar 1999).

RESULTS

Mortality and Tag Detection

A total of 634 crayfish were tagged over the course of the study, of which 17 (3%) succumbed to initial mortality and 146 (24%) were confirmed to have died or expelled their tag after release (Table 7). At least one observation was recorded for 462 different tags (75% of total number tagged).

Tags were encountered at 4,386 locations, of which 1,369 (206 separate tags) were eliminated from the analysis after failing to meet the validity requirements of the validity rule set leaving 3,017 valid choice sets from 364 different individuals (59% of tagged individuals contributed valid data). The average number of observations per tagged crayfish was 5.4 overall and ranged (excluding diel sampling) from averages of 3.0 observations per crayfish at 2ALLO in the summer to 6.6 observations per crayfish at 2ALLO in the spring (Table 8). The maximum number of times a single crayfish was encountered during a given season was 12 (excluding diel sampling). Twenty-three *O. hylas* and 14 *O. quadruncus* were encountered in both 200-m reaches of 1SYN. No crayfish tagged in 1SYN were encountered in 2ALLO and no crayfish tagged in 2ALLO were encountered in 1SYN.

Fine Scale Habitat Selection

Bar graphs of used and available habitat observations indicated some patterns in the data. At site 1SYN neither species used any of the three macrohabitat types more than 60% of the time and used each type at least 10% of the time (Figure 22). Each macrohabitat type was used similarly to its availability. At sites 2ALLO and 3ALLO, *O. quadruncus* used macrohabitat similar to its availability, but selected pools more than other types (Figure 23). Despite not being used in greater proportion to their availability, riffles were frequently used. Diel sampling showed that riffles were often not used in proportion to their availability, whereas pools were selected and runs used in proportion to availability (Figure 24). Macrohabitat use appeared similar during day and night samples.

Bar graphs showing the type of cover rock used clearly displayed selection of coarse particle sizes (Figures 25 and 26). Both species used coarse substrates as their cover rock over 50% of the time and no other particle class more than 25% of the time. Hard substrates were rarely used and although fine and pebble were used, they were never used in proportion to their availability regardless of site or season. Cover rock use was more varied for diel samples, but coarse substrate was used most often and in higher proportion than it was available (Figure 27). Surprisingly, crayfish used smaller cover rocks during the day than at night. Both species used cover rock size classes similarly across sites, seasons, and times of day.

Cover (organic detritus, woody debris, vegetation, leaves) was used in greater proportion than it was available in the autumn, but appeared less important during other

seasons and was commonly used less than 10% of the time (Figures 28 and 29). Cover use in the autumn was likely a result of high occurrence of freshly fallen leaves.

Plots of mean values and 95% confidence intervals for raw values of continuous variables displayed several patterns. Non-overlapping confidence intervals are defined as indicating significantly different use. *Orconectes hylas* used deeper water in autumn and summer at 1SYN than were available (Figure 30). In the summer, *O. quadruncus* used shallower areas than *O. hylas* at 1SYN (Figure 30). At site 3ALLO, *O. quadruncus* tended to use deeper areas than were available in spring, but this was not observed at 2ALLO (Figure 31). Both species used similar depths during the day and night (Figure 32). Overall, both species used mean depths ranging from 10 to 27 cm. At 1SYN, *O. hylas* and *O. quadruncus* used areas of similar current velocity and tended to use slower velocities in the spring and summer (Figure 33). Slower velocities were used at sites 2ALLO and 3ALLO by *O. quadruncus*, especially in the spring (Figure 34). Comparison of day and night observations indicated that both species used areas of similar velocity, regardless of time of day (Figure 35). Overall, both species commonly used velocities between 0.05 and 0.2 m/s.

Both species used higher values of the substrate metric (larger diameter substrates) than was available across all seasons and sites, except 2ALLO in the spring (Figures 36 and 37). Both species also tended to use larger substrates at night, but confidence intervals overlapped (Figure 38). Substrate heterogeneity was similar between species and among sites and seasons, with crayfish almost always using between two and three substrate types (Figures 39-41).

There was a great deal of model selection uncertainty (support for more than one candidate model) when the reach-level data were examined using separate seasons (proximate-level data were not examined individually by season) and this likely arose from small sample sizes (Table 9). This uncertainty made it difficult to compare parameter estimates among species and sites. The decision was made to only use the condensed models (referred to as seasonal and diel) to increase sample sizes and decrease difficulty in interpretation.

Model selection uncertainty existed in the condensed datasets, but was less prevalent for the reach-level availability models than the proximate-level availability models (Table 10). Site/species combinations with more than one model listed had support for multiple models and parameter estimates were averaged (Table 10). Overall, the global model (#20) received support (highest w_i) in all but two of the seasonal models. Other highly parameterized models also received support (models 17 and 19) for many of the species/site combinations. The only model with an interaction term that was supported in the seasonal model selection results was model 18 (interaction of S and cover rock type) for *O. quadruncus* from 3ALLO. The global model never received support in the diel models, but model 17 (velocity, depth, substrate cover rock type, and cover) received support in all but the *O. quadruncus* model from 3ALLO. Overall, the models supported for *O. quadruncus* were also supported for *O. hylas* indicating that similar types of variables are important for both species.

Seasonal models at the reach level of habitat availability had odds ratios ranging from 0.03 to 16.6 across the different variables (Table 11). When interpreting the odds ratios for V (current velocity), the estimate must be transformed, which results in the

number listed in the parameter estimates column actually representing the odds ratio (This is because of the negative exponential transformation done prior to data analysis for only this variable). For example, for *O. quadruncus* at site 1SYN, the odds ratio is actually 5.89 and the parameter estimate is 362.70. This means that for every 0.01 increase in current velocity, *O. quadruncus* is 5.89% less likely to be present in that habitat. If you expand this relation, *O. quadruncus* is 100 times more likely to be present in water velocities of 0.01 m/s than velocities of 0.17 m/sec. Categorical variables can be interpreted by comparing a given parameter estimate to the dummy variable category. For example, in the model for *O. quadruncus* at site 1SYN, an individual is 16.66% more likely to select a coarse cover rock (S_{Coarse} ; cobble or boulder) than a fine cover rock (S_{fine} ; sand or silt). In general, both species were more likely to be present in pools than riffles or runs, they positively selected cover, had weak relations with S, positively selected heterogeneous substrates, were negatively associated with increasing current velocities, had weak relations with depth, preferentially choose coarse cover rocks over pebble sized cover rocks, hard substrates, and fine substrates.

Few differences appeared between how the species selected habitats in reach-level seasonal models, with the most pronounced differences occurring in velocity and cover rock selection. *Orconectes quadruncus* choose slower water velocities when syntopic with *O. hylas* (5.89% per 0.01 m/s vs. 0.51% per 0.01 m/sec). *Orconectes quadruncus* choose slower velocities at the syntopic site compared to current velocity selection at allopatric sites. High level of selection for slow water by *O. quadruncus* at the syntopic site may indicate a response to the presence of *O. hylas*. *Orconectes quadruncus* also selected coarser substrate cover rocks (16% more likely than fine cover rocks) than did

O. hylas (8%) in the reach-level availability seasonal models. This high positive selection of coarse cover rock was also seen at one of the allopatric sites, which may indicate that *O. quadruncus* selects this type of cover rock regardless of *O. hylas* presence. The pattern did not continue at 3ALLO as selection of coarse cover rock was not as strong (3.4 % more likely than fine).

Odds ratios from the proximate-level availability models followed similar patterns to the reach-level availability models; however, selection was not as pronounced for most variables (Table 12). Macrohabitat type showed no real patterns, but this was expected because a random location within three meters is likely to be similar to the used macrohabitat type than macrohabitat at a point randomly selected within 20 m. The difference observed between species for velocity and cover rock category were the same as in the reach-level models.

At both levels of availability the relations at site 3ALLO were similar to those at 2ALLO, with the exception of velocity (proximate level) and cover rock (reach level). This indicates some variation based on stream, but overall similar habitat selection by the species. The weaker selection of velocity and cover rock at 3ALLO may indicate less habitat partitioning because fewer crayfish species are present.

Comparison of parameter estimates for diel models was more difficult because not all parameters were included in the supported models (Tables 12 and 14). Selection of coarse cover rock retained the same pattern as seasonal models, but was weaker (diel average 7.4% vs. seasonal average 11.4%). *Orconectes quadruncus* was 7.7% (reach) or 5.7% (proximate) more likely to choose a 0.01 m/s slower water velocity whereas *O. hylas* was 6.6% (reach) or 3.6% (proximate) more likely to choose a 0.01 m/s slower

water velocity. Selection of slower water velocities was not observed for *O. quadruncus* at the allopatric site. This may indicate that *O. quadruncus* selects faster current velocities at night than it would if *O. hylas* was not present.

Seasonal models were better able to correctly predict habitat selection than were diel models (Table 10). Reach-level models were the most accurate predictors overall, ranging from 72 – 84% correct classification rates. Diel proximate-level models were the worst predictors and ranged from 66 – 71% correct classification rates. No strong patterns were observed in model accuracy among sites or species.

Space Use Analysis

Results of space use analysis are presented based on the original site names because sites could not be combined for this analysis. Joint space use of *O. hylas* and *O. quadruncus* as calculated by the AI index was greatest in the autumn (AI = 0.81 at 1SYN (Site 1); AI = 0.79 at 1SYN (Site 2); Figures 42 and 43) and lowest in the summer (AI = 0.52 at Site 1; AI = 0.51 at Site 2; Figures 44 and 45). Joint space use in the spring was intermediate of the other two seasons (AI = 0.49 at Site 1; AI = 0.59 at Site 2; Figures 46 and 47), but closer to values from the summer than the fall. Diel comparisons (day vs. night) within species had high AI values (AI = 0.89 at Site 2 for *O. hylas*, AI = 0.90 at Site 2 for *O. quadruncus*, and AI = 0.83 at Site 3 (2ALLO) for *O. quadruncus*; Figures 48-50). The two species of crayfish overlapped more during the day (AI = 0.66) than at night (AI = 0.60) at Site 2 (Figures 51 and 52).

Growth Analysis

Values of IGR ranged from -0.00303 (which is approximately equal to -0.013 g per day) to 0.00940 (0.018 g per day) for all marked crayfish. Mean IGR of *O. quadruncus* was greater than *O. hylas*, but the associated 95% confidence intervals overlapped (Table 15). Male *O. quadruncus* grew faster than female *O. quadruncus*; however, 95% confidence intervals overlapped, albeit minimally (Table 16). There was little difference between IGR among sites (Table 17). These results should be interpreted with caution as they do not account for important sources of variation (e.g, differences in IGR between species does not account for variation caused by site, species, or season).

A one-way ANOVA was used to test for IGR differences among four seasons. Instantaneous growth rate differed significantly among the four seasons, $F(3, 141) = 15.55, p = 0.0001$. Tukey post-hoc comparisons of the four seasons indicate that autumn and winter growth rates were significantly lower ($p < 0.05$) than in spring and summer (Table 18). Water temperature averaged 20.5 °C in the summer, 13.0 °C in the fall, 8.6 °C in the winter, and 12.9 °C in the spring. This analysis combined species, sexes, and sites so caution should again be used when interpreting the results. However, exploratory analysis indicated that those factors accounted for a small portion of the variation compared to season.

Movement Analysis

The maximum movement rate observed was 10.6 m/hr by a male *O. hylas* during the diel sampling in the summer of 2009 (Table 20). Maximum movement rate estimates of seasonal sampling were lower than for diel sampling (Table 20). The minimum time

required for a crayfish to move one km was four days when diel data were considered, and 16 d using seasonal data. Overall, movement rates observed during diel sampling were greater than those observed during the seasonal sampling, likely an artifact of the time between encounters.

There was no relation ($R^2 = 0.0016$, $df = 1,489$, $p=0.37$) between the distance moved by crayfish and crayfish length (Figure 53). Truncated datasets using only those individuals that moved (Skalski and Gilliam 2000), or those that moved more than 10 meters, showed similar responses.

At 1SYN, crayfish average movement rate at night (0.54 m/hr) was greater than during the day (0.30 m/hr) ($df=1,67$, $F= 17.63$, $p= 0.0001$), but there were no statistical differences in sex ($p=0.06$) or species ($p = 0.65$). At 2ALLO, crayfish average movement rate at night (0.45 m/hr) was also greater than during the day (0.30 m/hr) ($df=1,20$, $F= 5.8$, $p= 0.03$), but no statistical difference was observed between sexes ($p = 0.16$). Despite the lack of statistical difference in movement rates between males and females, females did move at higher rates (Table 20).

Life-History Observations

Eight female *O. quadruncus* were ovigerous when tagged in the spring of 2010. Two of those individuals were recaptured (4/28 - 4/30/2010) and still had eggs. Five female *O. quadruncus* captured at the end of sampling in the spring of 2010 were ovigerous (one also had a sperm plug) and three had hatchlings (the reproductive status was not known for these individuals at the start of the spring sampling period because they were last captured in previous seasons). Three *O. hylas* recaptured in the spring of

2010 were ovigerous and two had hatchlings. All males of both species were form II (reproductively inactive) when they were tagged during the summer. Male crayfish tagged during other seasons were predominately form I, suggesting that they molt to form I sometime in mid August through early October and molt back to form II in late April or May. I witnessed mating between a marked and unmarked crayfish on several occasions ranging from August 12, 2009 through November 12, 2009 (Table 21). Most notable of the observed mating behavior occurred on August 12, 2009 at site 1SYN between a form I male *O. hylas* and a female *O. quadruncus* (which had a sperm plug). This was the first known occurrence of the two species exhibiting interspecific mating behavior. Of the crayfish tagged in the autumn of 2009, 18 female *O. hylas* had glair and 43 female *O. quadruncus* had glair (15 of which also had sperm plugs). Of the female crayfish tagged in the summer of 2009, 24 *O. quadruncus* had sperm plugs and one *O. hylas* had a sperm plug. The only other incidence of a sperm plug in *O. hylas* was seen in an individual recaptured on 11/12/2009. Six *O. quadruncus* recaptured in the autumn of 2009 had sperm plugs.

Several notable observations were made during the study not directly related to life history, but worthy of mention. On August 13, 2009 a live female *O. quadruncus* was found at site 3ALLO approximately 7 cm below the surface of the substrate after stream drying had occurred. The tag was no longer in the crayfish (the crayfish had a scar at the insertion location), but next to it in the moist gravel. One tag corresponding to a male *O. quadruncus* (25 mm CL) was located inside a common snapping turtle, *Chelydra serpentina*, during one occasion on June 8, 2009 at site 2ALLO. What appeared to be the same turtle was captured again on June 22, 2009 at 1SYN and had two tags in it

corresponding to a male *O. quadruncus* (24.3 mm CL) and a female *O. hylas* (23.3 mm CL). Finally, many crayfish of both species excavated burrows in the stream substrate down to and exceeding 15 cm. No quantitative data was collected related to this behavior, but it did appear to be a common behavior, regardless of season.

DISCUSSION

Mortality and Tag Detection

Mortality of tagged crayfish in this study was intermediate to rates observed in other studies. Bubb et al. (2002b) observed 93.3% survival of internally tagged *Pacifastacus leniusculus* over six months in the laboratory. Internal tagging of crayfish using PIT tags caused 14 – 22% initial mortality (within two hours of tagging) and 32 - 53% delayed mortality in *Orconectes compressus* (Black et al. 2010). I observed 3% initial mortality and 24% of the crayfish either expelled their tags or were delayed mortalities (either natural or tagging related causes).

Encounter rates for tagged individuals (75% encountered at least once) were lower than other crayfish studies using portable antennas. Bubb et al. (2006b) encountered 88% of tagged individuals over 17 d and Black et al. (2010) encountered 89% of tagged individuals over 10 d. Both studies were of much shorter duration which may explain the higher encounter rates and none of the crayfish in my study were searched for within the first 14 d of marking. Detection probability was influenced by observer, time of day, and discharge for fishes in small streams tagged with 23.1 mm long PIT tags (O'Donnell et al. 2010). Bubb et al. (2002b) found no difference in

detection probability between substrate sizes or burrow depths and located 80% of the hidden 12 mm PIT tags. No quantitative data on factors affecting tag detection were collected for my study, but several factors likely influenced tag detectability. Most notable of the factors that may have negatively affected detection probability was increased burrow depth in the substrate, observer error, and coarse (> 250 mm) substrate particles (J. Westhoff, personal observation). Habitat selection analyses could be biased by tag detection probabilities in that crayfish burrowing deep into substrate or hiding in large substrate may be underrepresented in the data.

Fine-Scale Habitat Selection

Orconectes hylas and *O. quadruncus* used a wide range of conditions for most habitat variables. It is important to consider that even though a habitat may not be preferentially selected, it may still be important if it is used (Rosenfeld 2003, Manly et al. 2010). This can occur when a resource is readily available and used in proportion to, or even less than, its availability. For example, at site 2ALLO in the summer, *O. quadruncus* was found in riffles 40% of the time, but riffles were also available approximately 40% of the time. It could be argued that despite *O. quadruncus* not selecting riffles, riffles are still a valuable macrohabitat type for the species. Therefore, consideration of both use and selection are important to understanding crayfish habitat relations.

Crayfish habitat associations are known to vary annually (DiStefano et al. 2003), seasonally (Gore and Bryant 1990), by time of day (Hill and Lodge 1994), and through ontogeny (Rabeni 1985, Gore and Bryant 1990, Flinders and Magoulick 2007a, Olsson

and Nystrom 2009). My study was not able to address juvenile habitat selection which may be an important life stage for interactions between *O. hylas* and *O. quadruncus*. The study was also not able to address annual variation; however, it did incorporate seasonal and diel variation. There was little evidence for seasonal or diel differences in habitat selection of these species. Some differences likely occurred, but were averaged out by combining seasons and combining day and night samples. Had these datasets not been combined, sample sizes would have been too small to make valid inferences, and model selection outcomes would have impeded the comparisons of parameter estimates.

There was little evidence that any one environmental variable associated with a fine-spatial scale (< 1 m) was able to explain the habitat selection of either *O. hylas* or *O. quadruncus* based on model selection results. Models that included one or two variables (models 1 – 12 and 14 -16) received little support overall during model selection procedures. Models with high number of parameters are penalized in AIC model selection (Burnham and Anderson 2003), so it was surprising that the most supported models in the seasonal analysis were models 17, 19, and 20. However, even though these models had many parameters, the variables that were included represented variables that I initially hypothesized as important and subsequently collected data on. Multiple variables are often viewed as important in microhabitat associations of crayfishes (Westhoff et al. 2006, Flinders and Magoulick 2007a, Johnston and Robson 2009).

Model selection procedures did not identify individual variables that were more or less important for habitat selection than others, but modeling did provide parameter estimates for each variable that could be interpreted and compared between *O. hylas* and *O. quadruncus*. Selection of cover rock type, as determined by odds ratios and bar

graphs, was strong for both species. The pattern of selection for cover rock was generally lowest for hard (bedrock and clay hardpan) and fine (sand, silt, and gravel) cover substrate, increased for pebble sized cover rocks, and was greatest for coarse cover rocks (cobble and boulder).

Substrate particle size is commonly related to crayfish habitat use (Bubb et al. 2006a, Jowett et al 2008, Olsson and Nystrom 2009, Westhoff et al. 2006). Riggert et al. (1999) observed the greatest densities of *O. quadruncus* on cobble and pebble substrates. Rabeni (1985) demonstrated that particle-size selection was dependent on the size and species of crayfish, and that it could be altered by the presence of predatory fish. Larger crayfish require larger interstitial spaces in the substrate to use as cover from predators (Gore and Bryant 1990). Crayfish in my study were of similar size (and all adults) so it may be expected that they use coarse substrates, which does not preclude the potential importance of different substrates for other life stages.

The mean substrate metric value for used locations was always greater than the mean value for available locations. This relation was not apparent in the discrete choice model results because the amount of variation surrounding the parameter estimates often overlapped with zero which gave little credibility to the direction of the relation (positive or negative). Although created specifically for this study, the substrate metric is closely related to the Fredle Index which combines observations on substrate characteristics to assign a single value to observations of substrate types in a given area (Lotspeich and Everest 1981). The importance of substrate size to crayfish is well established in other works (Bubb et al. 2006a, Jowett et al. 2008, Olsson and Nystrom 2009, Westhoff et al. 2006) and based on the importance of cover rock type it was surprising the relation with

this metric was not stronger. The reason for this may relate to the use of individuals instead of population metrics (like density) as the study unit. If crayfish rely on one or two large rocks to provide cover (as shown by the selection of coarse cover rocks) then the surrounding substrates may not be as important. For example, if each crayfish uses one cover rock, a 1-m² area that has lots of larger cover rocks may have a high density of crayfish, whereas if only a few larger cover rocks are present the crayfish density will be low. Analysis using crayfish density with a 1-m² area as the response variable would likely yield a positive relation between the substrate metric value (or percent coverage of large substrate) and crayfish selection. This hypothetical relation may be true, but what is needed by an individual adult crayfish may be one large rock and what is need for the population are many large rocks. This may also explain why substrate heterogeneity was not a strong predictor based on odds ratios or means and 95% confidence interval comparisons.

Orconectes hylas and *O. quadruncus* both selected slower current velocities based on parameter estimates. Although it was modeled as a linear relation, this relation may take a quadratic form in reality as evidenced by the plots of 95% confidence intervals and suggested by Gore and Bryant (1990). The average velocity used by crayfishes was often between 0.05 and 0.15 m/s, but they used a much wider range of values (0.0 – 0.91 m/s). *Orconectes hylas* and *O. quadruncus* may use slow velocities somewhat indiscriminately, but avoid faster velocities. Smaller crayfish species and those with dorsoventral flattening are thought to function better in faster water velocities than larger crayfish that have deeper bodies (Maude and Williams 1983). Both *O. quadruncus* and *O. hylas* are intermediate in these characteristics when compared to other crayfish present in the St.

Francis River drainage, but similar to each other (Pflieger 1996). The selection of slower velocity water may also reflect the biotic response of habitat partitioning among multiple syntopic crayfish species (Rabeni 1985, DiStefano et al. 2003). Water velocity is known to be important for other *Orconectes* species, as *Orconectes williamsi* were positively associated with faster current velocities (Westhoff et al. 2006) and adult *Orconectes neglectus* were more common in low velocity areas (Gore and Bryant 1990).

There was little evidence from model parameter estimates and bar graphs of use that either *O. quadruncus* or *O. hylas* selected specific macrohabitat categories. This may relate to the fact that macrohabitat variation in small streams (like Orr Hollow Creek and Captain's Creek) is less pronounced than in larger streams (J. Westhoff, personal observation). I did not collect habitat selection data on the other species of crayfish in the study streams so I cannot fully understand how biotic interactions may affect macrohabitat partitioning in the overall crayfish community. However, this crayfish community is comparable to those examined in other Ozarks streams (DiStefano et al. 2003) with regard to the presence of both *O. punctimanus* and *O. luteus*. The species that is not syntopic is *O. ozarkae* which is similar in appearance and size to both *O. hylas* and *O. quadruncus*. DiStefano et al. (2003) found that *O. ozarkae* was a macrohabitat generalist, but was more common in pools and backwaters. *Orconectes luteus* was common in all macrohabitat types and *O. punctimanus* was mostly found in pools, backwaters, and vegetation patches. If these relations are similar in St. Francis drainage, then *O. hylas* and *O. quadruncus* may select macrohabitat similar to *O. ozarkae*. Riggert et al. (1999) noted the higher densities of *O. quadruncus* in runs than in riffles, but were unable to discern the relation with pools.

Water depth was not a strong or consistent predictor in the discrete choice models as there was a great deal of variation compared to the magnitude of the parameter estimate. Both species generally used average depths from 10 to 27 cm, but were found in a much wider range of depths (0 - 161 cm). Depth has been related to crayfish habitat use in other species (Westhoff et al. 2006, Flinders and Magoulick 2007b). Englund and Krupa (2000) hypothesized that water depth affects predation rates on crayfish where large crayfish are susceptible to terrestrial predators (birds and mammals) in shallow water but may exceed the gape limitations of fish predators in deep water, whereas small crayfish display the opposite relation.

Cover (detritus, woody debris, aquatic vegetation, or freshly fallen leaves) was used from 15 – 40% of the time in the summer and autumn but was rarely used in the spring. Frequent use of cover in the autumn was likely related to the abundance of freshly fallen leaves in the system that covered about 1/3 of the stream bottom (J. Westhoff, personal observation). Biotic-derived cover (plant material) may temporarily act similarly to abiotic cover (rocks), but is expected to be less permanent in the system as it is more susceptible to transport by water. In addition to simply being associated with macrophytes (Garvey et al. 2003), crayfish are also known to consume detritus and macrophytes (Olsen et al. 1991).

Both adult *O. hylas* and *O. quadruncus* appear to be habitat generalists at a fine spatial scale in these small streams, with the exception of positive selection for slower water velocities and coarse substrate that is likely used for cover. There were few apparent differences in how the species selected habitats. If habitat partitioning was occurring between *O. hylas* and *O. quadruncus*, one would expect to see clear differences

in habitat selection (Rabeni 1985, DiStefano et al. 2003, Jones and Bergey 2007). Additionally, if *O. hylas* and *O. quadruncus* compete for the same limited resources and competitive exclusion was occurring, one would expect to see *O. quadruncus* habitat use differ between the syntopic and allopatric sites with habitat use at the allopatric site similar to that of *O. hylas* at the syntopic site. The strongest evidence for competitive exclusion was observed in current velocity at the reach and proximate levels of selection for the seasonal models, where *O. quadruncus* selected slower velocities than *O. hylas* at the syntopic site. Faster current velocities were selected by *O. quadruncus* at the allopatric sites compared to the syntopic site. Selection of resources was different between *O. hylas* and *O. quadruncus* for some of the models and sites (e.g., strength of selection of cover rock at allopatric site for seasonal reach-level model), but velocity was the only variable that suggested competitive exclusion along a niche axis may be occurring.

The common selection of resources in Orr Hollow Creek by *O. hylas* and *O. quadruncus* suggests that one species should exclude the other. Exclusion has not been observed in the 10 years that the two species have co-occurred in Orr Hollow Creek, although the densities of *O. quadruncus* have been reduced (DiStefano and Westhoff, in press). Four possible explanations for this are that interspecific competition is not occurring (Larson and Magoulick 2009), disturbance to the system is more influential in species persistence than competition (Ward and Stanford 1983, Light 2003, Larson et al. 2009), resources are not limiting which results in low levels of interspecific competition (Hutchinson 1957), or competitive exclusion is occurring slowly, but has not been fully achieved.

Competitive exclusion has been observed in crayfishes. Hill and Lodge (1994) demonstrated that despite a preference for cobble habitat when allopatric, *Orconectes propinquus* and *Orconectes virilis* were forced to use less desirable muck-macrophyte habitat when exposed to the invasive *O. rusticus*. Evidence of competitive exclusion of *Orconectes immunis* by *O. virilis* was observed whereby the latter would evict the former from crevices in the substrate (Bovbjerg 1970). Similarly, Gherardi and Daniels (2004) demonstrated that the invasive *Procambarus clarkii* was able to exclude the native *Procambarus acutus acutus* from shelters. Competitive exclusion may also be observed at coarser spatial scales in crayfish. *Orconectes rusticus* is believed to exclude *Orconectes sanbornii sanbornii* from larger streams, but not certain headwater systems (Flynn and Hobbs 1984).

Other studies have looked for evidence of competitive exclusion and found none. The invasive *P. leniusculus* preferred mud substrate and the native *Austropotamobius torrentium* preferred pebble substrates when in sympatry and allopatry suggesting that the presence of the invasive did not induce a habitat shift in the native in a laboratory setting (Vorburger and Ribi 1999). Larson and Magoulick (2009) did not observe evidence of competition between a native and invasive crayfish based on controlled field experiments.

Space Use

My study represents the first known attempt to apply the AI metric to crayfishes and no studies are known that compare home range overlap in crayfishes. The observed overlap in space-use between *O. hylas* and *O. quadruncus* does not explain why the

species use the same space or how they interact. I considered plotting locations of the various macrohabitat types to see if greater overlap occurred in certain types; however, statistical comparisons of these relations would not be valid because observations of the density value at a given point are highly correlated to the density value at nearby points.

The high amount of space-use overlap in the autumn suggests that individuals of the two species are within close proximity of each other nearly 80% of the time. Space-use overlap in the spring and summer was less, but was approximately 50 – 60%.

Reasons for differences in overlap among seasons are unknown. Space-use within species between day and night observations was from 83 – 90%, which may indicate crayfish do not spend time in different places during the day and night (e.g., individuals do not move from riffles to pools during the night). The space use overlap for these species may indicate a high potential for interspecific competition, but a lack of complete competitive exclusion. The only known study to use the AI index for aquatic organisms compared space use of flathead catfish (*Pylodictis olivaris*) and observed AI values from 0.03 to 0.21 for paired individuals (Vokoun and Rabeni 2005).

There is spatial error associated with AI index values based on location error of individuals and the metric does not account for the location across the stream channel, just the longitudinal location along the stream. This is also a static space use value so it does not take into account the temporal aspects of crayfish location (e.g., two crayfish may be located at the same distance along the stream, but one may have occurred there on day 1 and the other on day 2) (Vokoun and Rabeni 2005).

Growth

Interpretation of growth data were hindered by lack of independent observations of growth for every level of species, site, season, and sex. Mean IGR and associated 95% confidence intervals did not indicate any significant differences in growth rates between species or among sites. The lack of difference among sites indicates that *O. quadruncus* did not grow less (a symptom of competition) at the syntopic site than at the allopatric sites. There was some evidence that male *O. quadruncus* may grow faster than female *O. quadruncus*, but more data are required to fully understand this relation. Male crayfish are known to grow faster than females for some species (Brewis and Bowler 1982), but not always (Hill et al. 1993). Differences in growth rates between native and invasive species are thought to contribute to the displacement of some native crayfishes (Hill et al. 1993, Hill and Lodge 1999), but *O. quadruncus* actually grew at a faster mean rate than did *O. hylas* in this study. This contradicts suggestions by DiStefano et al. (2002) that *O. hylas* may have a growth advantage over *O. quadruncus* based on maximum attained size of juveniles. The discrepancy in findings may relate to the lack of spatial coverage by both studies and differences that occur at earlier, unstudied life stages.

Differences in growth based on season were observed to be statistically different. Crayfish growth rates (regardless of species, sex, or site) were greatest in the summer and spring when temperatures averaged 20.5°C and 12.9°C, respectively. Slower growth rates were observed in the winter and fall, when temperatures averaged 8.6°C and 13.0°C, respectively. Growth is incremental and thought to be greatest immediately after molting (Price and Payne 1984), which occurs primarily from April through September for both *O. hylas* and *O. quadruncus* (DiStefano et al. 2002). Whitledge and Rabeni

(2002) found that growth was maximized at 22°C for *O. hylas* which corresponds closely to average summer temperatures observed in this study.

Movement

Movement rates calculated in this study represent the first instance of movement information collected from individual crayfish for any endemic Ozark crayfish species. Dispersal rates observed by DiStefano and Westhoff (in press) based on yearly population level sampling showed that *O. hylas* advanced upstream in Orr Hollow Creek at a rate of 128 m/year (± 230 , 95% confidence interval). The maximum observed rate of upstream movement by *O. hylas* in Orr Hollow Creek was 10.6 m/hr in this study. Expansion of that rate (given the rate was sustainable over longer time periods) suggests that an individual *O. hylas* could disperse upstream 128 m in 12 h, or one km in approximately four days. Given this result, invasion rates could be much greater than suggested by DiStefano and Westhoff (in press).

Movement rates were lower for seasonal observations than for diel observations which may indicate that encountering crayfish every few days underestimates their movement capabilities compared to more frequent observations. Because of this bias, average movement rates for crayfish encountered during seasonal samples were not calculated and only average movement rates during diel sampling were used to look for differences between sexes, species, and time of day. Average movement rates during the night were one and a half to nearly two times greater than during the day. Crayfish are hypothesized to be more active at night (Hazlett et al. 1974), but until now no data existed to support this hypothesis for *O. hylas* or *O. quadruncus*. Higher movement rates

at night than during the day may be related to increased feeding behavior (Hazlett et al. 1974, Lorman 1975). No statistical differences were observed between the average movement rates of *O. quadruncus* or *O. hylas*, but some invasive crayfishes are known to have greater dispersal capability than natives (Bubb et al. 2006a). Average movement rates of *O. hylas* and *O. quadruncus* (0.3 – 0.54 m/hr) are slightly lower than those observed for the invasive *O. rusticus* (0.56 – 1.2 m/hr) in lentic environments (Byron and Wilson 2001). Movement rates are sometimes underestimated because long distance movements are difficult to detect (Byron and Wilson 2001); this may be the case for rates observed in this study. The layout of sample sites (1SYN downstream 600 m from 2ALLO) may provide additional insight to long distance dispersal of crayfish in Orr Hollow Creek. No crayfish was observed to have moved from the upstream site to the downstream site, or vice versa. If long distance dispersal or migration did occur, it was not observed with this study design.

Size of crayfish was not related to movement distance in this study, similar to findings by Byron and Wilson (2001). Byron and Wilson (2001) also noted that individual *O. rusticus* dispersal patterns fell into a group of non-dispersers and dispersers. The trend of differential dispersal was observed by others (Hazlett et al. 1974, Robinson et al. 2000) and was observed in this study (J. Westhoff, unpublished data).

Additional research is needed to better understand movement rates and dispersal trends for *O. hylas* and *O. quadruncus*. However, movement rates observed in this study can be used to better understand and predict the spread of *O. hylas* in the St. Francis River drainage. Predictions made from these rates and rates observed by DiStefano and

Westhoff (in press) can serve as predictors and can be tested with results of future research or monitoring efforts (Chapter 4).

Life History

Observations of life-history traits coincided with previous studies and supplement their findings (Riggert et al. 1999, DiStefano et al. 2002). Several female *O. hylas* and *O. quadruncus* were ovigerous in April which had been observed in other streams (Riggert et al. 1999, DiStefano et al. 2002). DiStefano et al. (2002) found that the number of *O. hylas* females with sperm plugs peaked in November and was never greater than about 15% of the population whereas Riggert et al. (1999) noted the peak number of sperm plugs (approximately 40% of captured females) for *O. quadruncus* was in December. At least some *O. quadruncus* females also had sperm plugs nine months of the year whereas *O. hylas* females had sperm plugs up to five months of the year (Riggert et al. 1999, DiStefano et al. 2002). Observations from this study support those findings as only two *O. hylas* females were observed to have sperm plugs over the course of this study whereas 30 *O. quadruncus* had sperm plugs. Effects of the difference in number and timing of sperm plugs between these species are not well understood.

The first observed occurrence of an *O. hylas* male engaged in mating behavior with an *O. quadruncus* female was observed. It is not known if the two species are able to successfully hybridize, but it has been hypothesized (Fetzner and DiStefano 2008) and observed to occur between other native and invasive crayfish (Perry et al. 2002). Regardless, evidence now exists that the mating behavior leading to hybridization has occurred and at a minimum may represent potential for reproductive interference.

Conclusions

Habitat use and selection data from my study suggest that at a fine spatial scale both *O. hylas* and *O. quadruncus* use and select similar habitat features. Coarse substrate particles were selected by individuals of both species and may represent the most important habitat feature investigated. Because of the high degree of habitat use overlap and space use overlap, the potential for competitive exclusion may be high. There was little evidence that competitive exclusion has occurred along any niche axis with the possible exception of selection of current velocity ranges. This was not surprising given the two species have co-occurred in Orr Hollow Creek for 10 years. Other streams in the drainage no longer harbor *O. quadruncus* following the appearance of *O. hylas* and habitat selection of crayfishes at those streams may differ. Competitive exclusion may also occur based on interactions that are important at other spatial scales or by means of competition for food or other resources not investigated in this study. Alternatively, interspecific competition with *O. hylas* may not be an important mechanism causing displacement of *O. quadruncus*. Other possibilities exist that may be the mechanism of displacement and are be discussed in Chapter 5.

Based on the similarity of habitat selection between *O. hylas* and *O. quadruncus* there does not appear to a feasible management strategy that could be enacted that would promote coexistence of these species at a fine spatial scale. Streams, like Orr Hollow Creek, with high amounts of coarse substrate may act as temporary refugia for *O. quadruncus* after invasion by *O. hylas* by reducing the intensity of competition for shelter. However, uncertainty about the potential for coexistence of *O. hylas* and *O.*

quadruncus will remain until more is known about the mechanism of species displacement.

Table 4. Range of dates crayfish were tagged and tracked during each sampling season along with the number of scanning events occurring at each sample site during that time span.

Season	Tagging date range	Scanning date range	1SYN (Site 1)	1SYN (Site 2)	2 ALLO	3ALLO
Pilot season	4/10/2009 – 4/23/2009	4/20/2009 – 4/30/2009	N/A	7	2	N/A
Diel 2009	6/2/2009 – 6/5/2009	6/22/2009 – 6/25/2009 and 6/29/2009 – 7/2/2009	N/A	19	19	N/A
Summer 2009	N/A	7/22/2009 – 8/13/2009	13	13	13	3
Autumn 2009	9/30/2009 – 10/15/2009	10/19/2009 – 11/12/2009	10	10	10	10
Spring 2010	3/17/2009 – 3/19/2009	4/5/2010 – 4/30/2010	10	10	10	10

Table 5. Variables used to assess fine-scale habitat selection of *Orconectes hylas* and *Orconectes quadruncus*. Abbreviation for the parameter (β), the description of the variable, and the observed range of values (0-1 indicates a categorical variable) are displayed.

β	Description	Observed range
M_{Rif}	Macrohabitat - Riffle (Dummy)	0-1
M_{Run}	Macrohabitat - Run	0-1
M_{Pool}	Macrohabitat - Pool/Backwater	0-1
D	Water Depth (cm)	0 -161
V	Water velocity at substrate surface (m/sec)	0 - 0.91
S	Substrate metric (unitless)	2 - 256
S_{Num}	Number of substrate types	1-5
U_{Large}	Upstream substrate type was cobble or pebble	0-1
S_{Fine}	Substrate – Gravel, sand, or silt (Dummy)	0-1
S_{Peb}	Substrate - Pebble	0-1
S_{Coarse}	Substrate – Cobble or boulder	0-1
S_{Hard}	Substrate – Bedrock or hardpan	0-1
C	Cover	0-1
I	Random effect for individual	

Table 6. List of *a priori* candidate models and the hypotheses they represent used for model selection in discrete choice analysis of habitat selection for *Orconectes hylas* and *Orconectes quadruncus*.

Model Number	Hypothesis	Model Structure	Expected Results
1	Negative effect of depth	$=B_1(D) + B_2(I)$	$B_1 < 0$
2	Negative effect of velocity	$=B_1(\exp(-V)) + B_2(I)$	$B_1 < 0$
3	Interaction of depth and velocity	$=B_1(D) + B_2(\exp(-V)) + B_3(D*\exp(-V)) + B_4(I)$	$B_1 < 0, B_2 < 0$
4	Positive effect of number of substrate types (heterogeneity)	$=B_1(N_{sub}) + B_2(I)$	$B_1 > 0$
5	Positive effect of substrate metric	$=B_1(S) + B_2(I)$	$B_1 > 0$
6	Positive effect of coarse cover rock substrate	$=B_1(S_{Hard}) + B_2(S_{Peb}) + B_3(S_{Coarse}) + B_4(I)$	$B_1 < 0, B_3 > B_2 > 0$
7	Positive effect of substrate metric and coarse cover rock	$=B_1(S) + B_2(S_{Hard}) + B_3(S_{Peb}) + B_4(S_{Coarse}) + B_5(I)$	$B_1 > 0, B_2 < 0, B_3 < B_4$
8	Effect of macrohabitat	$=B_1(M_{Pool}) + B_2(M_{Run}) + B_3(I)$	$B_1 < 0, B_2 > 0$
9	Effect of Substrate metric on macrohabitat	$=B_1(M_{Pool}) + B_2(M_{Run}) + B_3(S) + B_4(M_{Pool}*S) + B_5(M_{Run}*S) + B_6(I)$	$B_1 > 0, B_2 > 0, B_3 > 0, B_4 > B_1, B_5 > B_2$
10	Positive effect of large upstream substrate on velocity	$=B_1(U_{Large}) + B_2(\exp(-V)) + B_3(U_{Large} * \exp(-V)) + B_4(I)$	$B_1 > 0, B_2 < 0, B_3 > B_2$
11	Positive effect of coarse cover rock and number of substrate types (substrate heterogeneity)	$=B_1(S_{Hard}) + B_2(S_{Peb}) + B_3(S_{Coarse}) + B_4(N_{sub}) + B_5(I)$	$B_1 < 0, B_3 > B_2, B_4 > 0$
12	Macrohabitat positively influenced by substrate metric	$=B_1(M_{Pool}) + B_2(M_{Run}) + B_3(S_{Hard}) + B_4(S_{Peb}) + B_5(S_{Coarse}) + B_6(I)$	$B_1 < 0, B_2 > 0, B_3 < 0, B_4 > 0, B_4 < B_5$
13	Negative effects of velocity and depth and positive effects cover rock	$=B_1(\exp(-V)) + B_2(D) + B_3(S_{Hard}) + B_4(S_{Peb}) + B_5(S_{Coarse}) + B_6(I)$	$B_1 < 0, B_2 < 0, B_3 < 0, B_4 > 0, B_4 < B_5$

14	Effects of velocity on substrate metric	$=B_1(\exp(-V)) + B_2(S) + B_3(S*\exp(-V)) + B_4(I)$	$B_1 < 0, B_3 > 0,$
15	Effects of depth on substrate metric	$=B_1(D) + B_2(S) + B_3(S*D) + B_4(I)$	$B_1 < 0, B_2 > 0, B_3 < B_2$
16	Positive effect of cover and substrate	$=B_1(S_{Hard}) + B_2(S_{Peb}) + B_3(S_{Coarse}) + B_4(C) + B_5(I)$	$B_1 < 0, B_2 > 0, B_3 > 0, B_4 > 0$
17	High parameter model with cover	$=B_1(\exp(-V)) + B_2(D) + B_3(S_{Hard}) + B_4(S_{Peb}) + B_5(S_{Coarse}) + B_6(C) + B_7(I)$	$B_6 > 0$
18	Interaction of substrate metric and cover rock	$=B_1(S) + B_2(S_{Hard}) + B_3(S_{Peb}) + B_4(S_{Coarse}) + B_5(S*S_{Hard}) + B_6(S*S_{Peb}) + B_7(S*S_{Coarse}) + B_8(I)$	$B_1 > 0, B_2 < B_3 < B_4, B_5 < B_6 < B_7$
19	High parameter model with macrohabitat type	$=B_1(\exp(-V)) + B_2(D) + B_3(S_{Hard}) + B_4(S_{Peb}) + B_5(S_{Coarse}) + B_6(M_{Pool}) + B_7(M_{Run}) + B_8(I)$	$B_6 < 0, B_2 > 0$
20	Global	<i>All parameters (No interactions)</i>	<i>N/A</i>

Table 7. Numbers of *Orconectes hylas* and *Orconectes quadruncus* that were tagged over the course of the study (April 2009 – April 2010) displayed by site and sex. The number of initial mortalities (died within one hour of tagging) and delayed mortalities (tag recovered during sampling) are displayed.

Site	Species	Sex	Number tagged	Initial mortality	Delayed mortality
1SYN	<i>O. hylas</i>	F	89	2	22
		M	135	1	19
	Total		224	3	41
1SYN	<i>O. quadruncus</i>	F	86	1	24
		M	77	2	27
	Total		163	3	51
2ALLO	<i>O. quadruncus</i>	F	57	3	13
		M	67	5	20
	Total		124	8	33
3ALLO	<i>O. quadruncus</i>	F	62	1	10
		M	61	2	11
	Total		123	3	21
Grand Total			634	17	146

Table 8. Encounters of tagged *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) by sampling season at all sites.

Site	Season	Species	Number crayfish encountered	Number valid observations	Average observations per crayfish	Max number observations
1SYN	Diel	HY	51	243	4.8	16
	Diel	QU	41	331	8.1	14
	Summer	HY	45	151	3.1	9
	Summer	QU	38	228	5.4	12
	Autumn	HY	73	265	3.3	9
	Autumn	QU	36	201	4.6	10
2ALLO	Spring	HY	73	407	5.3	10
	Spring	QU	24	157	6.3	10
	Diel	QU	37	263	7.1	15
	Summer	QU	19	57	3.0	11
3ALLO	Autumn	QU	29	148	5.1	10
	Spring	QU	21	139	6.6	10
	Diel	QU	-	-	-	-
	Summer	QU	-	-	-	-
Total			562	3017	5.4	
	Average		28.1	107		

Table 9. Model selection results for reach-level models divided by site, species, and season. Model numbers correspond to candidate models and w_i indicates model weight values.

Site	Species	Season	Model	w_i	
1SYN	<i>Orconectes hylas</i>	Summer	17	0.66	
			13	0.23	
		Autumn	20	0.92	
			Spring	13	0.51
		17		0.32	
		19	0.16		
	<i>Orconectes quadruncus</i>	Summer	13	0.61	
			17	0.23	
			19	0.08	
		Autumn	17	0.91	
			Spring	13	0.65
		17		0.23	
19	0.12				
2ALLO	<i>Orconectes quadruncus</i>	Summer	9	0.97	
			Autumn	17	0.34
		20		0.30	
		16		0.21	
		11		0.11	
		Spring	17	0.49	
	20		0.34		
	19	0.11			
	3ALLO	<i>Orconectes quadruncus</i>	Autumn	19	0.34
				13	0.32
				17	0.14
				20	0.13
Spring		20	0.48		
		19	0.45		

Table 9 (continued). Model selection results for reach-level models divided by site, species, and season. Model numbers correspond to candidate models and w_i indicates model weight values.

Site	Species	Time of day	Model	w_i
1SYN	<i>Orconectes hylas</i>	Day	3	0.88
			Night	13
		20		0.27
		17		0.19
1SYN	<i>Orconectes quadruncus</i>	Day	13	0.50
			3	0.20
			17	0.18
			19	0.08
		Night	13	0.56
			17	0.30
			19	0.11
			2ALLO	<i>Orconectes quadruncus</i>
19	0.21			
Night	15	0.24		
	14	0.16		
	12	0.14		
	8	0.10		
	9	0.09		
	13	0.08		
	1	0.06		

Table 10. Model selection results for condensed (seasons combined and day and night combined) models for *Orconectes hylas* and *Orconectes quadruncus*. Model numbers correspond to candidate models, w_i indicates model weight values, and the classification rate (%) value indicates the predictive accuracy of the model averaged final model as determined by cross validation.

Site	Species	Proximate Level			Reach Level		
		Model	w_i	Classification rate (%)	Model	w_i	Classification rate (%)
Seasonal							
1SYN	<i>O. hylas</i>	20	0.99	75	20	0.90	77
	<i>O. quadruncus</i>	20 17	0.72 0.27	77	17	0.93	84
2ALLO	<i>O. quadruncus</i>	20	0.77	71	20	0.70	72
		16	0.16		17	0.24	
3ALLO	<i>O. quadruncus</i>	13	0.40	73	20	0.62	76
		18	0.31		19	0.38	
		17	0.21				
		19	0.07				
Diel							
1SYN	<i>O. hylas</i>	13	0.51	66	13	0.56	69
		19	0.21		17	0.19	
		17	0.21		3	0.17	
2ALLO	<i>O. quadruncus</i>	17	0.94	71	13	0.65	73
					17	0.22	
					19	0.17	
3ALLO	<i>O. quadruncus</i>	17	0.36	66	12	0.68	73
		16	0.21		19	0.28	
		13	0.13				
		6	0.08				
		11	0.07				
		7	0.06				

Table 11. Model averaged parameter estimates for variables (β) including standard errors (SE) and odds ratios (e^x) for both *Orconectes hylas* and *Orconectes quadruncus* at each of the three sample sites. Values were derived from the reduced set of models (summer, fall, and spring seasons combined) for the reach-level discrete choice models. Variables indicated by (*) represent dummy variables against which odds ratios for the associated variables should be compared for interpretation.

β^a	1SYN – <i>O. hylas</i>			1SYN – <i>O. quadruncus</i>			2ALLO			3ALLO		
	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}
M _{Rif} *	-	-	-	-	-	-	-	-	-	-	-	-
M _{POOL}	0.326	0.544	1.385	-	-	-	1.180	0.744	3.254	1.104	0.547	3.016
M _{RUN}	0.121	0.410	1.128	-	-	-	-0.051	0.527	0.951	0.942	0.513	2.565
C	0.683	0.443	1.981	0.719	0.956	2.052	1.076	0.656	2.932	-0.042	0.522	0.959
S	0.001	0.042	1.001	-	-	-	-0.002	0.055	0.998	0.008	0.054	1.008
S _{Num}	0.327	0.300	1.387	-	-	-	0.219	0.395	1.245	0.306	0.371	1.358
V	0.511	0.854	1.666	5.894	0.086	362.709	3.657	1.199	38.744	0.088	0.994	1.092
D	0.024	0.085	1.024	0.012	0.542	1.012	-0.017	0.129	0.983	0.031	0.085	1.032
S _{Fine} *	-	-	-	-	-	-	-	-	-	-	-	-
S _{HARD}	-1.193	0.666	0.303	0.037	0.522	1.038	-1.328	1.285	0.265	-0.765	0.752	0.465
S _{Peb}	0.450	0.465	1.569	0.746	0.741	2.108	1.279	0.612	3.594	0.007	0.477	1.007
S _{Coarse}	2.081	0.465	8.013	2.813	0.464	16.661	2.682	0.610	14.619	1.232	0.603	3.427
U _{LARGE}	-0.023	0.401	0.978	-	-	-	0.283	0.509	1.327	-0.232	0.476	0.793

Table 12. Model averaged parameter estimates for variables (β) including standard errors (SE) and odds ratios (e^x) for both *Orconectes hylas* and *Orconectes quadruncus* at each of the three sample sites. Values were derived from the reduced set of models (diel sampling, day and night combined) for the reach-level discrete choice models. Variables indicated by (*) represent dummy variables against which odds ratios for the associated variables should be compared for interpretation.

β^a	1SYN – <i>O. hylas</i>			1SYN – <i>O. quadruncus</i>			2ALLO		
	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}
M _{Rif} *	-	-	-	-	-	-	-	-	-
M _{POOL}	-	-	-	0.261	0.566	1.299	1.152	0.548	3.165
M _{RUN}	-	-	-	0.371	0.522	1.450	0.944	0.500	2.569
C	0.099	0.711	1.104	-0.201	0.662	0.818	-	-	-
S	-	-	-	-	-	-	-	-	-
S _{Num}	-	-	-	-	-	-	0.234	1.132	1.264
V	6.626	1.297	754.170	7.731	1.056	2277.243	0.234	0.133	1.264
D	-0.036	0.368	0.965	0.039	0.289	1.040	0.024	0.597	1.024
S _{Fine} *	-	-	-	-	-	-	-	-	-
S _{HARD}	-0.639	0.890	0.528	-0.346	0.857	0.707	-1.071	0.843	0.343
S _{Peb}	-0.119	0.562	0.888	0.176	0.658	1.193	0.387	0.595	1.472
S _{Coarse}	0.566	0.555	1.761	1.239	0.652	3.453	1.065	0.568	2.900
U _{LARGE}	-	-	-	-	-	-	-	-	-
V*D	0.514	0.569	1.671	-	-	-	-	-	-

Table 13. Model averaged parameter estimates for variables (β) including standard errors (SE) and odds ratios (e^x) for both *Orconectes hylas* and *Orconectes quadruncus* at each of the three sample sites. Values were derived from the reduced set of models (summer, fall, and spring seasons combined) for the proximate-level discrete choice models. Variables indicated by (*) represent dummy variables against which odds ratios for the associated variables should be compared for interpretation.

β^a	1SYN – <i>O. hylas</i>			1SYN – <i>O. quadruncus</i>			2ALLO			3ALLO		
	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}
M_{Rif}^*	-	-	-	-	-	-	-	-	-	-	-	-
M_{POOL}	0.285	0.534	1.330	-1.702	0.802	0.182	2.146	0.912	8.551	0.106	0.892	1.112
M_{RUN}	-0.139	0.628	0.870	-0.031	0.664	0.969	0.246	0.755	1.279	0.406	0.843	1.501
C	0.910	0.954	2.483	1.291	0.551	3.635	1.317	0.610	3.734	0.272	0.546	1.312
S	0.003	0.052	1.003	-0.003	0.047	0.997	-0.006	0.052	0.994	0.019	0.120	1.019
S_{Num}	0.374	0.612	1.454	0.093	0.340	1.097	0.039	0.372	1.040	-	-	-
V	1.851	1.360	6.365	4.218	1.073	67.886	0.945	1.061	2.573	1.040	1.071	2.829
D	0.024	0.156	1.025	0.036	0.117	1.037	-0.010	0.130	0.990	0.039	0.096	1.040
S_{Fine}^*	-	-	-	-	-	-	-	-	-	-	-	-
S_{HARD}	0.003	0.054	1.003	-0.347	0.830	0.707	-1.327	1.169	0.265	-0.862	0.754	0.422
S_{Peb}	0.024	0.156	1.025	0.876	0.502	2.400	0.448	0.579	1.566	0.329	0.650	1.389
S_{Coarse}	1.811	1.346	6.117	2.734	0.535	15.398	1.996	0.631	7.359	2.172	0.657	8.777
U_{LARGE}	0.133	0.365	1.142	0.217	0.425	1.242	0.242	0.465	1.274	-	-	-
S^*S_{COARSE}	-	-	-	-	-	-	-	-	-	-0.016	0.120	0.984
S^*S_{PEB}	-	-	-	-	-	-	-	-	-	-0.015	0.125	0.985
S^*S_{HARD}	-	-	-	-	-	-	-	-	-	0.279	0.284	1.322

Table 14. Model averaged parameter estimates for variables (β) including standard errors (SE) and odds ratios (e^x) for both *Orconectes hylas* and *Orconectes quadruncus* at each of the three sample sites. Values were derived from the reduced set of models (diel sampling, day and night combined) for the proximate-level discrete choice models. Variables indicated by (*) represent dummy variables against which odds ratios for the associated variables should be compared for interpretation.

β^a	1SYN – <i>O. hylas</i>			1SYN – <i>O. quadruncus</i>			2ALLO		
	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}	Estimate	SE ^a	e^{x^b}
M _{Rif} *	-	-	-	-	-	-	-	-	-
M _{POOL}	-1.080	0.889	0.340	-	-	-	-	-	-
M _{RUN}	-1.286	0.965	0.276	-	-	-	-	-	-
C	0.295	0.523	1.343	1.849	0.516	6.352	0.841	0.456	2.318
S	-	-	-	-	-	-	-0.003	0.002	0.997
S _{Num}	-	-	-	-	-	-	0.193	0.131	1.213
V	3.591	2.009	36.253	5.745	2.193	312.467	-1.472	0.177	0.229
D	0.042	0.15	1.04	0.066	0.015	1.068	0.034	0.015	1.034
S _{Fine} *	-	-	-	-	-	-	-	-	-
S _{HARD}	-0.542	0.747	0.582	0.198	0.970	1.219	-0.428	0.724	0.652
S _{Peb}	0.598	0.302	1.819	0.703	0.314	2.020	0.805	0.292	2.236
S _{Coarse}	1.62	0.347	5.040	1.951	0.328	7.033	1.683	0.327	5.384

Table 15. Mean instantaneous growth rates (IGR), standard deviation (σ), and upper and lower 95% confidence intervals for *Orconectes hylas* and *Orconectes quadruncus* by combining across seasons, sexes, and sites.

Species	N	Mean IGR	σ	Lower 95% CI	Upper 95% CI
<i>O. hylas</i>	63	0.00132	0.00165	0.00091	0.00174
<i>O. quadruncus</i>	82	0.00173	0.00265	0.00115	0.00232

Table 16. Mean instantaneous growth rates (IGR), standard deviation (σ), and upper and lower 95% confidence intervals for *Orconectes hylas* and *Orconectes quadruncus* by combining across seasons and sites.

Species	Sex	N	Mean IGR	σ	Lower 95% CI	Upper 95% CI
<i>O. hylas</i>	F	18	0.00132	0.00180	0.00043	0.00222
<i>O. quadruncus</i>	F	37	0.00091	0.00215	0.00020	0.00163
<i>O. hylas</i>	M	45	0.00132	0.00161	0.00084	0.00181
<i>O. quadruncus</i>	M	45	0.00241	0.00285	0.00155	0.00326

Table 17. Mean instantaneous growth rates (IGR), standard deviation (σ), and upper and lower 95% confidence intervals for *Orconectes hylas* and *Orconectes quadruncus* by combining across seasons and sexes.

Species	Site	N	Mean IGR	σ	Lower 95% CI	Upper 95% CI
<i>O. quadruncus</i>	2ALLO	24	0.00206	0.00317	0.00072	0.00339
<i>O. quadruncus</i>	3ALLO	26	0.00132	0.00228	0.00040	0.00225
<i>O. hylas</i>	1SYN	63	0.00132	0.00165	0.00091	0.00174
<i>O. quadruncus</i>	1SYN	32	0.00182	0.00254	0.00091	0.00274

Table 18. Mean instantaneous growth rates (IGR), standard deviation (σ), and upper and lower 95% confidence intervals for *Orconectes hylas* and *Orconectes quadruncus* combined across sites and sexes. Summer and spring IGR rates (^A) were significantly higher than autumn and winter (^B) IGR rates.

Season	N	Mean IGR	σ	Lower 95% CI	Upper 95% CI
^A Summer	41	0.00207	0.00159	0.00159	0.00254
^B Fall	39	0.00025	0.00200	-0.00039	0.00090
^B Winter	22	0.00033	0.00043	0.00014	0.00052
^A Spring	43	0.00288	0.00272	0.00204	0.00371

Table 19. Average movement rate (standard deviation) in m/hr for *Orconectes hylas* and *Orconectes quadruncus* combined at sites 1SYN and 2ALLO displayed by both gender and day or night.

	1SYN	2ALLO
Male	0.39 (0.51)	0.20 (0.30)
Female	0.50 (0.57)	0.47 (0.56)
Day	0.30 (0.36)	0.30 (0.43)
Night	0.54 (0.63)	0.45 (0.56)

Table 20. Maximum movement rates (m/hr) observed during each season for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU). The maximum movement rate was expanded to predict the time it would take a given individual to travel 1 km. Negative distance values represent downstream movements and (*) indicates movements known to occur at night.

Season	Species	Sex	Tag code	m/hr	Distance (m)	Hours	Days to travel 1 km
Diel*	HY	F	24429D	10.6	53	5	4
Diel*	HY	F	2CEDEE	9.8	49	5	4
Summer	HY	M	244676	1.4	-31	22	30
Summer	QU	F	240A9F	1.3	29	23	32
Fall	HY	M	244210	2.4	66	27	18
Fall	QU	F	23FF3E	2.4	-99	41.5	18
Spring	HY	M	343D04	2.6	72	47	16
Spring	HY	M	341A9A	2.5	70	47	17

Table 21. Instances of crayfish encountered while visible engaged in mating behavior. Crayfish indicated by ^A or ^B were observed mating with each other.

Site	Species	Sex	CL (mm)	Date
1SYN	<i>Orconectes hylas</i>	M	UK	8/12/2009 ^A
1SYN	<i>Orconectes quadruncus</i>	F	UK	8/12/2009 ^A
1SYN	<i>Orconectes hylas</i>	M	22.1	10/19/2009
1SYN	<i>Orconectes hylas</i>	F	24.6	10/20/2009
1SYN	<i>Orconectes quadruncus</i>	M	23.9	11/4/2009
3ALLO	<i>Orconectes quadruncus</i>	F	29.7	10/14 ^B
3ALLO	<i>Orconectes quadruncus</i>	M	24.9	10/14 ^B
3ALLO	<i>Orconectes quadruncus</i>	M	27.9	11/9/2009
3ALLO	<i>Orconectes quadruncus</i>	F	22.2	11/11/2009 11/12/2009

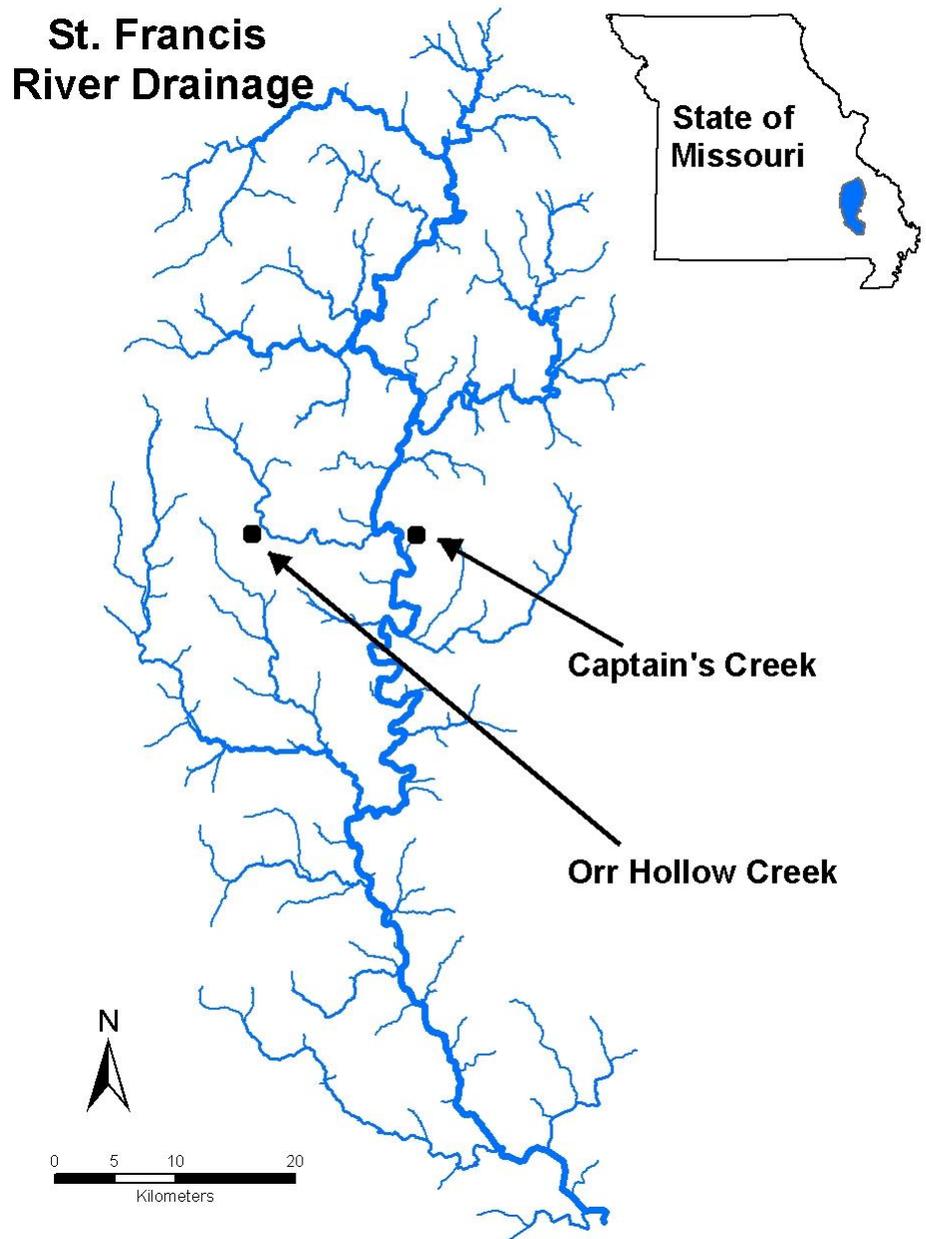


Figure 17. Location of the two study streams (Orr Hollow Creek and Captain's Creek) in the St. Francis River drainage, Missouri where fine-scale habitat selection was examined for *Orconectes hylas* and *Orconectes quadruncus*.

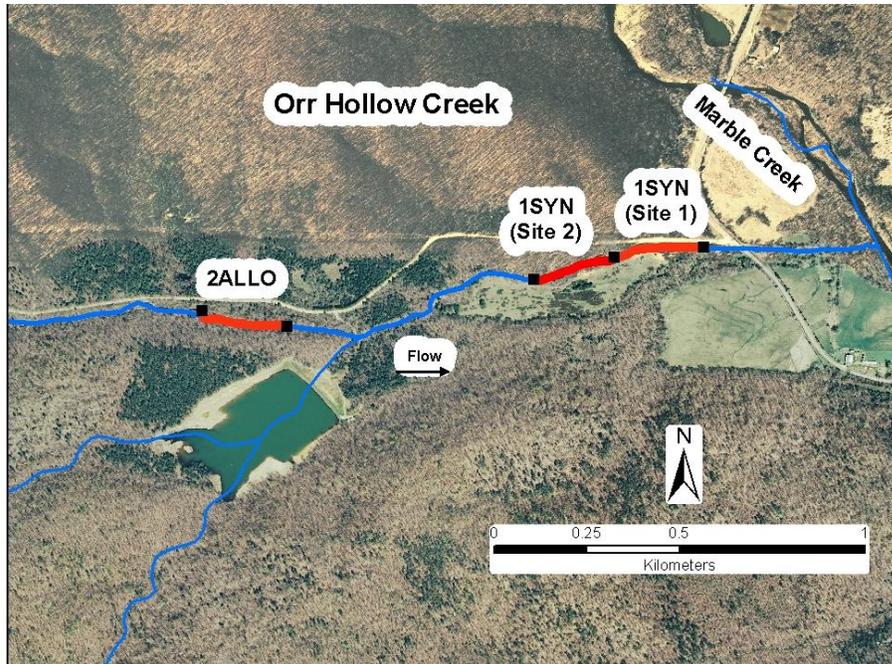


Figure 18. Location of sites 1SYN (*Orconectes hylas* and *Orconectes quadruncus* are syntopic) and 2ALLO (*O. quadruncus* is allopatric) on Orr Hollow Creek in the St. Francis River drainage, Missouri. Original site name for 2ALLO was Site 3.

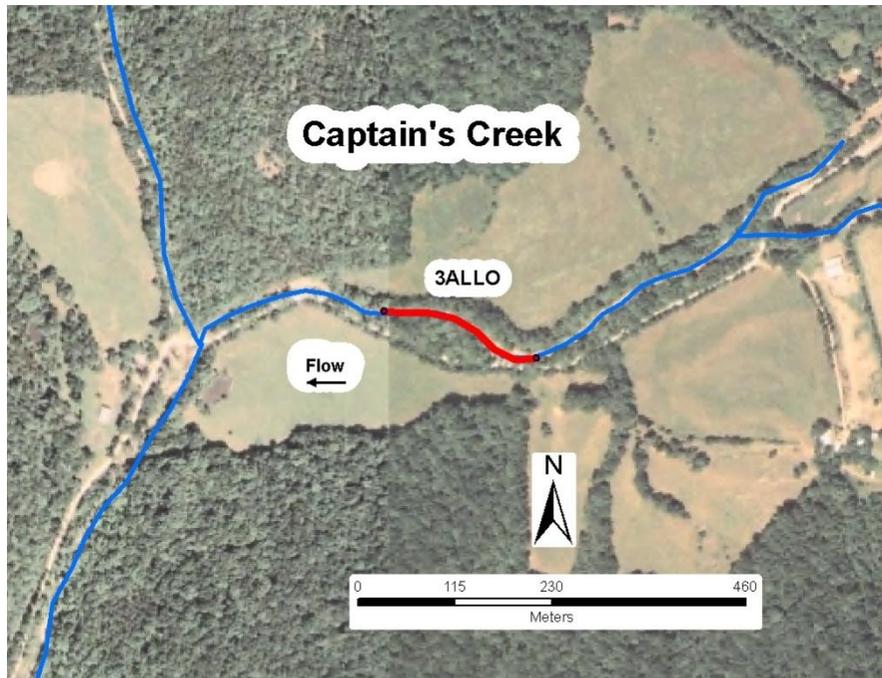


Figure 19. Location of site 3ALLO (*Orconectes quadruncus* is allopatric) on Captain's Creek in the St. Francis River drainage, Missouri. Original site name for site 3ALLO was Site 4.



Figure 20. Location of passive integrated transponder tag insertion during pilot work. Insertions were made between the pleopod I and II (one abdominal section in the caudal direction) during the actual study.

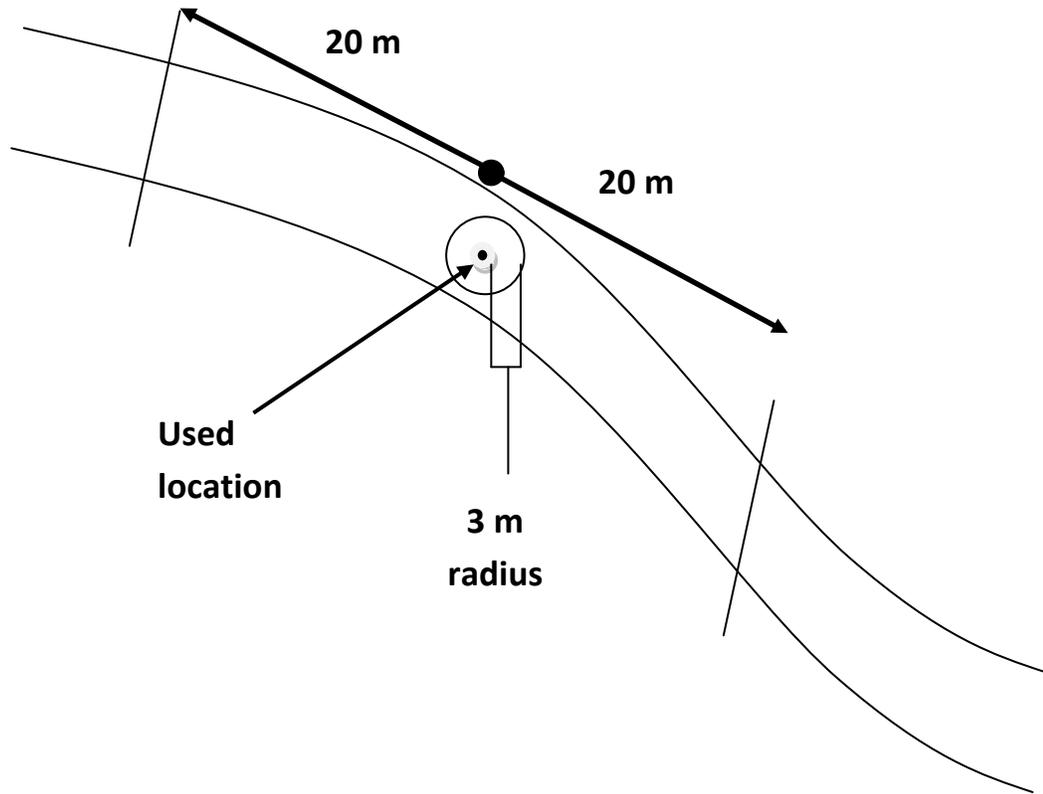


Figure 21. Depiction of method used to determine locations of available habitat measurements. The proximate-level availability location was randomly selected within a 3-m diameter of the used location and reach-level availability location was randomly selected within 3-20 m upstream or downstream of the used location.

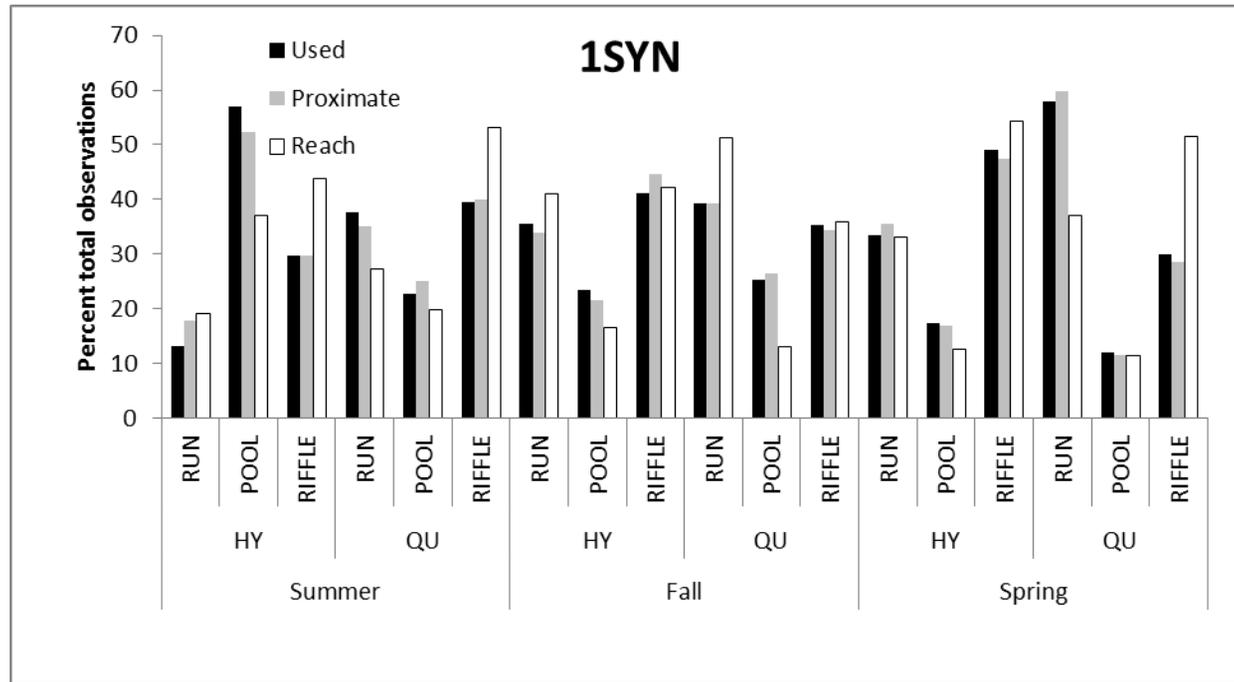


Figure 22. Percent of total observations in each macrohabitat category divided by season and species at site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the three macrohabitat types equals 100%, which allows for comparison among seasons and species.

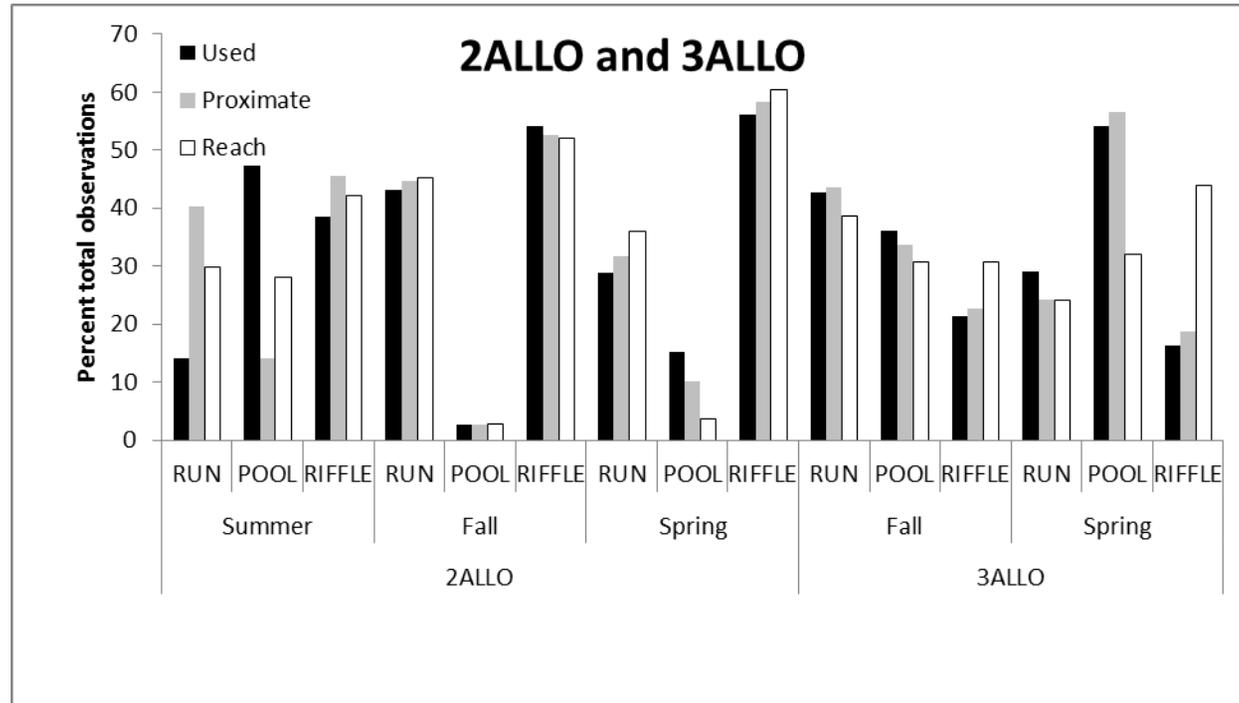


Figure 23. Percent of total observations in each macrohabitat category divided by season for sites 2ALLO and 3ALLO where *Orconectes quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the three macrohabitat types equals 100%, which allows for comparison among seasons and sites.

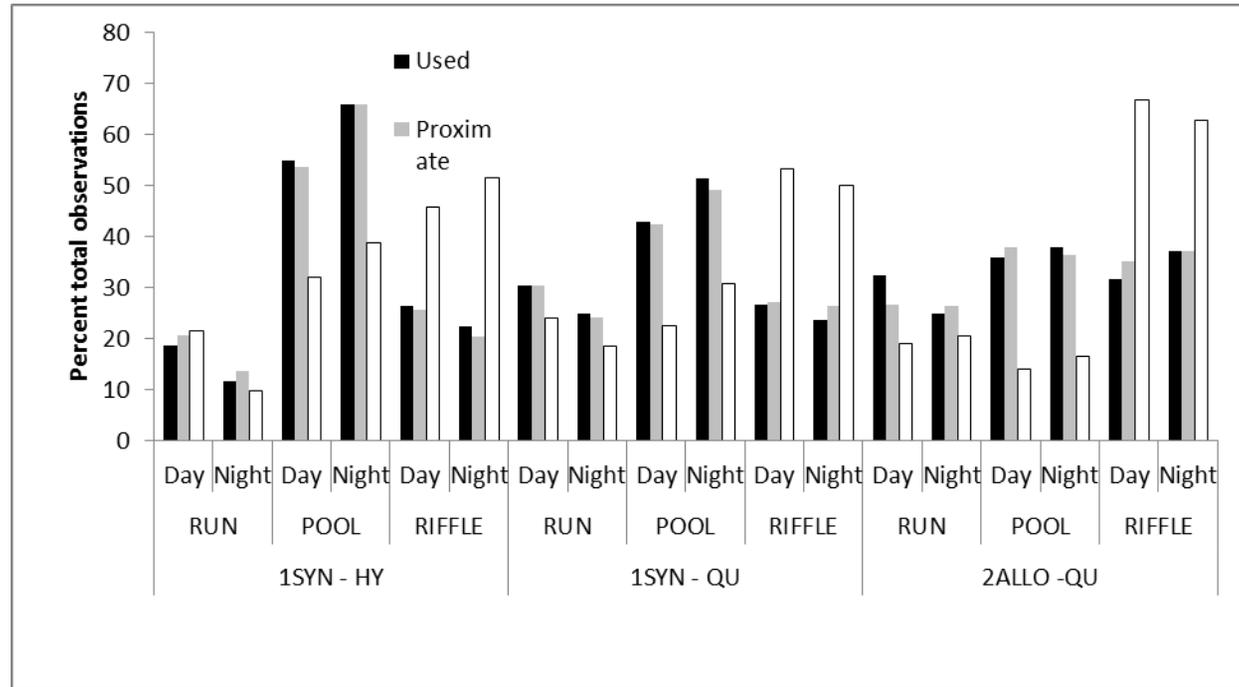


Figure 24. Percent of total observations in each macrohabitat category divided by species and time of day at site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic and site 2ALLO where *O. quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the three macrohabitat types equals 100%, which allows for comparison among time of day, site, and species.

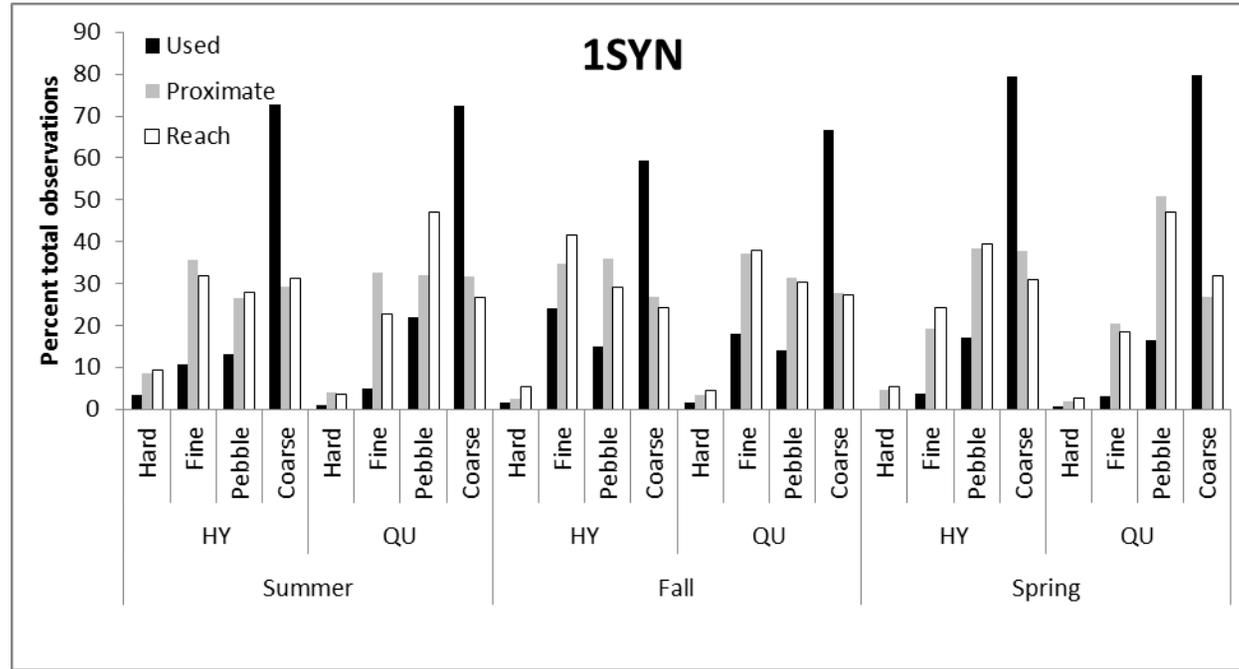


Figure 25. Percent of total observations in each substrate cover rock category divided by season and species at site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the four cover rock types equals 100%, which allows for comparison among seasons and species.

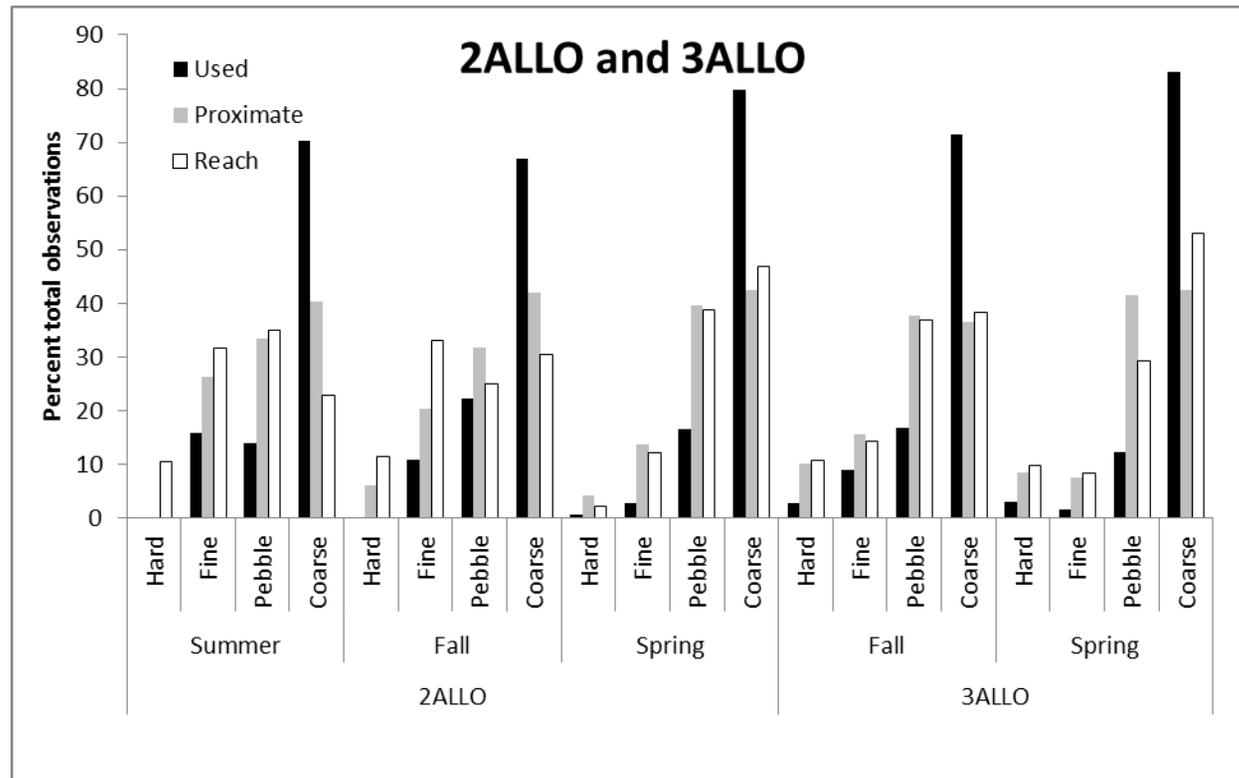


Figure 26. Percent of total observations in each substrate cover rock category divided by season for sites 2ALLO and 3ALLO where *Orconectes quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the four cover rock types equals 100%, which allows for comparison among seasons and sites.

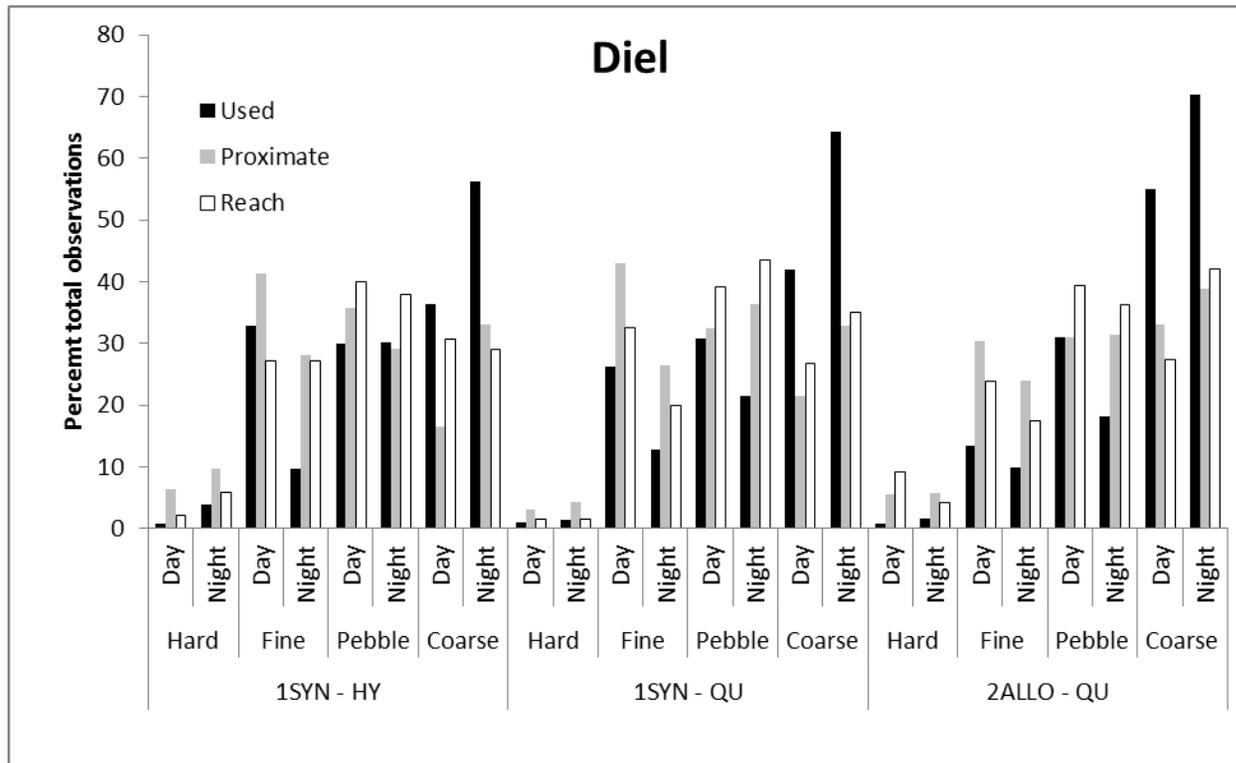


Figure 27. Percent of total observations in each substrate cover rock category divided by species and time of day at site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic and site 2ALLO where *O. quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow). Summing the bars for a given set of locations across the four cover rock types equals 100%, which allows for comparison among time of day, site, and species.

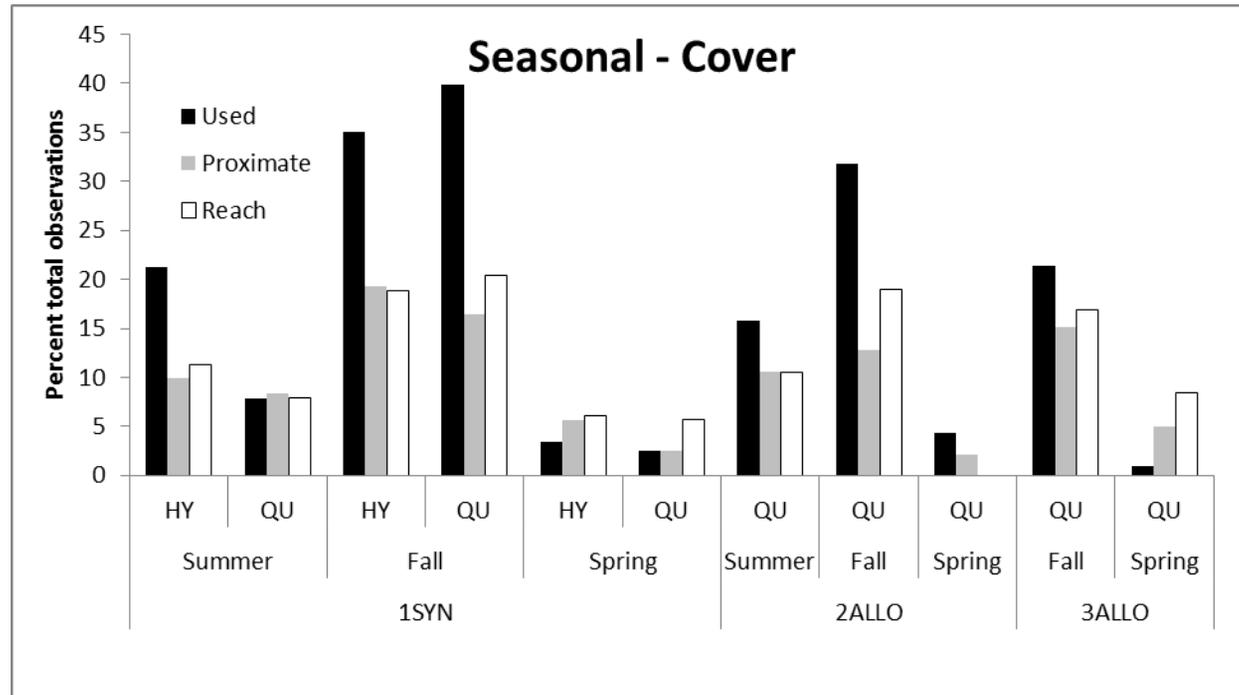


Figure 28. Percent of total observations for cover (combination of detritus, woody debris, vegetation, leaves) divided by season for site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic and sites 2ALLO and 3ALLO where *Orconectes quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow).

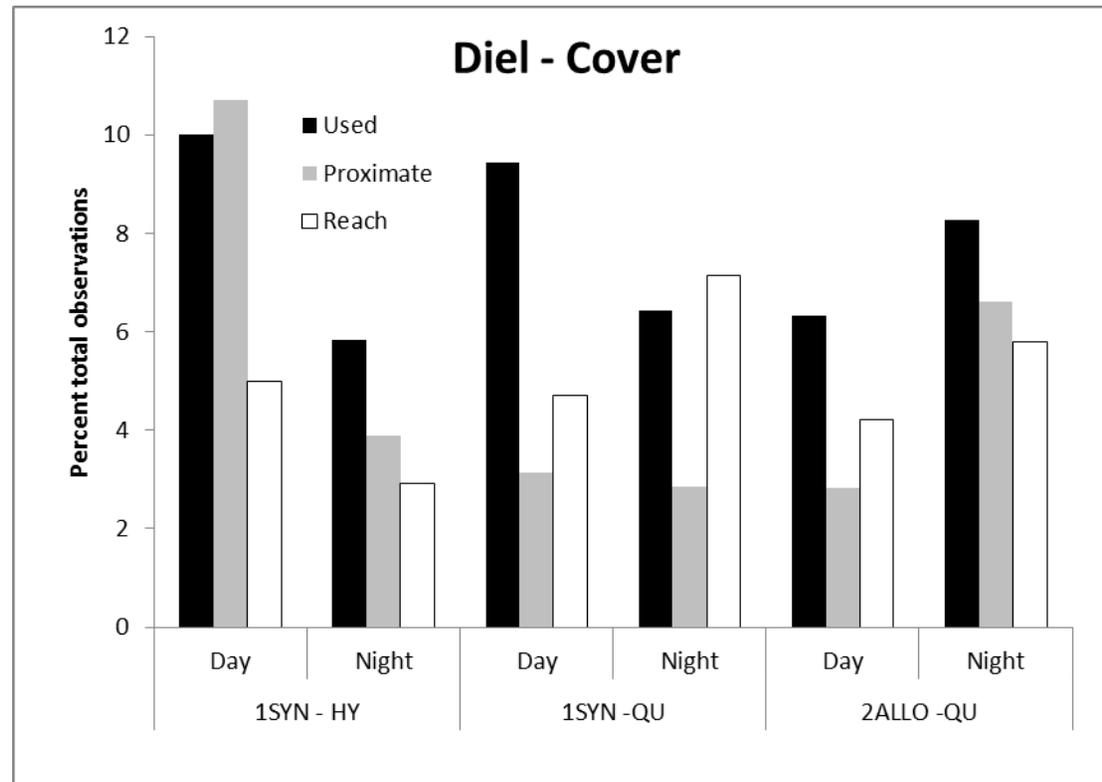


Figure 29. Percent of total observations for cover (combination of detritus, woody debris, vegetation, leaves) divided by species and time of day at site 1SYN where *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) were syntopic and site 2ALLO where *O. quadruncus* was allopatric. Bars represent used locations (solid black), proximate-level availability (solid gray), and reach-level availability (hollow).

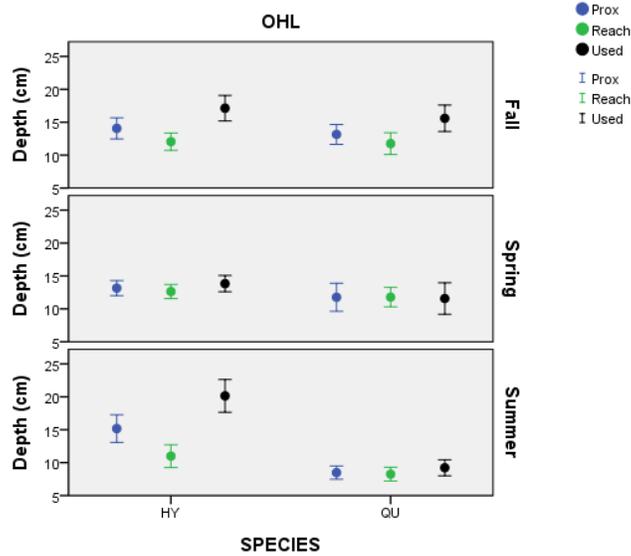


Figure 30. Means and 95% confidence intervals for depth observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 1SYN (OHL) during three seasons for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

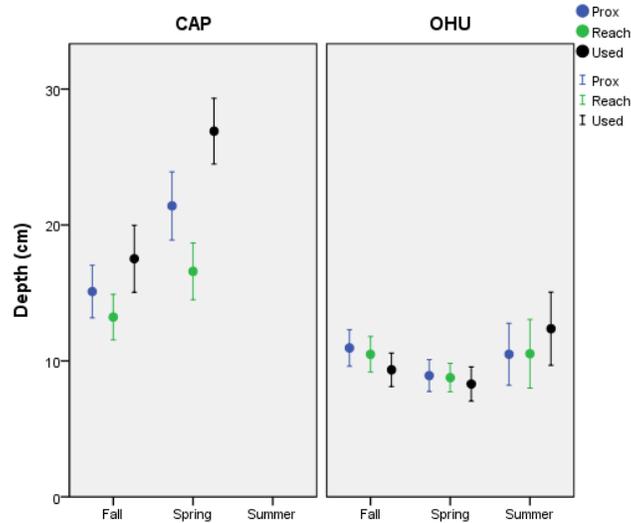


Figure 31. Means and 95% confidence intervals for depth observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 2ALLO (OHU) and 3ALLO (CAP) during three seasons for *Orconectes quadruncus*.

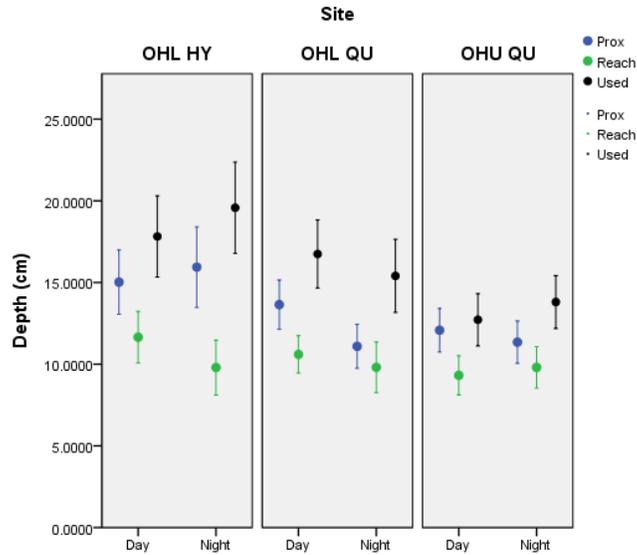


Figure 32. Means and 95% confidence intervals for depth observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at sites 1SYN (OHL) and 2ALLO (OHU) for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

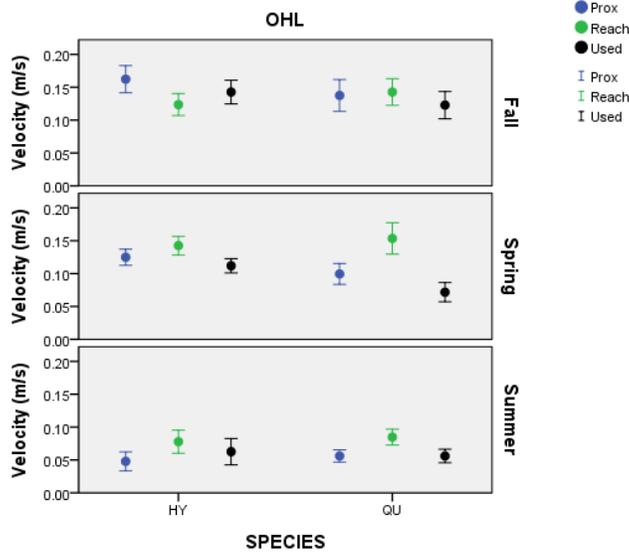


Figure 33. Means and 95% confidence intervals for current velocity observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 1SYN (OHL) during three seasons for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

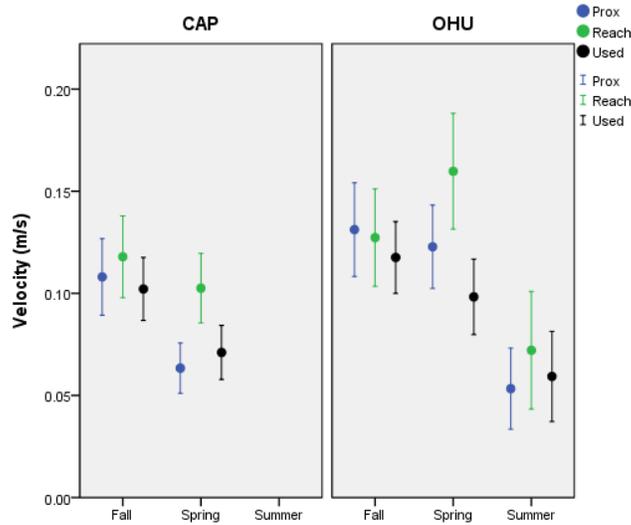


Figure 34. Means and 95% confidence intervals for current velocity observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 2ALLO (OHU) and 3ALLO (CAP) during three seasons for *Orconectes quadruncus*.

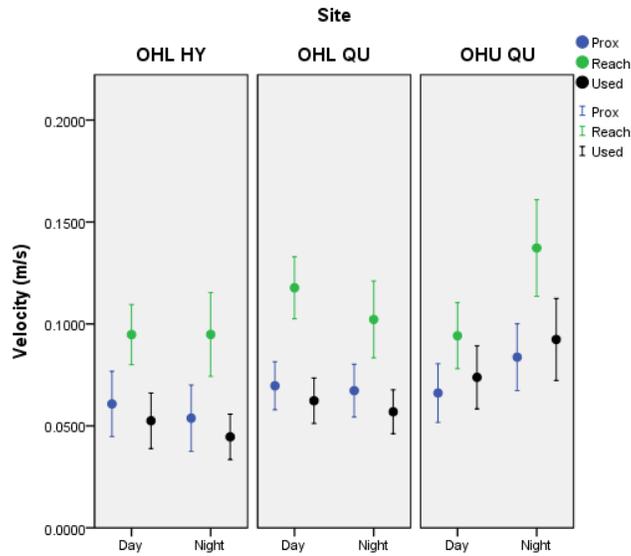


Figure 35. Means and 95% confidence intervals for current velocity observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at sites 1SYN (OHL) and 2ALLO (OHU) for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

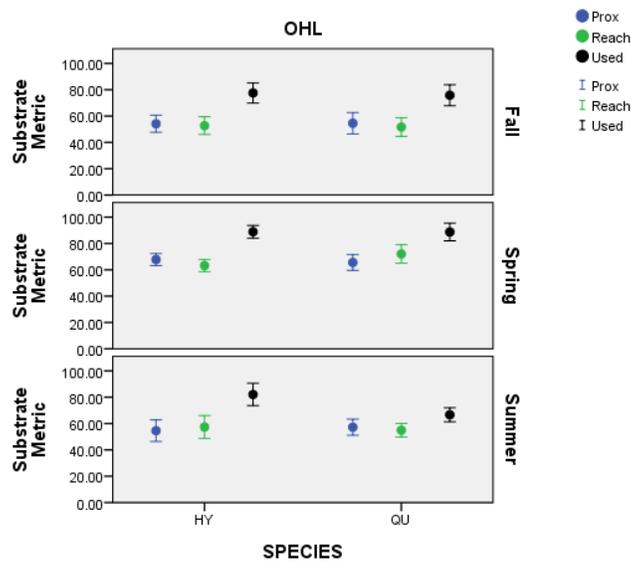


Figure 36. Means and 95% confidence intervals for the substrate metric observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 1SYN (OHL) during three seasons for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

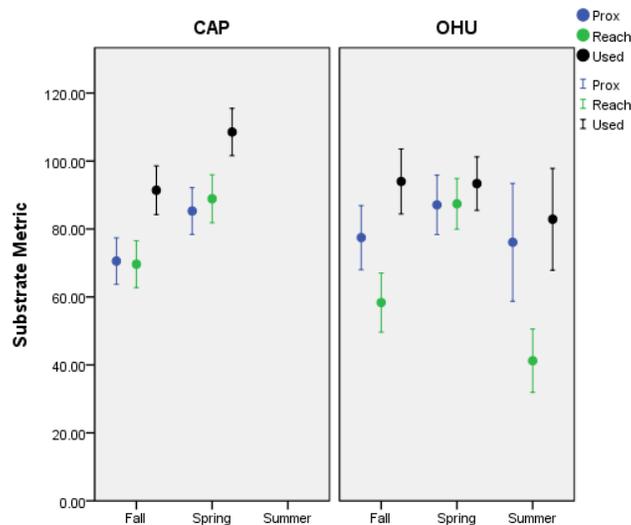


Figure 37. Means and 95% confidence intervals for the substrate metric observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 2ALLO (OHU) and 3ALLO (CAP) during three seasons for *Orconectes quadruncus*.

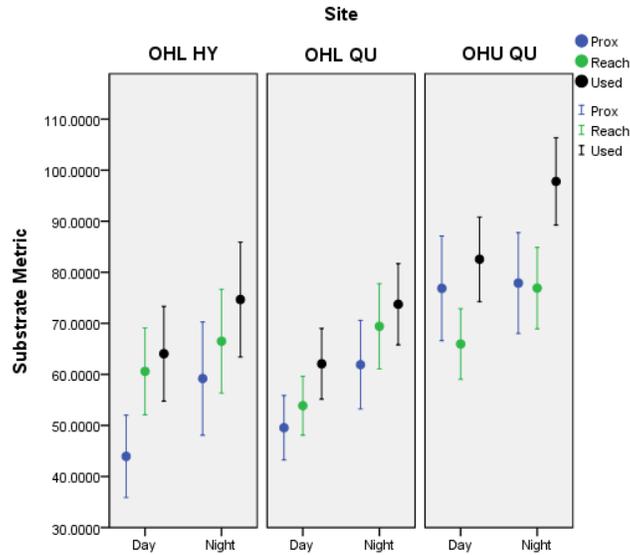


Figure 38. Means and 95% confidence intervals for the substrate metric observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at sites 1SYN (OHL) and 2ALLO (OHU) for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

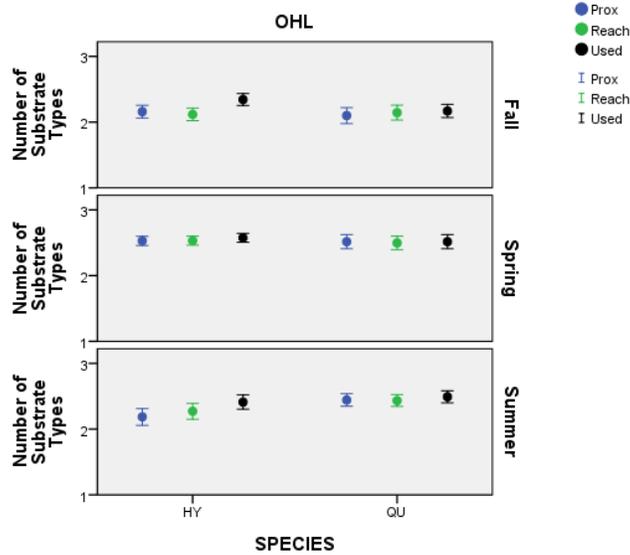


Figure 39. Means and 95% confidence intervals for the number of substrate types (heterogeneity) observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 1SYN (OHL) during three seasons for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

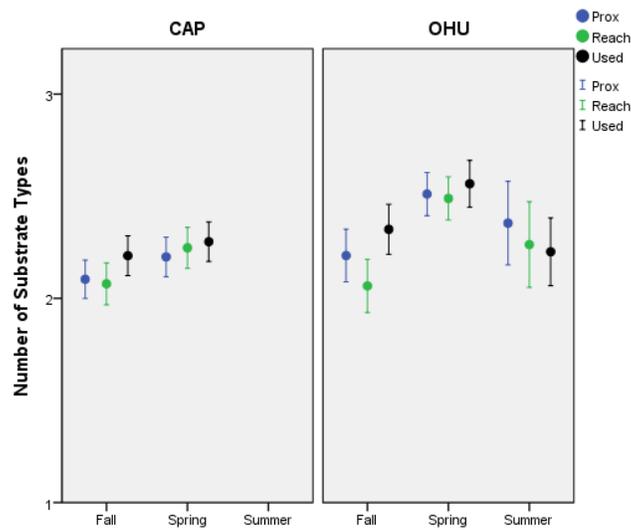


Figure 40. Means and 95% confidence intervals for the number of substrate types (heterogeneity) observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at site 2ALLO (OHU) and 3ALLO (CAP) during three seasons for *Orconectes quadruncus*.

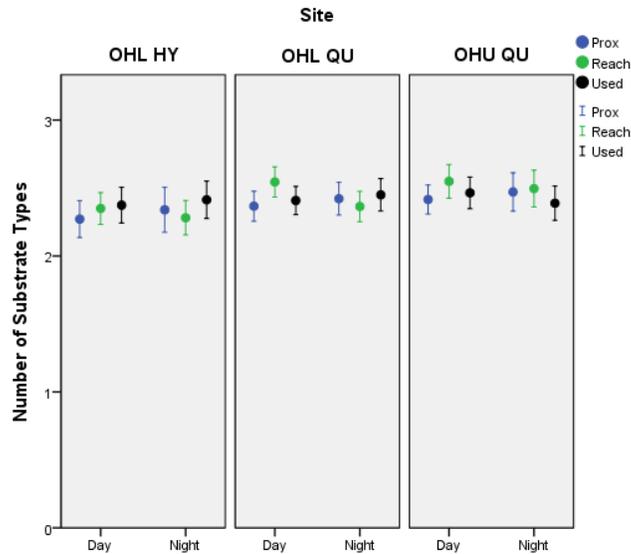


Figure 41. Means and 95% confidence intervals for the number of substrate types (heterogeneity) observations at proximate-level availability (left most), reach-level availability (middle), and used locations (right most). Data were collected at sites 1SYN (OHL) and 2ALLO (OHU) for *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU).

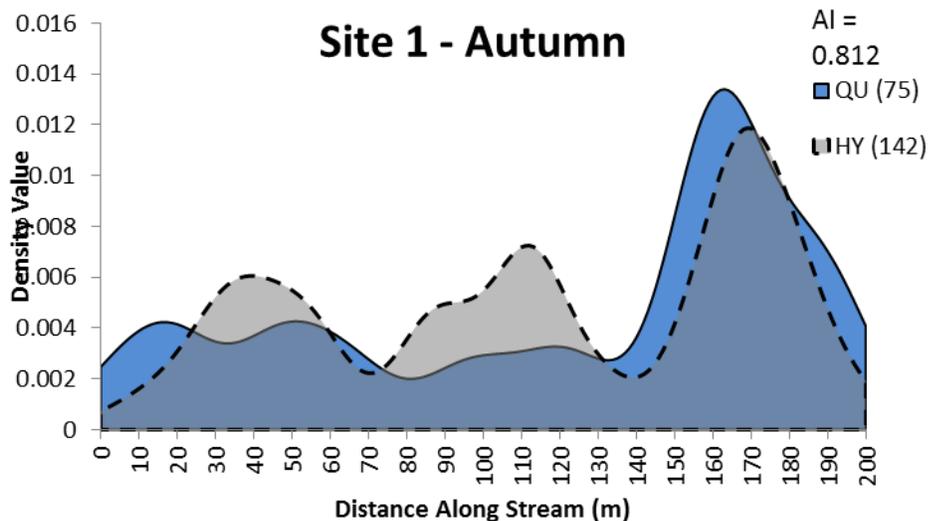


Figure 42. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 1 (downstream portion of 1SYN) during autumn sampling. Area of intersection index (AI) value represents the amount of space-use overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

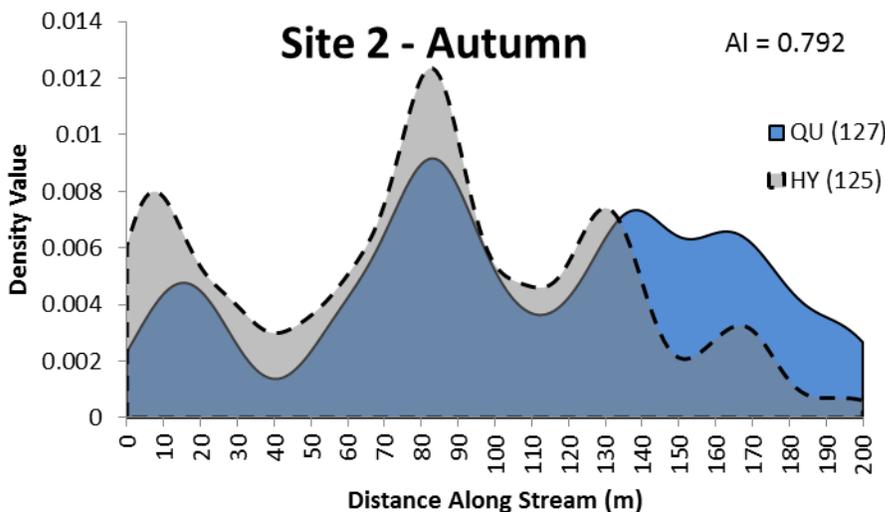


Figure 43. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 2 (upstream portion of 1SYN) during autumn sampling. Area of intersection index (AI) value represents the amount of overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

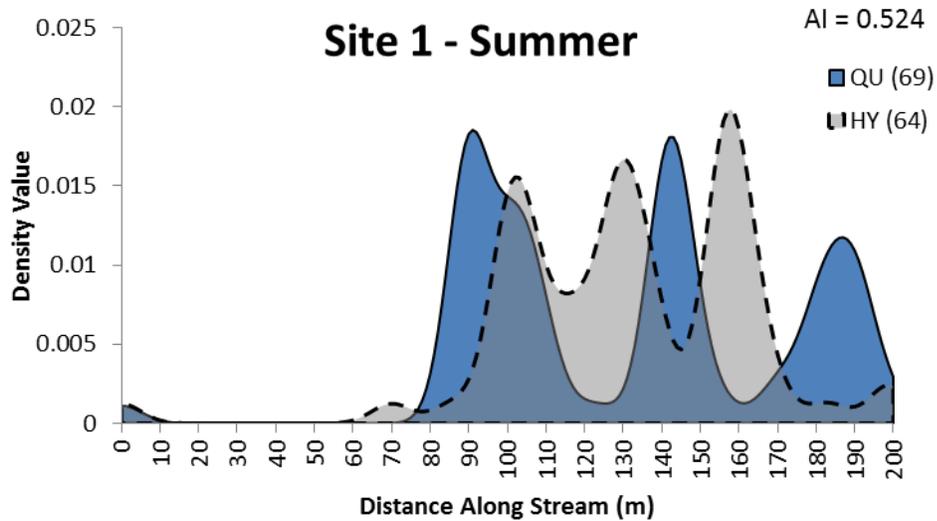


Figure 44. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 1 (downstream portion of 1SYN) during summer sampling. Area of intersection index (AI) value represents the amount of space-use overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

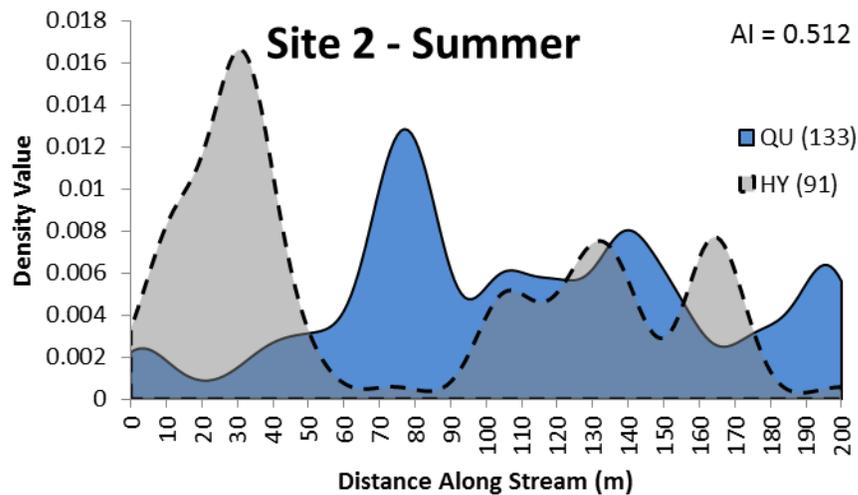


Figure 45. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 2 (upstream portion of 1SYN) during summer sampling. Area of intersection index (AI) value represents the amount of space-use overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

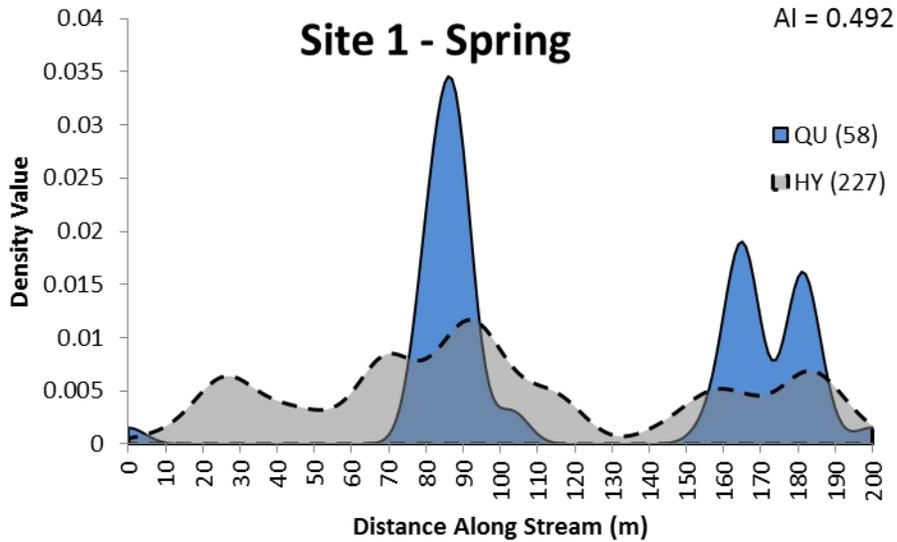


Figure 46. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 1 (downstream portion of 1SYN) during spring sampling. Area of intersection index (AI) value represents the amount of space-use overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

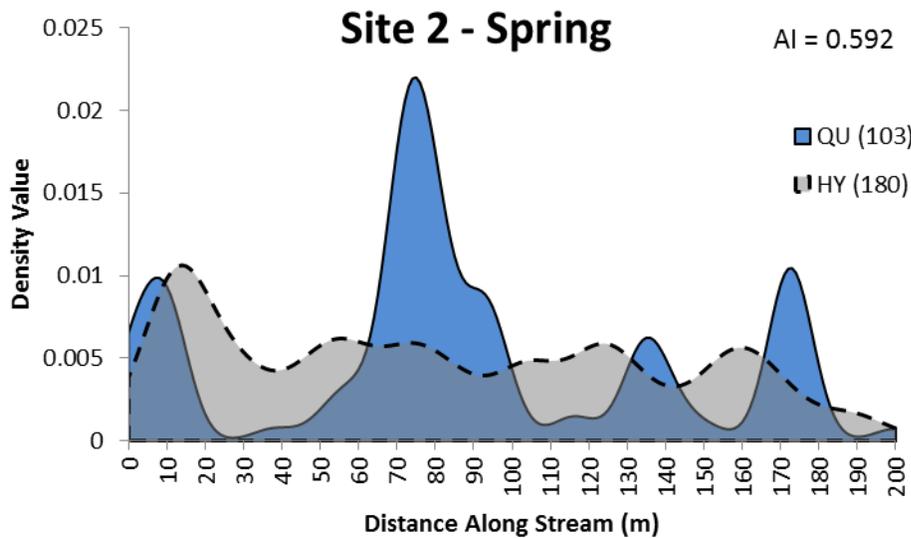


Figure 47. Utilization distributions for all *Orconectes quadruncus* (QU) and *Orconectes hylas* (HY) individuals located at Site 2 (upstream portion of 1SYN) during summer sampling. Area of intersection index (AI) value represents the amount of space-use overlap between the species. Numbers in parentheses represent the number of locations used to create the utilization distributions.

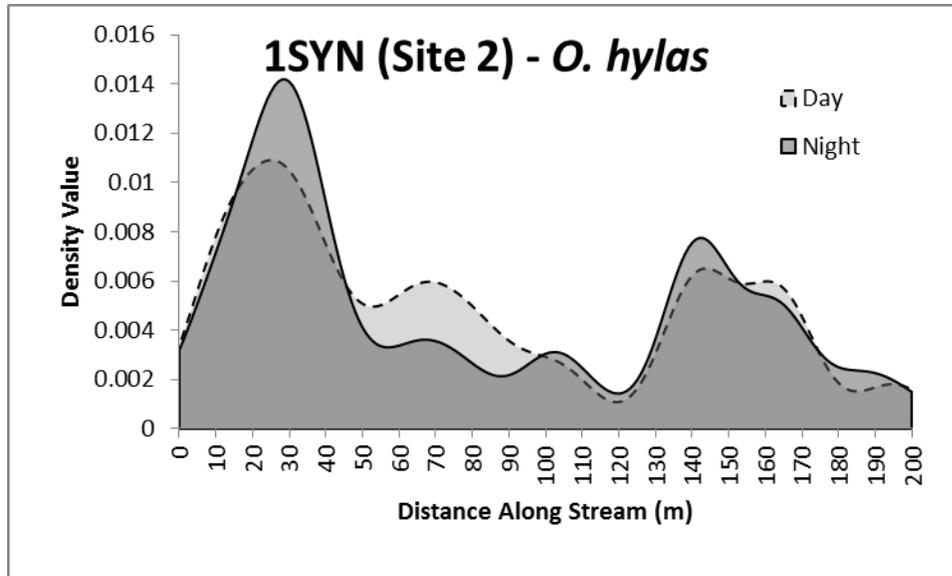


Figure 48. Utilization distributions for all *Orconectes hylas* individuals located at Site 2 (upstream portion of 1SYN) during diel sampling.

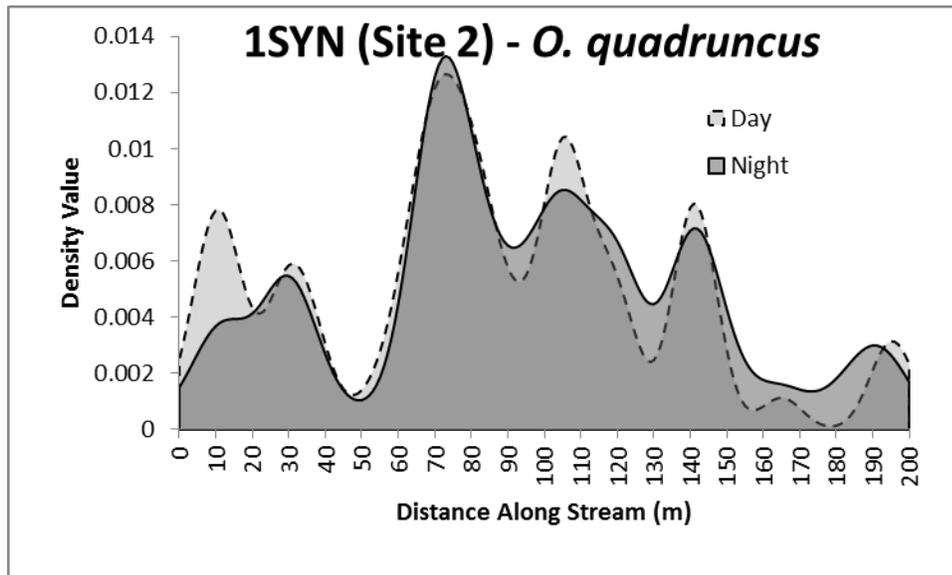


Figure 49. Utilization distributions for all *Orconectes quadruncus* individuals located at Site 2 (upstream portion of 1SYN) during diel sampling.

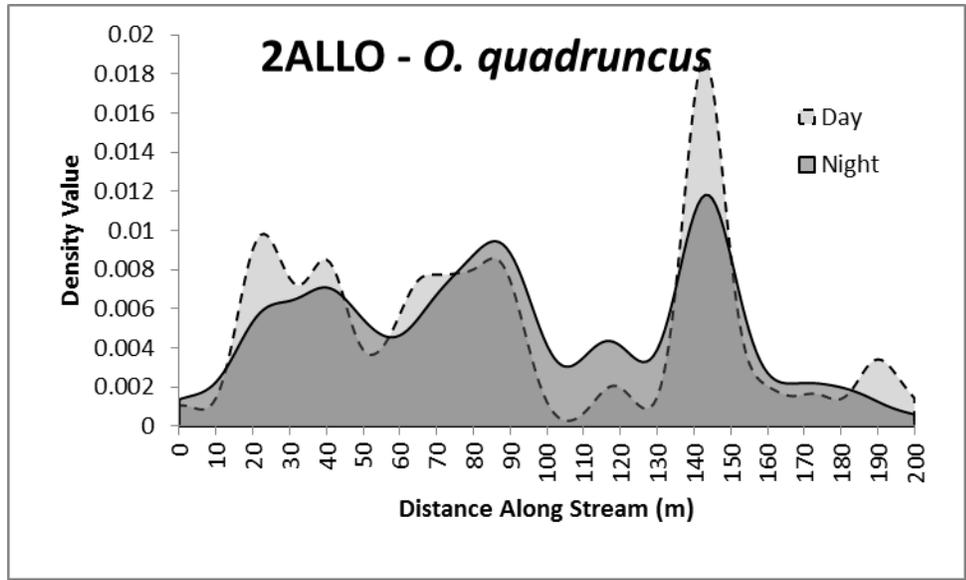


Figure 50. Utilization distributions for all *Orconectes quadruncus* individuals located at Site 2 (upstream portion of 1SYN) during diel sampling.

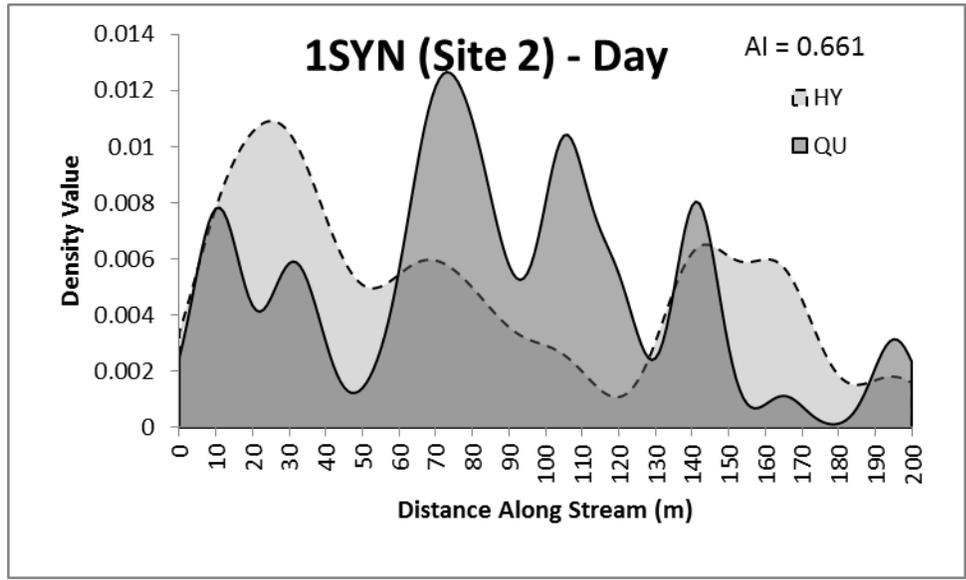


Figure 51. Utilization distributions for all *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) individuals located at Site 2 (upstream portion of 1SYN) during day sampling in the diel season. Area of intersection index (AI) value represents the amount of overlap between the species.

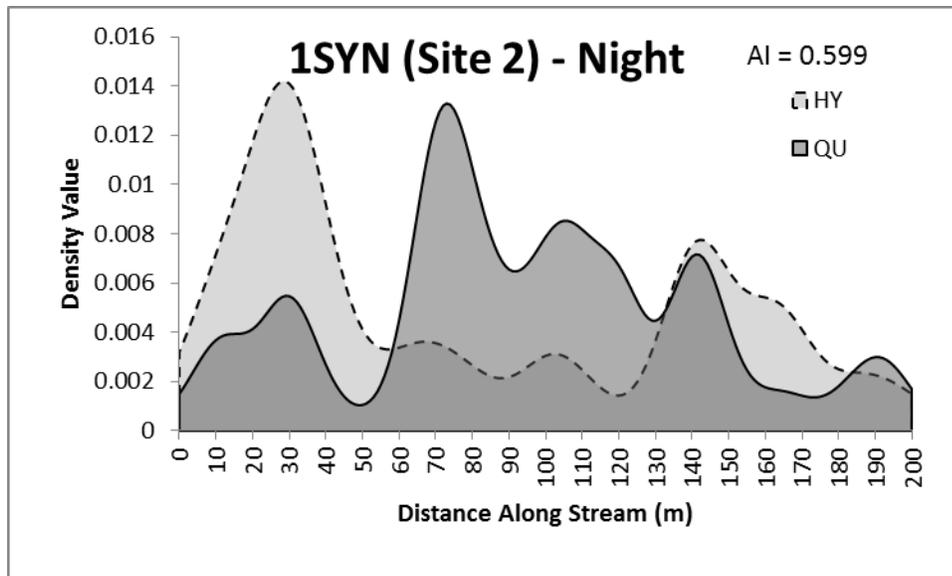


Figure 52. Utilization distributions for all *Orconectes hylas* (HY) and *Orconectes quadruncus* (QU) individuals located at Site 2 (upstream portion of 1SYN) during night sampling in the diel season. Area of intersection index (AI) value represents the amount of overlap between the species.

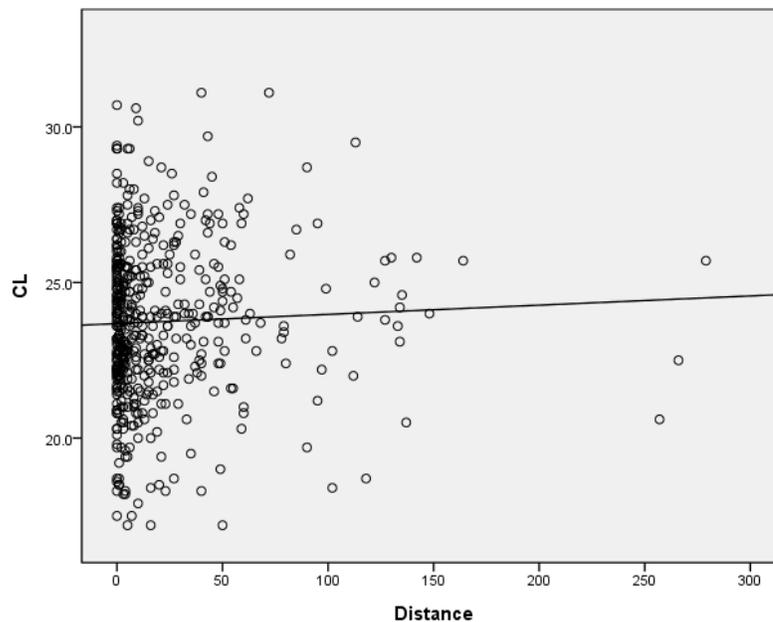


Figure 53. Relation between carapace length in mm (CL) and total distance travel by an individual crayfish during the seasonal portion of the study (summer, fall, and winter data combined).

CHAPTER 4

UPPER ST. FRANCIS RIVER DRAINAGE CRAYFISH FAUNA INTERIM MONITORING PLAN

INTRODUCTION

Definitions

Occupancy: Probability a randomly selected stream segment is occupied (i.e., contains at least one individual) by a species of interest (adapted from MacKenzie et al. 2006)

Detection probability: The probability that a member of the species of interest is included in the count (observed) at the time or location of interest (MacKenzie et al. 2006). A value of 1 indicates the species was always detected and a value of 0 indicates the species was never detected.

Upper St. Francis River Drainage (SFRD): Streams in the St. Francis River drainage of Missouri that are upstream of Wappapello Dam, excluding those impounded by Wappapello Reservoir.

Stream segment: Any section of stream between two tributary junctions as indexed by the segment identification codes used in the Valley Segment Type (VST) GIS data layers created by the Missouri Resource Assessment Partnership.

Sample Unit: Same as a stream segment from above. A stream segment in the St. Francis River drainage and the unit at which we wish to establish the presence or absence of an organism (equivalent to “site” as used in MacKenzie et al. 2006). Only a portion of a selected sample unit will actually be sampled, but the outcome of the sampling will be applied to the entire stream segment.

Survey Unit: One of three repeated surveys conducted within a sample unit. For this monitoring strategy the survey unit is replicated in space, not time.

Sub-sample: One of six, randomly located 1-m² seine samples taken within a survey unit.

Background

The goal of monitoring is often to gather information needed to detect changes in trend or process that can be used to guide decisions intended to conserve a species. Monitoring the status of rare or endangered organisms is used as a way to track population trends, evaluate conservation or management actions, or provide baseline information for future study (Zielinski and Stauffer 1996, Bisbal 2001). Factors threatening rare or endangered organisms are also monitored and can include the spread of invaders or diseases and the alteration of biotic or chemical conditions (Campbell et al. 2002). Information on threats to species persistence can be more informative than species status, especially when the threat causes rapid decline of a population (Campbell et al. 2002).

Monitoring efforts are commonly suggested but are not always implemented due to lack of funding, manpower, or taxonomic bias (Campbell et al. 2002). Other times monitoring effort is made, but not at a level that provides sufficient data to detect important trends or otherwise meet monitoring goals (Bisbal 2001, Maxwell and Jennings 2005). Effective monitoring efforts clearly identify achievable goals that are biologically relevant and set quantitative benchmarks for those variables that once reached initiate management actions (Bisbal 2001). Failure to address these components results in ineffective monitoring that may waste resources better directed toward other endeavors.

The invasion of *Orconectes hylas* in the upper St. Francis River drainage (SFRD) is an example of a situation whereby monitoring may be a viable tool to assist managers in

taking appropriate and timely conservation action. Monitoring populations of *Orconectes peruncus* and *Orconectes quadruncus* was identified as a priority research and inventory need by the Missouri Department of Conservation for the St. Francois Knobs Conservation Opportunity Area which overlaps much of the native range of these two species (Missouri Department of Conservation 2005).

Methodological Considerations

Many potential informative metrics exist that are of interest to managers and could be obtained through monitoring crayfishes in the SFRD, but it is important to choose monitoring metrics that are most informative to monitoring and management goals. Nowicki et al. (2008) recommend a mark-recapture approach to monitor population size of crayfishes. This approach was appropriate for their goals, but may not be appropriate for other goals as it is limited by the required high sampling effort and a limited number of sites. For instance, monitoring crayfish abundance or estimating population size at a small number of sites may not fully represent changes that occur range-wide for a given species and limit inference. It is already known that populations of *O. peruncus* and *O. quadruncus* decline or are eliminated at locations where *O. hylas* has established; thus, further documentation of these processes at select sites yields less information than an approach that considers range-wide changes. Peay (2003) conducted monitoring at a coarser spatial scale where white-clawed crayfish *Austropotamobius pallipes* abundance (number of crayfish observed per 10 refuges searched) was used as a monitoring metric. This approach allowed for a greater spatial inference, but was constrained because it lacked the ability to estimate crayfish density, did not account for

the effects of species using different habitats on relative abundance estimates, and did not account for detection probability. Addressing these issues is not important for all monitoring plans, but can be done with a different sampling design.

Management goals for crayfishes in the SFRD can be informed through estimating the proportion of stream segments occupied by a species of interest and trends of occupancy (ψ) over time. Occupancy estimation and modeling provides a framework for estimating the percentage of stream segments occupied by a species of interest given detection probability is less than one (MacKenzie et al. 2002). Detection probability is estimated in this framework and indicates how likely the sampling was to detect the species. Understanding detection probabilities is important for the SFRD situation because knowing the likelihood of encountering a species given it was present can determine confidence in distributional data for both the invasive and native crayfishes. In addition to accounting for imperfect detection, the covariates affecting detection and occupancy can be modeled in an information theoretic framework allowing for greater understanding of factors that influence these parameters (MacKenzie et al. 2002). Occupancy estimation has become a common monitoring tool (MacKenzie et al. 2006, Mattfeldt et al. 2009) and is the metric selected for the SFRD crayfish situation.

Management and monitoring objectives for crayfishes in the SFRD are listed below. These objectives are based on my suggestions alone and would benefit from further refinement based on discussion of all parties involved in managing the species.

Management Goals

1. Ensure wild populations of *Orconectes peruncus* and *Orconectes quadruncus* exist in the Upper St. Francis River drainage in at least 25% of their historical range.
2. Reduce or eliminate the spread of *Orconectes hylas* throughout the Upper St. Francis River drainage.

Monitoring Goals

1. Regularly estimate the occupancy parameter for *Orconectes peruncus* and *Orconectes quadruncus* in the Upper St. Francis River drainage.
2. Document changes in range size of *O. peruncus* and *O. quadruncus* in the Upper St. Francis River drainage.
3. Document changes in range size of the invasive *Orconectes hylas* in the Upper St. Francis River drainage.
4. Determine if *O. hylas* is able to establish in an upstream or downstream tributary of the St. Francis River from the confluence of a current occupied stream.

Successful establish of such locations indicates the ability of *O. hylas* to disperse though large lotic systems.

Part I: Background Sampling

Naïve Occupancy Estimation

Data were used from other crayfish sampling efforts in the drainage and dedicated pilot work to establish several parameters used to design monitoring protocols (Riggert et al. 1998, J. Westhoff, unpublished data). Existing presence and absence data were used to estimate naïve occupancy (ψ_n) for *O. hylas*, *O. peruncus*, and *O. quadruncus* (Table 22; see Appendix D for calculation details). Naïve occupancy estimates do not account for detection probability and are expected to underestimate true occupancy (MacKenzie et al. 2006); however, ψ_n may serve as a rough baseline against which qualitative comparisons can be made. Naïve occupancy was estimated for both the known species compositions in 2008 (ψ_n *O. hylas* = 0.20; ψ_n *O. peruncus* = 0.37; ψ_n *O. quadruncus* = 0.39) and hypothesized compositions had *O. hylas* not been introduced into the SFRD (ψ_n *O. peruncus* = 0.42; ψ_n *O. quadruncus* = 0.48). Formal comparison of ψ_n to occupancy estimates from the proposed monitoring framework is not advised because the methods to determine them differ. Estimation of ψ can also be useful when determining sample size requirements and sampling strategy.

Gear Selection

Several gear types exist that are suitable for sampling lotic crayfishes in rocky-bottomed streams (Rabeni et al. 1997, DiStefano et al. 2003). Historically, 1-m² kick seine samples, 1-m² quadrat samples, overnight trapping with conical wire-mesh fish traps, and hand searching with aquarium nets have been used to collect crayfishes in the drainage (Riggert et al. 1998, DiStefano and Westhoff in press). Each gear type has

biases, time requirements, and costs. Data collected from pilot sampling efforts were used to compare gear types and select the most appropriate sampling strategy. The average number of samples taken to detect a species was compared between 24 sample sites where 1-m² quadrat samples were taken and 27 sample sites where 1-m² kick seine samples were taken. Similar numbers of samples were required to detect each species regardless of gear type based on overlapping 95% confidence intervals (Table 23). Estimates for seine samples may be artificially low because sample location was not random within a site and “good” habitat was likely sampled first, followed by less desirable habitats; however, effort was made to sample all available habitats. Quadrat sample locations were random.

Each gear type also has biases associated with crayfish characteristics such as species, sex, and activity, as well as habitat biases related to water depth, current velocity, and complexity. Data were not collected to address these biases quantitatively, but traps are able to sample deep water (>0.5 m) and structurally complex (i.e., boulder or woody debris present) habitats more effectively than seines. Traps require at least 10 cm of water and are biased towards capturing larger, more active individuals (Rabeni et al. 1997, J. Westhoff, personal observation). The number of crayfish captured per trap (0.34 ± 1.0 ; mean \pm standard deviation) was significantly lower (t-test; $p < 0.001$, 103 df) than the number captured in 1-m² seine samples (6.4 ± 6.3) at sites where both gears were used. Quadrat samples are thought to be more effective at sampling deep into the substrate (15 cm) than are seines and were 69% efficient at capturing riffle-dwelling *Orconectes* crayfish in the Ozarks (Larson et al. 2008).

Information on time required for each of the two gears was recorded to estimate and compare person-hours per sample required for each gear. Four, 1-m² seine samples could be obtained in one person hour, whereas only a single 1-m² quadrat sample could be obtained in one person hour. Similar data were collected for trapping efforts and it was estimated that 12 traps could be set in one person hour. Trapping requires a return visit to the site which takes one person approximately two hours to collect traps and record crayfish data. Estimates for all gear types can be influenced by crayfish density, personal experience, environmental conditions, and many other factors. Hand collecting was most reliant on these factors and thus was not used considered appropriate for this monitoring strategy.

1-m² seine samples represent the most feasible gear type to use for monitoring efforts in wadeable streams in the SFRD. Four times as many 1-m² seine samples can be taken for every one 1-m² quadrat sample, and the number of samples need to detect a given species was nearly identical for both gears. Trapping takes a similar amount of time per person, but often produces lower numbers of individuals and rarely produces young-of-the-year individuals. Seining efforts may be supplemented with traps in large, non-wadeable streams. Hand collecting should only be used to supplement seining, as it is the most qualitative method and provides the least amount of information.

Part II: Establishing a monitoring framework

Sample Unit Selection

There are three categories of sample units within the SFRD that are of interest regarding monitoring objectives.

1. ***Orconectes hylas* suspected or occupied sample units (SOS)** – Stream segments that are known to harbor *O. hylas* or in unsampled stream segments that are within areas known to have *O. hylas*.
2. **High invasion risk of *O. hylas* sample units (HRS)** – Stream segments upstream or downstream of known edges of *O. hylas* occupied segments extending to the headwaters of the given stream or to the confluence of that stream with the St. Francis River. Additionally, segments of the St. Francis River upstream and downstream to the next major tributary junction, as well as the first segment of those tributaries.
3. **Low invasion risk of *O. hylas* sample units (LRS)** – Any stream segment not included in the previous two categories.

Each category of sample units contains unique information that can be applied to the monitoring objectives. Monitoring of SOS provides information on changes in relative abundance of native species exposed to the invader and confirms that populations of *O. hylas* are persisting. Information gained from HRS includes range expansion and colonization rates for *O. hylas* and range contraction and extirpation rates for the native

species. Monitoring of LRS provides background relative abundance data and provides a mechanism to identify previously unknown invasions. Information from all sample units can be combined to determine drainage-wide occupancy and relative abundance and insures that all three categories of sample units are included for those inferences.

There are 32, 12-digit hydrologic units (HUs) comprising 2,159 stream segments in the SFRD (Table 22). *Orconectes peruncus* has been collected from 14, *O. quadruncus* from 15, and *O. hylas* from eight HUs. Eight HUs are not known to harbor any of these three species, leaving 24 HUs harboring species of interest. Two sampling units will be randomly selected from each of these 24 HUs. At least one sample unit must be from the HRS category if any stream segments meet those criteria in the HU. The other randomly selected sample unit can be of any category. The first site selected in each HU will be a permanent monitoring location. The second sample unit in each HU will be randomly selected prior to each sampling season. This approach allows for estimation of colonization and extinction rates from the permanent sites and allows more extensive sampling throughout the drainage. Monitoring will begin in 2012 and will occur every third year. More frequent sampling is thought to be cost prohibitive and less frequent sampling may reduce the ability to detect trends (see Adaptation of monitoring strategy section). Given that two sample units can be completed in one day and a crew must travel to the drainage, six sample units could be done in one week (Table 24). A total of 48 sample units can be sampled in eight weeks of effort during a season. Based on simulations run using standard error calculation procedures for standard designs (MacKenzie and Royle 2005), this number of sample units is expected to yield a standard error value of approximately 0.075 based on suspected detection probabilities (0.5 to 1),

naïve occupancy values (0.3 – 0.4), and four surveys per season (Table 25). Lower standard error values are desired to better detect trends, but logistical constraints will likely not allow for more sampling. Other studies using occupancy modeling report standard error values from 0.07 – 0.22 (Mattfeldt et al. 2009) and from 0.07 – 0.13 (MacKenzie et al. 2002).

Field sampling rarely works out as planned (J. Westhoff, personal observation) and thus the schedule may be altered somewhat by several factors. First, the proposed schedule does not account for sample unit scouting efforts. It can take several weeks to locate suitable sample units and secure landowner permission. It is best if these activities can be done prior to the main sampling effort. If sampling is completed early one day, the remainder of the day can be used to scout additional sample units. Second, the estimate of two sample units a day is conservative and sampling will likely take less than three hours at a given sample unit if a crew of three or four people is used. The time required per sample unit relies heavily on the number of crayfish that must be processed. Sample units with low densities of crayfish will likely require less time, and those with high densities may require more. On days when sampling is finished early, a third sample unit may be sampled allowing for more than six per week. Third, crew experience should increase with time as personnel become familiar with methods and crayfish identification. Sampling rate may increase by the third or fourth week of sampling, potentially allowing for three sample units a day. Forth, driving time will vary for sample units as they are spread over the drainage. This will not likely reduce the number of sample units below two per day, but may increase the number of sample units per day when sample units are close. Fifth, this schedule assumes the sampling crew will

have to travel four hours to reach the drainage (Table 24). If a local crew can conduct sampling then it is likely that at least eight sample units can be sampled during a week. This also impacts the monitoring budget as a local crew does not have many associated travel expenses. A distant crew would require funding for lodging, food, and additional mileage (see budget section).

Sampling Methods

Sampling will occur from May through August and will constitute one season. These dates align with the time when personnel are often available and when crayfish are thought to be active and most effectively captured (J. Westhoff, personal observation). Sampling done near the end of the season may be limited by low water levels in many small streams in the SFRD. Conclusion of sampling prior to July 15, if possible, may reduce this problem. All sampling should occur during daylight hours to avoid any differences in crayfish activity level or sampling efficiency that may affect detection probabilities (Hamrin 1987).

Upon arrival at a previously un-sampled monitoring sample unit, four independent stream reaches each consisting of one discrete high-flow habitat (i.e., riffle and/or run) connected to one discrete low-flow habitat (i.e., pool) will be identified (Figure 54). The downstream most point in sample unit will be located at the downstream end of the lowest riffle and marked using a GPS unit (Figure 54). Three of the four reaches will be randomly selected (with replacement) in order to meet closure assumptions (MacKenize et al. 2003) and will serve as the repeat surveys of the sample unit. Spatial replication of survey units instead of temporal replication is intended to

reduce the amount of time required to complete sampling at a sample unit over the season. Three repeat surveys are recommended and exceed the optimum number suggested by MacKenzie and Royle (2005) based on expected high (>0.7) detection probabilities and mid-range (0.25 – 0.75) occupancy estimates. This level of effort exceeds recommendations from simulations by Guillera (2010) and should maximize detection probability.

Six, 1-m² seine samples will be randomly conducted in each of the three selected reaches beginning in the most downstream unit and will serve as sub-samples. Sample location within a habitat unit will be determined by starting on the right-bank descending side along the downstream edge and using the random number table to select the location (Figure 55). The first random number represents the percentage of the way across the stream the sample will occur and the second number represents the percentage of the way along the stream the sample will occur. Three sample locations will be located in high-flow habitat (riffle or run) and three in low-flow habitat (pool). Sample location will be determined prior to any sampling in the reach and regardless of selection order, the most downstream location should be sampled first. Any randomly selected samples located in unsuitable habitat (silt, sand, bedrock, or immovable boulder) should be discarded and re-selected. This is done to avoid sampling habitats where crayfish are almost certainly absent or where seining is not effective for capturing crayfish. No 1-m² seine sample should be taken within 0.5 meters of a previous sample. If a given habitat unit is selected for multiple sampling events it should be large enough to accommodate all seine samples. If it is not, another habitat unit should be selected. This process will be repeated for each selected habitat unit for a total of 18, 1-m² seine samples at each monitoring unit and

should take approximately two hours. Conducting 18 samples within a sample unit should result in high detection probabilities for the three species of interest based on pilot work results (Table 23) in which no more than 10 samples were required to detect a species at any site.

Sample unit information will be recorded and will include nearby land cover, riparian condition, visible stream disturbance, landowner name, start and end time, and weather conditions (Table 26; Appendix E). Readings for water temperature, dissolved oxygen, and pH will be taken at one location in the downstream most riffle and pool. At each sample location habitat information will be recorded comprising water depth (taken using marked seine brail or depth rod), dominant and sub-dominant substrate types (modified Wentworth scale), and relative current velocity (high, medium, or low)(Table 27; Appendix F). This information can be used to incorporate heterogeneity in occupancy modeling and serves as a record of sampling conditions.

All crayfish captured will be identified to species and enumerated. If species cannot be determined, the individual will be recorded as unknown (UK) on a datasheet (Appendix F). Sex, form, and adult or young-of-year (YOY) status will be recorded. As a general rule, crayfish will be considered adults if carapace length (CL) exceeds 12 mm (Riggert et al. 1999). This break-point can be altered in the field if evidence exists that suggests it is not applicable. Data will be recorded separately by seine sample.

Methodological Flexibility

There is variation in the SFRD among and within potential sampling units which requires the adaptation of sampling methods. Several potential issues are listed below and solutions are presented.

1. If the sample unit is on a small stream it may be difficult to conduct six random samples in a single survey unit. In this case, potential survey units can be combined to ensure adequate spacing for all samples (Figure 54). This approach will require additional survey units to be identified.
2. If the sample unit is on a large stream it may be difficult to locate and identify five survey units. To avoid having to traverse long pools in these situations, one survey unit can be divided into two survey sections and counted towards the five survey units (Figure 54).
3. If the sample unit is on a larger stream and 1-m² seining is not effective, sampling can be supplemented with baited traps set overnight. I recommend setting 12 traps in each survey unit following the same sub-sample location methods described for the seines, but increasing the minimum distance between traps to five meters increase the independence of the sub-samples. Traps should be baited with canned dog food and left overnight as described by DiStefano and Westhoff (in press).
4. Other sampling gear types can be considered in situations where large streams are not effectively sampled with traps or 1-m² seines. Some options include hand collection of crayfishes with the aid of SNUBA , large seines (3-5 m long), or a modified Missouri trawl method (Herzog et al. 2009).

5. Sampling should not occur if water levels are high due to a recent rain event (based on sampling crew discretion).
6. It is expected that as *O. hylas* expands in the drainage, some stream segments will need to be redefined according to the classification criteria (SOS, HRS, LRS) prior to sample unit selection for the following year.

Part III: Interpretation and application of monitoring results

Data Entry and Analysis

Each sample unit will be given a unique 8-digit number following a specific format. The first four digits represent the year (e.g., 2011), the last four represent the valley segment type (VST) segment identification number (excluding the 8020202 that precedes all segment identification numbers in the SFRD) from the VST shapefile (i.e., 0001 - 2166). The example number 20110001 represents a sample unit visited in 2011 and located on VST segment 0001. This numbering system provides spatial and temporal information about each sample.

All data will be entered into a MS Access database consisting of six tables. The SURVEY table will contain data on the number of crayfish captured in each subsample and the sub-sample covariate information. The COORDINATES table will contain all GPS readings for the sample unit, and the SAMPLE_UNIT table will contain the information about the sample unit and the sample unit covariates. The other three tables will serve as the occupancy modeling input tables for each species.

Occupancy estimates and detection probabilities for the three species of interest are the most informative metrics for the SFRD monitoring strategy; however, sampling design allows for collection of information related to other metrics that may be informative to management goals. Crayfish density can be used as a proxy for the ecological impact of *O. hylas* on the system. Crayfish densities that are higher when the invasive is present as compared densities prior to invasion may indicate alteration of energy flow in the system as secondary production may be effected by the altered biomass structure (Hall et al. 2006). Crayfish density is known to vary temporally so careful consideration should be given to analysis and interpretation of density results (DiStefano et al. 2003). Relative abundance information can be used to look for associations between the presence of the invader and declines in the native crayfish fauna. The distributional data collected from this monitoring effort can also be used to verify and update the probability of species presence models described in Chapter 2 of this dissertation.

Occupancy modeling will be conducted in program PRESENCE. Occupancy will be estimated for *O. hylas*, *O. peruncus*, and *O. quadruncus* separately after the first sampling season using a single-season model. This will be done to determine if the sampling strategy is effective and will provide baseline occupancy estimates for each species. Upon completion of subsequent sampling, multiple season models will be used to estimate occupancy. Multiple season models allow for the inclusion of explicit dynamics related to colonization and extinction parameters (MacKenzie et al. 2003, Mattfeldt et al. 2009). Occupancy of sample units in this study should not vary randomly over time, thus colonization and extinction should respond as a Markov Chain Process

where occupancy at time T_1 is partially dependent on occupancy at time T_0 . Trends in these parameters can be investigated by including a linear time covariate in the parameters as a candidate model. Information will exist to estimate occupancy for other species in the drainage and can be done if desired. Occupancy modeling requires training and should be conducted by an individual with prior experience or training.

An advantage to using an occupancy modeling approach to monitoring is the ability to use sample unit and sampling covariates to estimate occupancy and understand what factors (natural or sampling artifacts) affect occupancy and detection probability. Suggested methods for this monitoring plan reduce heterogeneity that influence these parameters, but some heterogeneity remains and can potentially be modeled. Predicted relations between covariates and estimated parameters, along with hypothesized reasons, are presented in Tables 26 and 27. Any covariate that affects detection probability indirectly affects occupancy estimation due to the relation of the parameters. Covariates affecting occupancy don't necessarily affect detection probability.

Covariates can be incorporated into candidate models to account for heterogeneity and used in a model selection framework (Burnham and Anderson 2002) within PRESENCE . Numerous candidate models can be formulated and a few of the more likely models for the SFRD monitoring strategy are listed in Table 28. Many of the covariates will likely have little influence on occupancy and detection probability estimates because of the way in which they were collected (e.g., qualitative estimates of depth and current velocity) or from limited temporal relevance (e.g., one observation of temperature during a year). It is also likely that information on factors potentially related to occupancy will not be collected (e.g., density of fish predators). The main purpose of

this monitoring is not to understand what influences occupancy rates, rather what those rates are and if they are changing. Information gained through modeling covariates can increase the accuracy of parameter estimates and provide “bonus” information on species ecology. Sampling techniques can be adjusted to maximize detection probability based on the relation of covariates to that parameter. Information collected on covariates that is not used for modeling can be used to describe sampling conditions and better frame the inference of results. Future work may require the collection of different covariates or creation of alternate candidate models.

The stratification approach to sample unit selection requires an extra step be taken prior to determining drainage wide occupancy for any species (MacKenzie and Royle 2005). Each 12-digit HU has a unique number of stream segments (32 – 117) so occupancy must be calculated for each HU and weighted by the percentage of stream kilometers in that HU before combining the estimates into one estimate for the area of inference (see Appendix D for details). Failure to do this step may bias the estimate by giving greater influence to HU with fewer stream segments than those with many stream segments. The individual HU occupancy estimates can be determined in PRESENCE, but the weighting must be done outside of that program. If the best overall model for occupancy did not include any covariates for occupancy (i.e., $\psi(\cdot)$ model) then simply summing the weighted HU occupancy estimates is appropriate. However, if the best model has covariates for occupancy, then the regression coefficients from the model must be used to further weight the individual HU occupancy estimates prior to determining the overall estimates.

Alternatively, this problem could be addressed by adding a candidate model where the number of segments in the HU are used as a covariate (e.g., $\Psi(\text{Segments in HU})p(\cdot)$ from Table 28). Little or no support for that model indicates that occupancy varied randomly with the number of stream segments in the HU and thus does not significantly bias the overall occupancy estimate. Support for that model indicates that the stratification method did bias the estimates and the method described in the preceding paragraph should be used.

Management Thresholds

Monitoring is not effective as a management tool if thresholds and benchmarks are not set as trigger points for conservation action (Bisbal 2001). This monitoring strategy provides a method in which trends in occupancy can be detected, but at some level of occupancy action should be taken beyond just further documenting the demise of a species. I suggest setting conservation action thresholds based on changes in occupancy estimates over time. Naïve occupancy estimates of the hypothesized compositions had *O. hylas* not been introduced into the SFRD (ψ_n *O. peruncus* = 0.42; ψ_n *O. quadruncus* = 0.48) may serve as a baseline for these recommendations. Occupancy estimates less than 0.20 for either species may be an appropriate threshold as these values represent a greater than 50% decrease in species distribution. This threshold value lacks stringent biological significance, but may represent a significant societal benchmark that can act as a catalyst for management or further refinement of biologically significant thresholds as determined using population demographics in a population viability analysis type approach.

Actions meant to protect *O. peruncus* or *O. quadruncus* may include listing under the U.S. Endangered Species Act, protection of existing wild populations through the installation of dispersal barriers, removal of *O. hylas* from strategic locations (e.g., leading edge of invasion, stream segments where species displacement is slow), or captive breeding and reintroduction efforts (Further discussed in Chapter 5). The efficacy of management actions can be assessed by observing occupancy patterns post-implementation. This approach informs decision makers about not only when to take action, but also if those actions are successful.

Adaptation of Monitoring Strategy

It is possible that the methods proposed in this monitoring plan will not be applicable given unforeseen budget constraints or shifting agency priorities. The proposed monitoring approach attempts to maximize information and minimize expenditure. In a scenario where additional resources are available there are two changes that maximize information. The first is to conduct the sampling on a shorter rotation than every third year. This provides better temporal resolution for occupancy estimates and should enable managers to detect trends more quickly. It also provides a greater presence of scientists in the area which can further education campaigns and enables more frequent contact with private landowners. The second change that maximizes information is to increase the number of sample units visited during a sampling season. This provides greater spatial coverage of the drainage and should increase the ability to detect changes in occupancy estimates. Increasing the sampling effort within a sampling unit (e.g., more sub-samples, more surveys) may decrease the uncertainty of occupancy estimates, but

significant gains will be made only if detection probabilities are low (MacKenzie et al. 2006) and is not recommended for this situation.

A reduction of resources dedicated to this monitoring effort will result in a loss of information relative to the proposed plan. Less stringent sampling than what is proposed may render the occupancy estimation approach inappropriate. However, the occupancy approach may work given longer periods between sampling seasons (5 years as opposed to 3 or less). This reduces the temporal resolution of the sampling and may allow for significant declines to go undetected. Less frequent sampling also introduces greater risk of agency personnel turnover and shifting agency priorities that may result in the termination of the monitoring activity. It may be feasible to reduce the number of sample units visited during a sample season, but that change would likely yield high standard error values associated with occupancy estimates which may make trend detection more difficult (Table 25). Reducing sampling effort within a sample unit (e.g., fewer surveys or fewer sub-samples) is not expected to save significant time or resources as the resource consumptive aspect of this sampling is getting to sample units, not sampling once there. However, if detection probabilities are near one, a reduction in the number of surveys to two per sample unit may save some time without increasing variance significantly.

Reductions more severe than those mentioned above may result in an approach to monitoring not related to occupancy modeling. The spread of *O. hylas* in the SFRD is believed to be the greatest threat to the persistence of *O. peruncus* and *O. quadruncus* and so the minimum monitoring effort should focus on documenting the spread of the invasion. This can be accomplished by visiting stream segments adjacent to or nearby the known leading edges of the invasion (i.e., stream segments categorized as SOS).

Sampling protocols described in this plan can be used, but the metric of interest is no longer occupancy, rather detection probability within a sample unit. Detection probability can be calculated with the reduced dataset and will provide managers information about how effective the sampling was at detecting the invasive, given it was not observed.

Part IV: Proposed Budget

The following section includes estimates of financial costs associated with implementation of this monitoring strategy. All estimates will likely vary from what is presented and do not include overhead charges. Estimated salary expenses for a distant crew of four people (travel status required) are \$11,520 and the associated food and lodging fees are estimated to be \$6,080 (Table 29). If a local crew can be used (no travel status required), the costs of food and lodging are unnecessary and sampling could occur for 12 weeks for roughly the same expense of eight weeks from a distant crew (Table 29). Additional sampling time could be used to visit more sampling units or directed towards other goals not affiliated with this monitoring strategy. Expenses will be incurred each sampling year, but some items will not have to be purchased after the initial sampling season (Table 30). Equipment could be borrowed from other biologists and projects as it is likely not feasible to purchase some expensive items (e.g., GPS unit or vehicles) solely for the purpose of this project.

Conclusions

Orconectes peruncus and *O. quadruncus* are cryptic species in the sense that much of the general public does not know of their existence and most biologists rarely encounter or recognize them, even when sampling other aquatic species in the SFRD. It is unrealistic to depend on casual observations from the public or private sector to inform managers of the status of either species. A dedicated monitoring strategy should be adopted to follow distributional trends and threats for both species. Implementation of this flexible monitoring strategy should provide the required information for managers and decisions makers to best conserve *O. peruncus* and *O. quadruncus* given the available options.

Table 22. Categorization of stream segments in the Saint Francis River drainage (SFRD) by 12-digit hydrologic unit code (HU). Columns represent characteristics of the stream segments in each HU and are; the number of stream segments, the percentage of total stream segments, the total kilometers (km) of stream, segments suspected or occupied by *Orconectes hylas* (SOS), segments at high risk of *O. hylas* invasion (HRS), segments with low risk of *O. hylas* invasion (LRS), the number of segments where *O. hylas* (HY) has been recorded, the number of segments where *Orconectes peruncus* (PE) has been recorded (with the number of segments that PE has been extirpated from in parenthesis), the number of segments where *Orconectes quadruncus* (QU) has been recorded (with the number of segments that QU has been extirpated from in parenthesis), and the total number of segments that have been sampled. HUs denoted by * indicate that no species of interest have been collected from that HU.

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12-Digit HU	# stream segments	% of total drainage	Total KM stream	SOS	HRS	LRS	# stream segments w/HY	# stream segments w/PE	# stream segments w/QU	Total stream segments sampled
080202020101	99	0.046	1683	0	0	99	0	0	1	1
080202020102	93	0.043	1490	0	0	93	0	1	2	4
080202020103	39	0.018	721	0	0	39	0	0	6	6
080202020201	89	0.041	1407	0	0	89	0	1	1	3
080202020202*	56	0.026	977	0	0	56	0	0	0	0
080202020203*	51	0.024	836	0	0	51	0	0	0	0
080202020204	83	0.038	1105	0	0	83	0	0	1	1
080202020205*	64	0.030	936	0	0	64	0	0	0	1
080202020206*	32	0.015	542	0	0	32	0	0	0	1
080202020207	38	0.018	664	38	0	0	7	0	0 (7)	7
080202020208	66	0.031	1166	34	18	14	3	0	4 (2)	8
080202020209	88	0.041	1129	0	4	84	0	0	1	1
080202020210	53	0.025	812	0	12	41	0	1	3	3
080202020301	98	0.045	1408	65	33	0	11	2 (10)	0	20

12-Digit HU	# stream segments	% of total drainage	Total KM stream	SOS	HRS	LRS	# stream segments w/HY	# stream segments w/PE	# stream segments w/QU	Total stream segments sampled
080202020302	94	0.044	1451	67	27	0	7	6 (2)	0	10
080202020303	65	0.030	1182	23	42	0	3	9	0	9
080202020304	39	0.018	718	38	1	0	4	1(2)	0	5
080202020401	87	0.040	1347	63	24	0	5	0	4 (1)	7
080202020402	64	0.030	914	0	4	60	0	5	4	6
080202020403	36	0.017	526	0	0	36	0	1	0	1
080202020404	37	0.017	789	0	0	37	0	3	0	3
080202020405	37	0.017	481	0	3	34	0	0	5	5
080202020406	45	0.021	817	0	0	45	0	3	1	3
080202020407	90	0.042	1206	0	19	71	0	2	0	2
080202020501	75	0.035	1260	0	0	75	0	1	0	1
080202020502	75	0.035	968	0	12	63	0	0	2	3
080202020503	117	0.054	1381	1	83	33	1	2	0	3
080202020504*	49	0.023	896	0	0	49	0	0	0	0
080202020505*	57	0.026	825	0	0	57	0	0	0	0
080202020506*	102	0.047	891	0	0	102	0	0	0	0
080202020507	89	0.041	1178	0	0	89	0	0	3	3
080202020508*	52	0.024	919	0	0	52	0	0	0	0
Total	2159	1	32625	329	282	1548	41	38 (14)	38 (10)	117

Table 23. Average (95% CI)(maximum number of samples taken before first detection) number of samples performed for first detection of adults of each species from multiple sites in the St. Francis River drainage. *Orconectes virilis* and *O. punctimanus* were combined and labeled as *O. virilis*. *Cambarus diogenes* and *O. harrisoni* were omitted from analysis because of a lack of information resulting from low encounter rates.

Species	1-m ² kick seine (n=27)	1-m ² quadrat sample (n=24)
<i>Orconectes hylas</i>	2.9 (2.2)(10)	2.1 (1.2) (9)
<i>O. peruncus</i>	1.7 (0.65) (4)	2.3 (1.4) (9)
<i>O. quadruncus</i>	3.0 (1.4) (7)	2.3 (1.0) (5)
<i>O. luteus</i>	1.4 (0.4) (4)	2.5 (1.0) (8)
<i>O. virilis</i>	7.9 (2.6) (20)	8.3 (3.5) (19)
<i>Cambarus hubbsi</i>	3.8 (2.3) (9)	4.4 (2.4) (17)

Table 24. Example weekly field schedule for a distant crew (travel status required).

Monday		Tuesday		Wednesday		Thursday	
AM	PM	AM	PM	AM	PM	AM	PM
Travel	One sample unit	One sample unit	Two sample units	One sample unit	Two sample units	Two sample units	Travel

Table 25. Simulation results used to determine sample size needs for desired standard error (SE) values of 0.05, 0.075, 0.1, and 0.125 across multiple occupancy estimate values (0.1 – 0.8) at two detection (p) rates (0.5 and 1.0). Simulations were based on conducting three or four surveys (K) at each sample unit. Methods follow those of MacKenzie and Royle 2005 for standard designs.

p	SE	K	Occupancy estimate							
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
0.5	0.05	3	46	84	114	136	150	156	154	144
0.5	0.05	4	40	72	95	111	119	118	110	94
1	0.05	3,4	36	64	84	96	100	96	84	64
0.5	0.075	3	21	38	51	61	67	70	69	64
0.5	0.075	4	18	32	43	50	53	53	49	42
1	0.075	3,4	16	29	38	43	45	43	38	29
0.5	0.1	3	12	21	29	34	38	39	39	36
0.5	0.1	4	10	18	24	28	30	30	28	24
1	0.1	3,4	9	16	21	24	25	24	21	16
0.5	0.125	3	8	14	19	22	24	25	25	23
0.5	0.125	4	7	12	16	18	19	19	18	15
1	0.125	3,4	6	11	14	16	16	16	14	11

Table 26. Sample unit covariates and their expected relation (+, positive; -, negative; quadratic; N/A, does not apply; unknown) with occupancy and detection probability estimates. One hypothesized reason for the relation is given, but others may exist.

Sample unit covariate	Expected relation with occupancy	Reasoning	Expected relation with detection	Reasoning
Year (yr)	+,-	Occupancy should increase for the invader and decrease for the two natives over time	Unknown	Personnel changes over time may influence detection, but the direction is unknown
Date	N/A	These species are not known to migrate, thus their presence is independent of sample date	+	Sampling crew may become more effective at sampling over time
Sampling Time	N/A	Presence of a species is independent of how much time is spent looking for it	+	More time spent sampling may relate to more effective sampling
Personnel	N/A	Presence of a species is independent of the people looking for it	N/A	One crew will likely conduct all sampling
Land Use – Forested	+	Forested stream segments may be related to higher quality habitat	N/A	This has little bearing on the ability to detect a species
Land Use – Row Crop	-	Row cropping near stream segments may be related to lower quality habitat	N/A	This has little bearing on the ability to detect a species
Land Use - Pasture	-	Pasture near stream segments may be related to lower quality habitat	N/A	This has little bearing on the ability to detect a species
Land Use - Urban	-	Urbanization near stream segments may be related to lower quality habitat	N/A	This has little bearing on the ability to detect a species
Riparian Zone Intact	+	Intact riparian zone may be related to higher quality habitat	N/A	This has little bearing on the ability to detect a species
Stream Disturbance Present	-	Disturbance of stream segments may relate to lower quality habitat	-	Frequent disturbance may force crayfish to habitats that are more difficult to sample

Sample unit covariate	Expected relation with occupancy	Reasoning	Expected relation with detection probability	Reasoning
Temperature	N/A	These species are not known to respond to temperature gradients	+	Sampling may be more effective in water temperatures comfortable for sampling crew
DO	+	Crayfish may be unable to persist at low DO levels	N/A	This has little bearing on the ability to detect a species
pH	quadratic	Crayfish may be unable to exist at extreme pH levels (low or high)	N/A	This has little bearing on the ability to detect a species
Stream Order	N/A	The species of interest are known to occur all stream orders present in the drainage	-	Suggested sampling methods can be less effective in larger streams
Segments in HU	Unknown	Included to assess the effect of sample stratification on occupancy	N/A	This covariate has no effect on detection probability

Table 27. Survey covariates and their expected relation (+, positive; -, negative; quadratic; N/A, does not apply) with occupancy and detection probability estimates. One hypothesized reason for the relation is given, but others may exist.

Survey covariates	Expected relation with occupancy	Reasoning	Expected relation with detection probability	Reasoning
Survey #	N/A	Surveys within a site have an random probability of species presence	N/A	All surveys within a site should have an equal (but random) probability of species presence
Mean Depth High-flow (DHF)	N/A	The species of interest are considered generalists	+	Extremely shallow areas may be less effectively sampled by seining
Mean Depth Low-flow (DLF)	N/A	The species of interest are considered generalists	-	Extremely deep areas may be less effectively sampled by seining
Mean Current Velocity High-flow (CVHF)	N/A	The species of interest are considered generalists	+	Swift areas may be more effectively sampled by seining
Mean Current Velocity Low-flow (CVLF)	N/A	The species of interest are considered generalists	+	Swift areas may be more effectively sampled by seining
Dominant Substrate High-flow (DSHF)	quadratic	Small and larger substrates may harbor fewer crayfish than medium substrates.	-	Seining may not be as effective in large substrates
Dominant Substrate Low-flow (DSLFL)	quadratic	Small and larger substrates may harbor fewer crayfish than medium substrates	-	Seining may not be as effective in large substrates
Sub-dominant Substrate High-flow (SDSHFL)	quadratic	Small and larger substrates may harbor fewer crayfish than medium substrates	-	Seining may not be as effective in large substrates
Sub-dominant Substrate Low-flow (SDSLFL)	quadratic	Small and larger substrates may harbor fewer crayfish than medium substrates	-	Seining may not be as effective in large substrates

Table 28. Example candidate models for occupancy (Ψ) and detection probability (p) estimation for both single season analysis and multiple season analysis in program PRESENCE. Other candidate models exist and may be considered.

Model	Model interpretation
Single Season Modeling	
$\Psi(.)p(.)$	Occupancy and detection probability constant
$\Psi(.)p(\text{date})$	Occupancy constant and detection probability varies by date
$\Psi(.)p(\text{stream order})$	Occupancy constant and detection probability varies by stream order
$\Psi(.)p(\text{CVLF})$	Occupancy constant and detection probability varies by mean current velocity in low-flow samples
$\Psi(\text{land use})p(.)$	Occupancy varies by land use (insert forest, row crop, pasture, or urban) and detection probability constant
$\Psi(\text{Segments in HU})p(.)$	Occupancy varies by the number of segments in the hydrologic unit (i.e., the stratification method biased the estimate) and detection probability constant
$\Psi(\text{riparian zone})p(.)$	Occupancy varies by riparian zone and detection probability constant
$\Psi(\text{riparian zone})p(\text{date})$	Occupancy varies by riparian zone and detection probability varies by date
$\Psi(.)p(\text{CVLF, DSLF})$	Occupancy constant; detection probability varies by mean current velocity and dominant substrate in low-flow samples
$\Psi(\text{riparian zone})p(\text{CVLF, DSLF})$	Occupancy varies by riparian zone; detection probability varies by mean current velocity and dominant substrate in low-flow samples
Multiple Year Model	
$\Psi(.)\gamma(.)\epsilon(.)p(.)$	Occupancy, colonization extinction, and detection probability constant
$\Psi(.)\gamma(.)\epsilon(.)p(\text{year})$	Occupancy, colonization, and extinction constant; detection probability varies by year
$\Psi(\text{yr})\gamma(\text{yr})\epsilon(\text{yr})p(.)$	Detection constant, occupancy, colonization, and extinction vary by year
$\Psi(\text{riparian zone, yr})\gamma(\text{yr})\epsilon(\text{yr})p(\text{CVLF, DSLF})$	Occupancy varies by riparian zone and year; occupancy and colonization vary by year; detection probability varies by mean current velocity and dominant substrate in low-flow samples

Table 29. Estimated expenditures related to salary and travel needs for one sample season in US dollars. Estimates for a distant crew (one that requires travel status) and a local crew (one that does not require travel status) are presented.

Distant crew				
Number of people	Hourly salary	Number of hours per person each week	Number of sampling weeks	Total
4	\$9.00	40	8	\$11,520
Number of people	Average food allowance per day	Number of Days in Field	Number of sampling weeks	Total
4	\$25	4	8	\$3,200
Number of motel rooms (2 people per room)	Average price per night	Number of nights in field	Number of sampling weeks	Total
2	\$60	3	8	\$2,880
Total per season				\$17,600
Local crew				
Number of people	Hourly salary	Number of hours per person each week	Number of sampling weeks	Total
4	\$9.00	40	12	\$17,280

Table 30. Estimated equipment needs and associated costs in US dollars. Total marked by (*) indicate equipment that may only have to be purchased one time. Gasoline costs were not estimated because of varying prices and too much uncertainty about sampling needs.

Equipment	Number	Price per unit(\$)	Total (\$)
1-m ² seine	3	50	150*
Wading boots	4	100	400
Waterproof paper	1	60	60
Calipers	1	45	45*
Miscellaneous field supplies	N/A	N/A	100
GPS unit	1	Borrow	0
Wire minnow traps	50	Borrow	0
Vehicles	1 or 2	Borrow	0
Gasoline	N/A	N/A	UK
Total			755

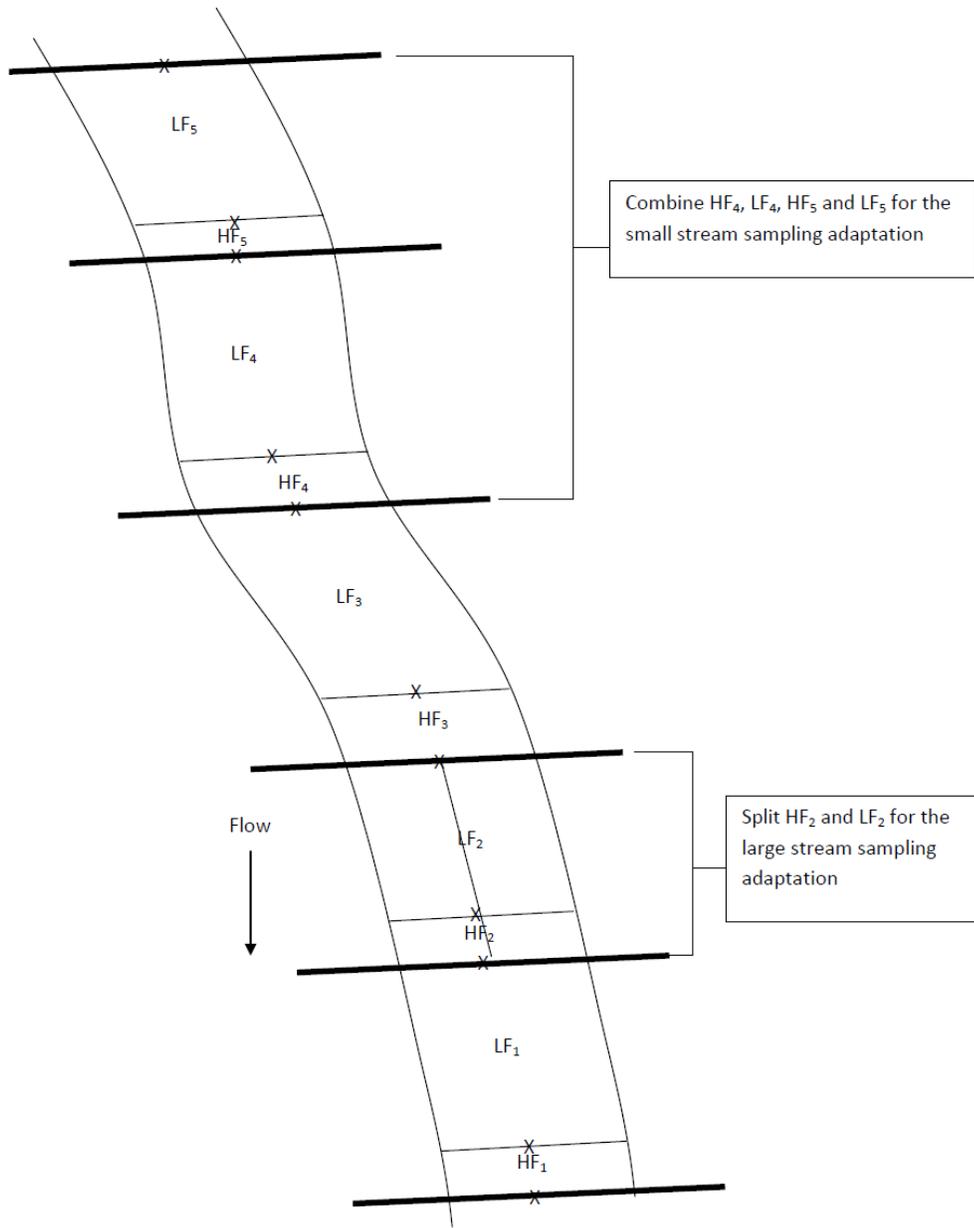


Figure 54. Example layout of sample unit. Potential survey units (area between bold lines comprising one high-flow (HF_i) and one low-flow (LF_i) habitat) will be identified starting with the downstream most riffle (HF₁). Four survey units will be randomly selected (with replacement) from the five survey units. Coordinates will be recorded with a GPS unit halfway across the stream on the downstream edge of each high-flow or low-flow habitat (locations marked with X).

Sample (S _i)	% Across	% Along	Sample Order
S ₁	70	50	2
S ₂	25	25	1
S ₃	60	80	3
S _x	85	70	N/A
S ₄	45	20	4
S ₅	65	80	6

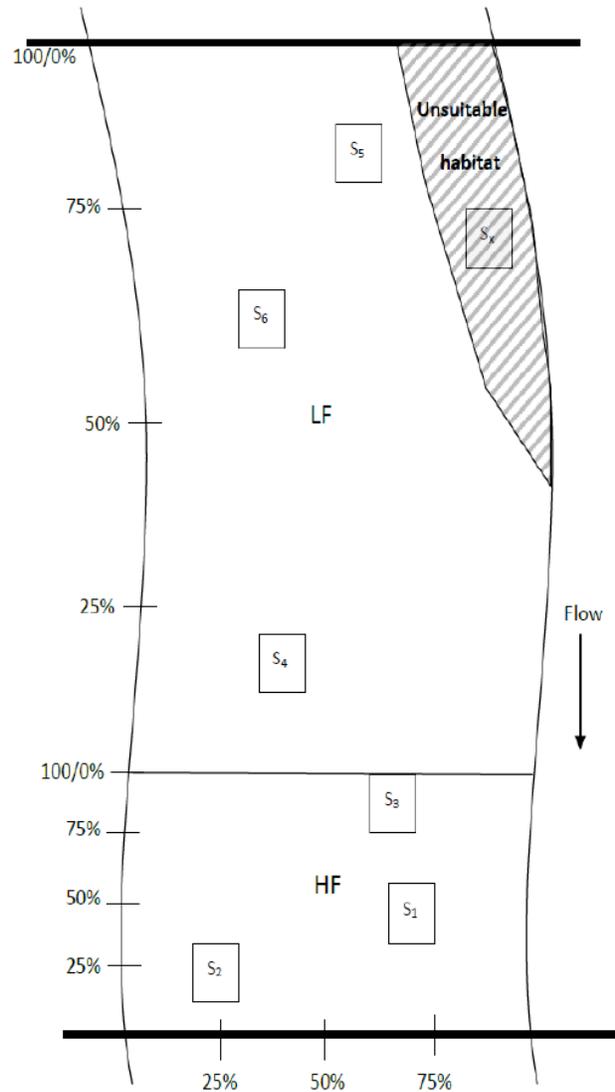


Figure 55. Example survey unit layout depicting the combination of one high-flow (HF) and one low-flow (LF) habitat. Percentages along the right bank descending margin of the stream represent the distance along the stream where a sample can occur and percentages across the bottom represent the distance across the stream where a sample can occur. The location of 1-m² quadrat samples (S_i) correspond to values in the table.

CHAPTER 5
GENERAL CONCLUSIONS

CONCLUSIONS

Multi-Scale Habitat Associations

Stream habitats are thought to be organized in a hierarchical pattern whereby species pass through a series of habitat filters that operate at various spatial and temporal scales (Frissell et al. 1986, Tonn 1990, Poff 1997, Brewer et al. 2007). The results of this study can be viewed in a hierarchical nature regarding *O. hylas* and *O. quadruncus*. I did not investigate fine-scale habitat associations of *O. peruncus* in this study, so inference based on empirical evidence about the hierarchical nature of its habitat use are restricted to what was discussed in Chapter 2. Inferences about *O. hylas* and *O. quadruncus* at fine-spatial scales may also be somewhat limited because data at that scale were collected from only one (*O. hylas*) or two (*O. quadruncus*) streams within the overall range of each species. Fine scale habitat selection likely varies spatially for both species.

Orconectes hylas (Figure 56) and *O. quadruncus* (Figure 57) respond to similar habitat filters at the coarse (Chapter 2) and fine (Chapter 3) spatial scales. Geology and soil texture shape the hydrologic and chemical features of certain stream systems in the St. Francis River drainage in such a way that *O. hylas* and *O. quadruncus* are able to occupy those streams. For *O. quadruncus*, there was evidence that stream size affects suitability of a stream, but overall the species was found in streams of all sizes. Within streams with favorable conditions shaped by proper geology and soil conditions, it may not matter what kind of macrohabitat (riffle, runs, pools) are present as both species occupied all types of macrohabitat, at least in the small streams examined. My analysis indicated velocity and substrate size were the two habitat variables that best reflected

habitat suitability at the fine-spatial scale. The amount of coarse substrate in the stream is related to the amount and size of coarse particles in the soils, which may also be related to the geology of the catchment (Frissell et al. 1986, Boone 2001). If *O. hylas* and *O. quadruncus* occupy streams with flashy runoff, large substrate particle size may act to armor the bed against high shear stress that would alter bed characteristics and could be disruptive to crayfish.

This multi-scale approach to habitat association and organization of hypothesized habitat filters can be an example of for others examining habitat associations of other crayfishes. Additionally, my study demonstrates that even though the potential for competitive exclusion is thought to be high, it may not always be the answer in species displacement scenarios.

Future Research

This study, and several others (DiStefano et al. 2002, Rahm et al. 2005, Westhoff et al. in review), have investigated potential mechanisms of species displacement or *O. peruncus* and *O. quadruncus*, but have not identified an empirically supported causal link. The introduction and subsequent spread of *O. hylas* coincides almost completely with the declines of *O. peruncus* and *O. quadruncus*, a pattern that has been observed in several unconnected stream segments throughout the western portion of the St. Francis River drainage. The presence of *O. hylas* is undoubtedly connected to the declines, but the mechanism(s) causing species displacement remain unknown. Knowledge of the mechanism of species decline could shape conservation actions directed towards species persistence. For instance, conservation actions could be aimed at promoting coexistence

of the species at syntopic sites or directed towards keeping populations of *O. quadruncus* and *O. peruncus* physically separated from *O. hylas*. If hybridization or disease is the mechanism of displacement, actions that promote coexistence of syntopic populations would likely be wasteful. If competition is the mechanism, perhaps habitat can be altered or *O. hylas* population size reduced to promote coexistence. Without knowledge of the mechanism, the safest conservation action is to segregate the species with barriers to dispersal and protect existing populations of *O. peruncus* and *O. quadruncus* that physically isolated from *O. hylas*.

Anthropogenic alterations to the landscape do not appear to be highly associated with the declines of the natives or spread of *O. hylas* at a coarse spatial scale; however, they may cause localized population declines (Allert et al. 2008). The potential for interspecific competition between *O. hylas* and either *O. peruncus* or *O. quadruncus* has been discussed throughout my study as a mechanism of displacement, but several other possibilities exist and include hybridization, reproductive interference, disease, differential predation, reciprocal predation, exploitative competition, and differential response to anthropogenic alteration of the landscape (Figure 58). Many links and interactions among these potential mechanisms likely exist (Figure 58). I focused on other potential mechanisms not directly related to interspecific competition or anthropogenic alteration of the environment for the remainder of this discussion; however, not all interactions shown in Figure 58 were discussed.

Hybridization is known to occur between native and invasive crayfishes (Perry et al. 2002) and the copulatory behavior associated with hybridization has been observed between *O. hylas* and *O. quadruncus* (Chapter 3). Genetic relatedness of *O. hylas* and *O.*

peruncus may enable successful hybridization (Fetzner and DiStefano 2008). Male crayfishes captured in recently invaded (by *O. hylas*) streams inhabited by *O. peruncus* sometimes exhibit gonopods that appear to be intermediate in morphology between *O. peruncus* and *O. hylas* (J. Westhoff, personal observation). Regardless of the production of viable offspring, inter-specific mating behavior could lead to reproductive interference which has been demonstrated in other invasive crayfish scenarios (Butler and Stein 1985, Söderbäck 1994). I feel the anecdotal accounts of possible hybridization and reproductive interference observed in this study warrant further investigation as a potential mechanism of displacement.

Crayfish diseases are understudied in North America, but have received much attention in Europe regarding their role in the decline of native crayfishes (Lodge et al. 2000, Edgerton et al. 2004, Dunn et al. 2009). Differential response among species to similar diseases has been established as a cause of the decline of European crayfishes (Edgerton et al. 2004), but it has not been studied as a possibility for the decline of *O. quadruncus* or *O. peruncus*. Although there are no observations of diseased individuals of these native species, the fast rate (often within five years; J. Westhoff, personal observation) at which they are extirpated in many locations and the lack of evidence of strong competitive advantage for *O. hylas* suggests a mechanism like disease could have potential.

Many studies have shown that predators preferentially consume native crayfishes opposed to some invasive crayfishes (Mather and Stein 1993, Garvey et al. 1994, Garvey et al. 2003, Dunn et al. 2009). These studies often occur in laboratory settings or lentic environments and may not translate well to lotic systems with more complex predator

assemblages. Fish predation of crayfish is thought to be closely related to body size, whereby smaller bodied crayfish are consumed in greater numbers than larger bodied crayfish (Garvey et al. 1994). *Orconectes hylas* does achieve a slightly larger body size than either native (DiStefano et al. 2002); however, this difference is less pronounced than in other invasive crayfish species situations. This mechanism may occur, but given the variety of predators found in the various sized streams invaded by *O. hylas*, it seems unlikely that the information could be used by managers to promote the persistence of the natives.

Species displacement may occur due to reciprocal predation whereby introduced adult crayfish prey upon the juveniles of a native species in greater proportion than adult natives prey on introduced juveniles (Begon et al. 2006). To my knowledge this has not been demonstrated in crayfishes, but does occur in other crustaceans (Omori et al. 2006). Crayfish are known to consume other crayfish, so the potential exists for this mechanism (Momot 1967, Alcorlo et al. 2004). Relative abundance is important concerning species replacement in this instance because low numbers of invaders likely would not have a large effect on a more abundant population of natives. Hence, if this mechanism is occurring, it is likely in conjunction with another mechanism that allows for high abundance of the invader.

Another possible mechanism of the displacement of *O. peruncus* and *O. quadruncus* by *O. hylas* is related to life-history characteristics including fecundity, annual timing of reproduction, and growth rate. DiStefano et al. (2002) described how *O. hylas* may be more fecund, reproduce earlier, and grow faster than both natives and how this may dictate competitive ability. It is also possible that these traits are not linked

directly to interspecific competition, but are instead related to exploitative competition. Individual *O. hylas* may not be competitively superior to individual *O. peruncus* or *O. quadruncus* but may simply consume resources before the natives are able to do so. Exploitative competition has not been specifically identified as a mechanism of displacement in crayfishes to my knowledge, but is known to occur in other aquatic organisms such as larval bullfrogs *Rana catesbeiana* (Kupferberg 1997). A related life-history trait, the age of first reproduction, could be investigated using population growth models to predict population growth over time under various biologically realistic population parameters.

My study was concerned only with the effects of *O. hylas* on *O. peruncus* and *O. quadruncus*; however, the invasion of *O. hylas* may have affected other crayfishes, other faunal groups, and the ecological processes of the lotic environment. Crayfish introductions are known to affect amphibians (Gamradt and Kats 1996, Cruz et al. 2006), fishes (Guan and Wiles 1997, Taylor and Soucek 2010), other invertebrates (Crawford et al. 2006), plants (Nyström and Strand 1996), and foodwebs or ecosystem function (Lodge et al. 1994, Saito et al. 2007). *Orconectes hylas* appears to be similar morphologically and likely functionally to the natives it displaces, but slight differences may have large effects. For instance, *O. hylas* may increase the total biomass accounted for by crayfishes in a system and to attain that biomass, they may consume more energy and prey than the native crayfishes did historically. Changes to energy flow are known to cause trophic cascades or lead to the energetic “dead ends” if the invasive crayfish are not themselves consumed (Saito et al. 2007). Therefore, it is important to investigate the effects of *O. hylas* on other organisms to better understand the effects of the invasion beyond crayfish.

Little is known about the potential effects of the *O. hylas* invasion on humans and the local economy. Invasive species are known to cause billions of dollars in damage every year in the United States alone (Pimentel et al. 2005). There are likely few industries that derive direct economic benefit from natural crayfish communities in the St. Francis River drainage. Recreational fishing occurs on some of the larger streams in the drainage and could potentially be affected by the invasion; however, this is not known. *Orconectes hylas* may alter ecosystem services (e.g., energy processing or water quality) provided by the natural community which can be assigned monetary values (Howarth and Farber 2002). Bioeconomic risk analysis (*sensu* Leung et al. 2002) for this invasion may be a way to determine what level of spending is appropriate for control of *O. hylas*.

Conservation Actions

Costs of conservation actions directed towards elimination or management of established invasive species can be prohibitively high (Peters 2010). Policy changes and education represent the most feasible and cost-effective strategies for preventing future invasions (Lodge et al. 2000, Leung et al. 2002). Crayfish introduction vectors have received much attention recently (Lodge et al. 2000, Larson and Olden 2008, DiStefano et al. 2009) but some governments have not taken steps towards reducing the risk of further introductions. Several species of crayfish are currently legal to purchase or collect for use as bait in the state of Missouri, but crayfish bait has been fully or partially banned in other states and provinces (DiStefano et al. 2009). It is clear that current education and policy efforts are not effective at preventing the introduction of non-native

aquatic organisms based on the continued identification of new introductions (Peters and Lodge 2009). Personal conversations evidence the shortcomings of current prevention techniques (J. Westhoff, personal observation). One individual admitted to repeatedly catching crayfish in the Black River drainage for use as fishing bait and releasing the remaining live crayfish into streams in the St. Francis River drainage. This, and similar instances, represent failure of past and/or current educational efforts directed towards preventing the introduction of alien species. Collaboration between trained educators, advertising professionals, and scientists is needed to relay the importance of introduced species prevention.

For established invaders, like *O. hylas* in the St. Francis River drainage, prevention of additional introductions is important; however, even if no additional introductions occur the impacts of *O. hylas* on the native aquatic community will continue as *O. hylas* expands its range. There are no known conservation actions that would feasibly eliminate *O. hylas* from the entire St. Francis River watershed; however, some strategies may exist that could be used on a finer scale. Hyatt (2004) reviewed strategies for crayfish eradication and concluded that most are ineffective to apply at meaningful scales. Abundance reduction efforts have been taken to address invasive crayfish populations, and include chemical (Bills and Marking 1988, Peay et al. 2006, Sandodden and Johnsen 2010), biological (Freeman et al. 2010), and mechanical (Hein et al. 2007) control strategies. Chemical strategies involve using known chemicals to kill crayfish inhabiting the treatment area (Eversole and Sellers 1997, Holdich et al. 1999). Peay et al. (2006) successfully treated several small ponds with the biocide Pyblast (Agropharm Ltd.) to eliminate populations of the introduced *Pacifastacus leniusculus* in

Scotland but concluded that large scale use of the biocide was not feasible and long-term monitoring is required to ensure successful treatment. Chemical treatment of water bodies entails ethical and legal considerations. Other difficulties may exist when using biocides including unintended mortality of non-target species, difficulty of delivering a lethal dose to all areas harboring crayfish (e.g., the benthic environment), and long-term effects of residual chemicals (Peay et al. 2006). Eradication of *O. hylas* using biocides is not feasible throughout the entire St. Francis River drainage but may be an option if new populations are discovered and treated while they are restricted to small areas.

Biological control methods often involve the use of predators to reduce crayfish densities, but may also include the use of pathogens (Freeman et al. 2010). Several studies in lentic environments demonstrated reductions in invasive crayfish abundance associated with predation from fishes (Hein et al. 2007, Peters 2010, Tetzlaff et al. 2011). It should be noted that similar results have not been tested in lotic environments. Manipulation of the predator community in the St. Francis River drainage is likely not a feasible method to control *O. hylas*. Predators would have to demonstrate preferential selection of *O. hylas* over native species, which is not likely given the similarities between the species of interest. Further, large scale manipulation of predators over a heterogeneous stream system would be difficult to achieve. Biological control of *O. hylas* using pathogens suffers many of the same problems related to large scale application of chemicals and the assumption of differing responses among native and invasive crayfishes.

The most popular abundance reduction method, mechanical removal of invasives using traps or other methods, has met with poor success in some instances (Peay and

Hiley 2001) and higher success rates in others (Bills and Marking 1988, Hein et al. 2007). Most successful removal efforts have been implemented in relatively small lentic environments where crayfish populations are isolated from source populations (Bills and Marking 1988, Hein et al. 2007). In lotic environments, trapping efforts must address the greater potential for re-colonization of treated areas from immigration of individuals from untreated areas. Because of this, removal efforts in streams may be less successful. Mechanical removal methods are also often not effective at removing all individuals because of crayfish behavior, gear biases, and insufficient time and resources for proper application (Rabeni et al. 1997, Peters 2010, Ogle and Kret 2008). Incomplete removal of introduced crayfishes is known to release remaining individuals from density dependent effects of intraspecific competition which can result in greater growth of the remaining individuals (Moorhouse and Macdonald 2011). Additionally, because crayfish are highly fecund, they are able to maintain high densities despite periodic density reductions (Momot 1998).

Mechanical removal efforts for *O. hylas* throughout the St. Francis River drainage are not likely to be successful because the amount of effort need to overcome methodological shortcomings likely exceeds available resources; however, targeted population reductions of *O. hylas* may be useful to managers. Streams where *O. hylas* has co-occurred with *O. quadruncus* (Marble Creek and Orr Hollow Creek) for several years may represent potential areas of long-term co-occurrence. If competition with *O. hylas* is the mechanism of species displacement for *O. quadruncus*, reductions in the densities of *O. hylas* may reduce interspecific competition. Reduced competition, in conjunction with favorable habitat for *O. quadruncus*, may slow the displacement of *O.*

quadruncus. The long-term success of this approach would require either perpetual management from humans (i.e., *O. hylas* removal) or the natural selection of *O. quadruncus* traits that allow for successful competition with, or niche differentiation from, *O. hylas*. This is based on the hypothesis that in favorable environmental conditions where *O. hylas* is not abundant, *O. quadruncus* will be able to successfully compete with *O. hylas* for essential niche components.

An alternative strategy for application of *O. hylas* removal efforts is to target the leading edges of the *O. hylas* invasion. Some introduced species are thought to spread in a pulsed pattern whereby individuals remain in a newly established area until a threshold abundance is achieved and density dependent effects illicit dispersal behavior of a portion of the population (Hastings et al. 2005). If pulsed range expansion is occurring, maintaining a density of *O. hylas* below the threshold for dispersal may slow the spread of the invasion. There is some evidence that range expansion of *O. hylas* is highly variable on an annual basis, which may indicate a pulsed dispersal pattern (DiStefano and Westhoff in press). For *O. hylas*, density reduction efforts targeted at the leading edges are more likely to succeed when the spread is in an upstream direction than density reductions of *O. hylas* in well established stream reaches. *Orconectes hylas* expansion is thought to occur faster in a downstream (active and passive dispersal) direction opposed to an upstream (active dispersal only) direction (DiStefano and Westhoff in press), so upstream leading edges likely represent more feasible locations for this conservation action. This method alone would not prevent the expansion of *O. hylas*, but may slow it giving managers time to apply other management strategies.

Effective reduction of *O. hylas* abundance would likely require much involvement from managers for an extended number of years. An alternative method that may slow or prevent the spread of *O. hylas* in the St. Francis River drainage is to create physical barriers to crayfish dispersal (Kerby et al. 2005). In addition to the physical barrier presented by the dam, the resulting reservoir creates a lentic environment thought not to favor *O. hylas* both in habitat conditions and predator interactions with centrachids. It should be noted that dams are known to have negative impacts on native aquatic biota as well (Baxter 1977, Tiemann et al. 2004).

Impoundments represent possible existing barriers to the spread of *O. hylas*. Lake Killarney, created in 1911 on Stouts Creek, may serve as barrier to dispersal of *O. hylas* as *O. hylas* has not been known to have established a population downstream of the dam but has completely displaced *O. quadruncus* above the dam. Crane Lake on Crane Pond Creek is another potential barrier to the dispersal of *O. hylas* individuals which are currently expanding upstream towards the impoundment. *Orconectes peruncus* was found both upstream and downstream of the dam on Crane Pond Creek. If the dam acts a barrier to *O. hylas*, the population of *O. peruncus* upstream of the dam warrants additional protection as a refuge population. Potential conservation actions that could protect this population include the ban of live crayfish as bait in Crane Lake, coordination with current land owners in the drainage to raise awareness of the issue, purchase of land in the catchment to prevent possible unfavorable habitat alteration, and placement of signs at Crane Lake and at the bridge crossing on CR 124 asking people not to release crayfish or other organisms into the stream. Other impoundments exist in the drainage and should be considered as possible management tools. Reliance on isolated

populations of *O. quadruncus* and *O. peruncus* will not provide for natural gene flow and is not ideal, but may represent a feasible way to ensure the persistence of the species.

In light of the threats to *O. peruncus* and *O. quadruncus*, protection under the U. S. Endangered Species Act (ESA) may be appropriate. Listing of these species may provide additional resources that could be used to support conservation actions, shape beneficial policy measures, or support continued research. Despite the benefits, challenges exist even if the species are listed under the ESA. Many of the success stories of the ESA are associated with species that are declining from activities that can be directly regulated or controlled such as overharvest and application of chemicals (Abbitt and Scott 2001). Unfortunately, the reasons for the declines of *O. peruncus* and *O. quadruncus* are not as directly manageable. Listing a species as threatened or endangered under the ESA also brings the risk of decreased landowner cooperation (Leigh and Olive 2008). Much of the St. Francis River drainage is privately owned and landowners may be hesitant to cooperate with researchers and managers if they feel their property rights may be affected.

Uncertainty remains surrounding the causes of, and solutions to, the decline of *O. peruncus* and *O. hylas* in the St. Francis River drainage. Additional research, monitoring, and management are required to prevent further declines, and potential extinction of these species. Continued effort, innovation, and collaboration are the keys for ensuring the persistence of *O. peruncus* and *O. quadruncus*.

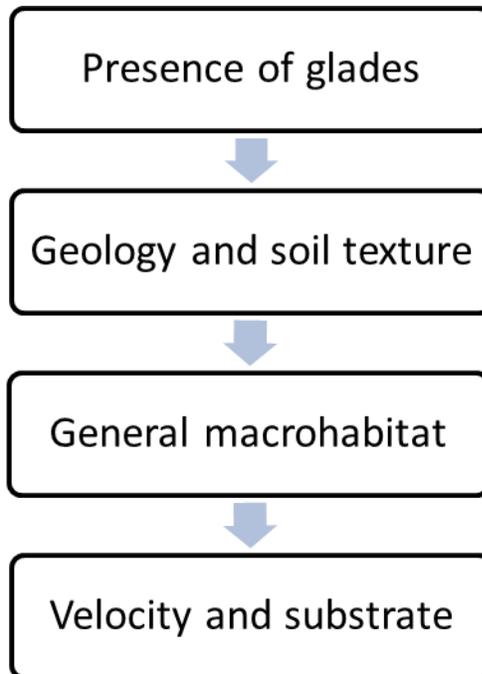


Figure 56. Conceptual model of the hierarchical filters that influence the potential of a stream to harbor *Orconectes hylas*. Filters related to glades, geology and soil texture are associated with the watershed scale, macrohabitat is associated with the reach scale, and current velocity and substrate are associated with the macrohabitat scale.

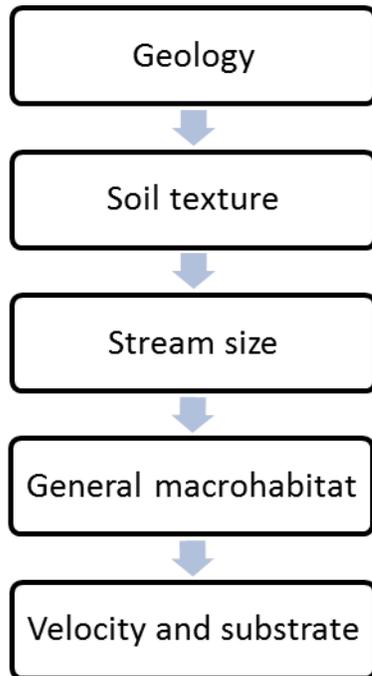


Figure 57. Conceptual model of the hierarchical filters that influence the potential of a stream to harbor *Orconectes quadruncus*. Filters related to geology, soil texture and stream size are associated with the watershed scale, macrohabitat is associated with the reach scale, and current velocity and substrate are associated with the macrohabitat scale.

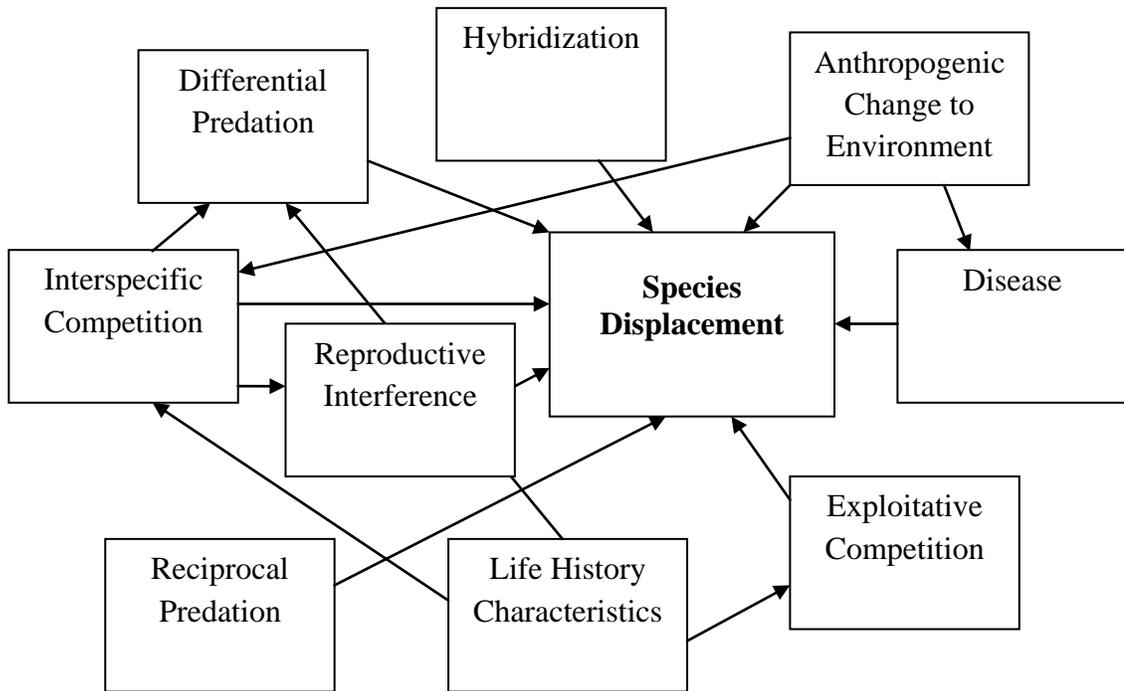


Figure 58. Conceptual diagram of potential mechanisms of displacement for crayfishes exposed to introduced crayfishes. Arrows represent relations of individual mechanisms to each other and species displacement.

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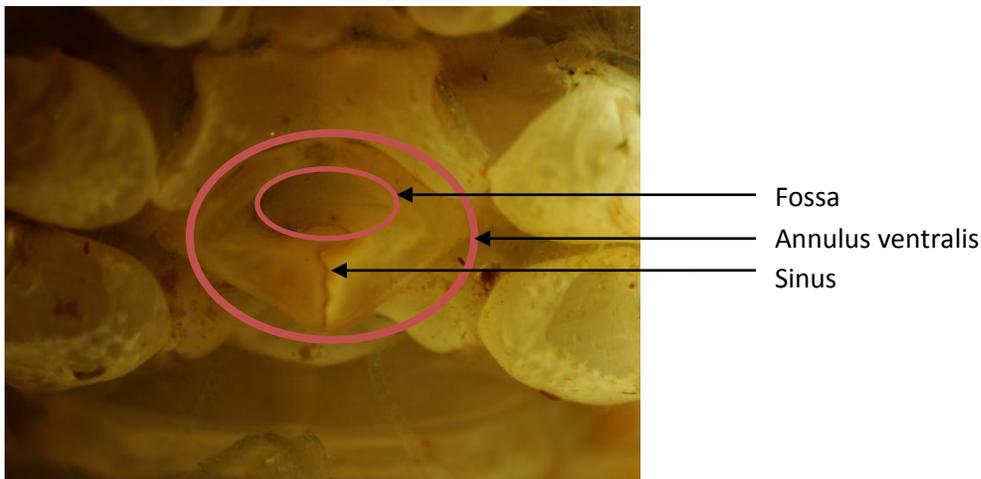
Appendix A. Species Identification

The following characteristics represent morphological features that may be useful for taxonomic identification of *Orconectes hylas*, *Orconectes peruncus*, and *Orconectes quadruncus*. The most established and reliable character is the shape of the male gonopods and is recommended as the primary identifying characteristic. Cyclic dimorphism of the gonopods occurs as the individual molts from form I (reproductively active; indicated by the letter I on the diagrams) to form II (reproductively inactive; indicated by the letter H on the diagrams). This change should not impede identification efforts as differences among species are retained, albeit slightly less distinct in form II males.

Females present a greater identification challenge, but are distinguishable. Some factors can make the identification of females more complex. The amount of sculpture in the annulus ventralis can change as the crayfish ages and the fossa is known to occur in mirror image within a species. Additionally, some females can have annulus ventralis structure that is markedly pointed on the caudal portion during the reproductive cycle.

Other characteristics appear to differ among species and are outlined, but a high degree of variation in color and mottling pattern exists among populations of the same species. These characteristics should be used with caution and only to assist with identification after examination of the reproductive structures.

There is no known method for distinguishing young-of-the-year crayfish among these species if reproductive structures are not developed.



Photos by: Michael Craven, Chris Lukhaup, Jacob Westhoff

Diagrams from: Pflieger 1996

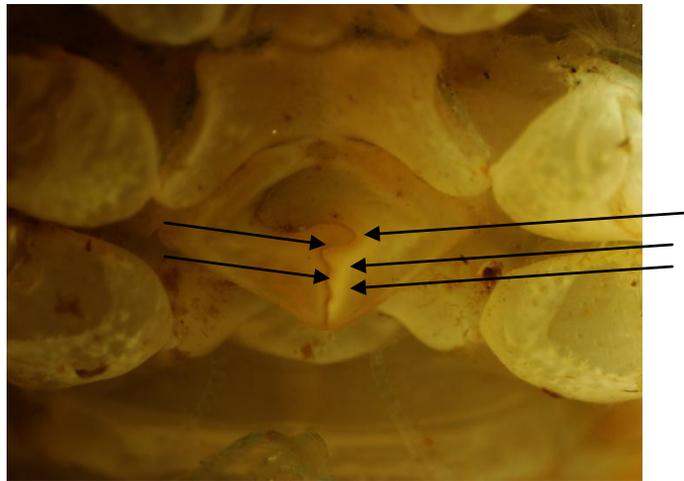
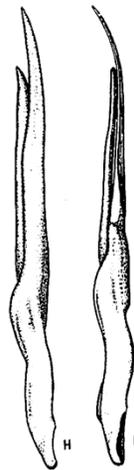
Orconectes hylas

Identifying Characteristics:

Males - Long and slender gonopods that have primary (longer) and secondary (shorter) processes of approximately equal thickness that touch along their length and both terminate in sharp points.

Females – Annulus ventralis is generally more compressed in the rostral –caudal direction and the sinus has five direction changes (arrows) that appear smoother (less angular) than *O. peruncus* and looks similar to a question mark.

General – The saddle mark on the carapace is not well defined from the rostral direction and consists of larger mottling than *O. peruncus* and *O. quadruncus*. Mottling occurs over entirety of the carapace and the underside of the chelae. Overall color is a light tan, most similar to *O. peruncus*.



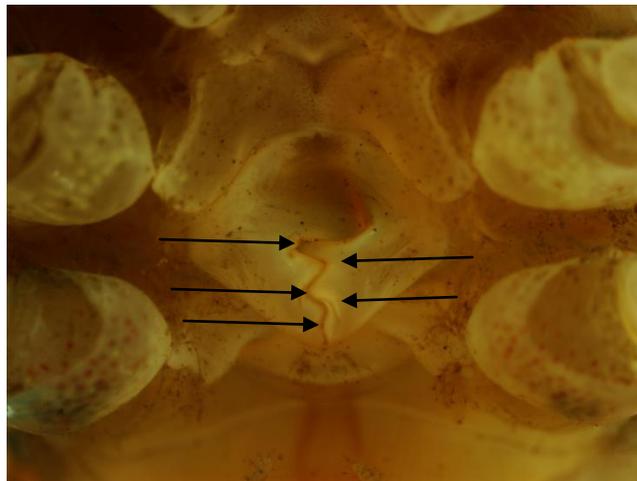
Orconectes peruncus

Identifying Characteristics:

Males - Long and slender gonopods with primary and secondary processes tend to be more separated and the secondary process is thicker and curves away from the primary process at its terminus. Sometimes the secondary process has a flattened shape at its distal point.

Females – Annulus ventralis is generally more elongated in the rostral –caudal direction and the sinus has five direction changes (arrows) that appear sharper (more angular) than *O. hylas*.

General – The saddle mark on the carapace is not well defined from the rostral direction and consists of slightly smaller mottling than *O. hylas* but larger than *O. quadruncus*. Mottling occurs over entirety of the carapace. Overall color is a light tan, most similar to *O. hylas*.



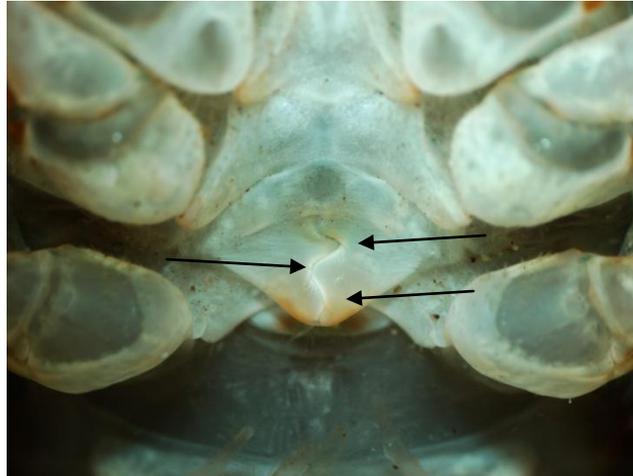
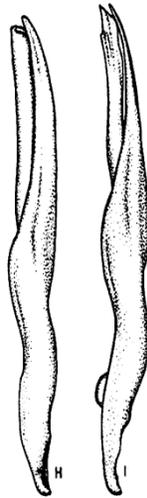
Orconectes quadruncus

Identifying Characteristics:

Males - Gonopods with primary and secondary processes equal length and much shorter than those of the other two species.

Females – Annulus ventralis is generally less sculpted and fossa much shallower than *O. hylas* or *O. peruncus*. The sinus has three directional changes which are most smooth (least angular).

General – The saddle mark on the carapace is often defined and consists of smaller mottling, which is lacking in the mid-section of the carapace. No mottling occurs on the underside of the chelae. Overall color is a darker tan or greenish-brown.



Appendix B. Data validity rule set.

1. Was the tag encountered during the sampling season?
 - a. If yes, then step 2
 - b. If no, then **do not** use data
2. Was the crayfish captured alive at the end of the sampling season?
 - a. If yes, then use all data
 - b. If no, then step 3
3. Was the tag recovered without an associated live crayfish during the sampling season?
 - a. If yes, then **do not** use data
 - b. If no, then step 4
4. Was the crayfish seen alive at any point earlier in the season?
 - a. If yes, then use data
 - b. If no, then step 5
5. Were any movements greater than five meters?
 - a. If yes, then use data
 - b. If no, then step 6
6. Were any records from the “distance from previous marker” greater than 50 cm?
 - a. If yes, then use data
 - b. If no, then step 7
7. Were there three or fewer encounters for that tag?
 - a. If yes, then use data
 - b. If no, then step 8
8. Was the crayfish determined to be alive in a subsequent season?
 - a. If yes, then use data
 - b. If no, then **do not** use data

Rationale:

The five-meter movement cutoff (Step 5) was established based on the range of distance estimation error observed from field approximations of tag location. No distance estimate was determined to be greater than three meters based on the marker number system (markers were left at the location of an encounter and used over consecutive days if the same tag was encountered repeatedly). The use of a five-meter cutoff eliminated estimation error as possible evidence that a tag moved. I believe the value of five meters is conservative as it includes a two-meter buffer on top of the observed error.

The 50-cm movement cutoff (Step 6) was established to use the field data collected based on fine-scale movements observed. When a tag was encountered within two meters of a

marker from a previous sampling event the marker was moved to the new location and the distance between the two points was estimated to the nearest 10 cm. The records were checked to ensure the tag code matched both markers and eliminate the possibility that the original crayfish moved and a new crayfish was detected nearby. The 50-cm cutoff was established as a conservative measure based on the detection location error which sometimes, but rarely, reached 20 cm. It is possible that downstream movement could occur for tags that were no longer associated with crayfish and had simply washed downstream with the current. I felt this was not a major issue based on my observations and was willing to accept that source of error.

The three or fewer encounters rule (Step 7) accounts for situations where no other information was available to confirm the validity of the record. The value of three was somewhat arbitrary but no precedent was available. This rule is meant to eliminate records that are associated with tags that did not move and were never found. It may eliminate valid data from live crayfish that were stationary and detected numerous times or it may allow for inclusion of data from disassociated tags that were only detected a few times. I felt that three false locations per tag were acceptable but four or more were not. Elimination of all records in this category was considered but not done to avoid eliminating many likely valid encounters as tags that were only detected a few times were more likely to have moved than to be detected in a few scanning events and missed in all other scanning events.

Appendix C. Substrate Metric Calculation Methods

Substrate calculations in the individual habitat selection analysis used a substrate grid centered on the rock determined to be above the marked crayfish (cover rock). The grid was further situated so observation 1 was upstream and parallel with flow. Each of the four grid arms was 12.5 cm in length. Substrate values were recorded at each grid node (5) by visually estimating size classes using a modified Wentworth Scale (Figure 1, Table 1).

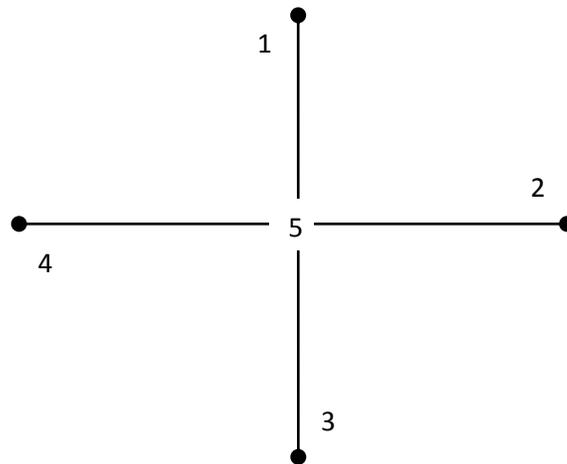


Figure 1. Substrate grid used in the individual habitat selection study.

Geometric mean was calculated by multiplying across all substrate categories

$$\prod_{i=1}^n S_i^{P_i},$$

where S_i = the midpoint diameter of the substrate class i and P_i = the percentage of substrate class i in the sample. The midpoint diameter of a substrate class was determined using a modified Wentworth Scale (Bovee and Milhous 1978). The maximum value in the range of sand and silt was used instead of the midpoint diameter value because sand was more common in the study streams than silt and using the midpoint would underestimate the substrate size. The size class of boulder starts at 256 mm but has no upper limit. Because of this, the lowest value in the range for boulders was used in the calculations. Most boulders in the study reaches were relatively small and it was hypothesized that larger boulders actually are less valuable to crayfish as

shelter than small boulders. Using a larger value for these calculations would likely force the relation between crayfish use and substrate metric to take a quadratic form. Bedrock and hardpan have no size ranges on the Wentworth Scale; thus, the value of 5.5 was used for the midpoint diameter because it was hypothesized that the cracks and interstitial spaces usable by crayfish in those two substrate types was intermediate in value between sand and gravel.

Two sample calculations of geometric mean are detailed below in words and in Table 1. The method described is an adaptation of the Fredle Index (Lotspeich and Everest 1981) that does not require sieving and weighing of substrate. In addition to the using a grid to determine substrate types, the method can be used without a grid where percent occurrence of each substrate class is recorded in a given area. Those analyses follow the same methods and do not require the first step of converting counts to percentages. Percent composition is likely more accurate than using a grid to sample, but requires more time in the field.

Example 1: For a sample containing the following records, 1= pebble, 2 = cobble, 3 = pebble, 4= pebble, and 5 = cobble the records are displayed as three observations of pebble and two observations of cobble. Next, the percentage of each substrate type is calculated by dividing each total by the number of total substrate observations (5). This results in a value of 0.6 for pebble, 0.4 for cobble, and zeros for all other size classes. The value of the midpoint diameter of the associated size class is raised to percentage of that substrate type in the sample ($40^{0.6} = 9.1$ and $160^{0.4} = 7.6$). Those values are then multiplied to calculate the geometric mean ($9.1 * 7.6 = 69.2$).

Example 2: For a sample containing the following records, 1= cobble, 2 = cobble, 3 = boulder, 4= cobble, and 5 = cobble the records are displayed as four observations of cobble and one observation of boulder. Next, the percentage of each substrate type is calculated by dividing each total by the number of total substrate observations (5). This results in a value of 0.8 for cobble, 0.2 for boulder, and zeros for all other size classes. The value of the midpoint diameter of the associated size class is raised to percentage of that substrate type in the sample ($160^{0.8} = 58.1$ and $256^{0.2} = 3$). Those values are then multiplied to calculate the geometric mean ($58.0 * 3.0 = 174.0$).

Table 1. Values used in geometric mean calculations. Midpoint values indicated by * do not represent true midpoint values for the substrate class and are explained in the text.

	Silt and Sand	Gravel	Pebble	Cobble	Boulder	Bedrock and Hardpan	Geometric Mean
Range	0-2	2-16	16-64	64-256	256+	N/A	
Midpoint	2*	9	40	160	256*	5.5*	
Example 1	0	0	0.6	0.4	0	0	
Midpoint^ percentage	1	1	9.1	7.6	1	1	69.2
Example 2	0	0	0	0.8	0.2	0	
Midpoint^ percentage	1	1	1	58.0	3.0	1	174.0

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Appendix D. Calculations used to determine naïve occupancy estimates.

The general equation for estimating the naïve occupancy parameter (ψ_n) is:

$$\Psi_n = \frac{x}{s},$$

where x is the number of stream segments where the species was present and s is the total number of sampled stream segments. The samples used to estimate ψ_n were not randomly selected from within the area of inference (i.e., combined range of *O. peruncus* and *O. quadruncus*) and each HU had a unique number of stream segments. To address this issue, ψ_n was calculated for each 12-digit HU in the area of inference and multiplied by the quantity of the total number of stream segments in that HU (S_{HU}) divided by total number of stream segments in the inference area (S_{AI}) and then summed across all HUs:

$$\Psi_n = \sum \left(\left(\frac{x}{s} \right) * \left(\frac{S_{HU}}{S_{AI}} \right) \right).$$

Had this correction not been applied, ψ_n would have been heavily influenced from the targeted sampling of sites where species presence was known *a priori* and the uneven sampling effort in the various HUs. This correction does not account for all of the bias, but it weights occupancy by the number of stream segments in a HU to provide a better estimate (MacKenzie and Royle 2005).

Ex: There are two HUs and HU 1 has a true ψ of 0.8 and HU 2 has a true ψ of 0.2. The species is detected at 12 of the 15 samples (0.8) taken in HU 1 and at one of the five (0.2)

samples taken in HU 2. Without considering the differences between the numbers of stream segments in each HU, the estimate for ψ in the area of interest (i.e., combined HUs) would be 0.65. However, if HU₁ comprises 100 stream segments (i.e., 67% of total) and HU₂ comprises 50 (i.e., 33% of total), then weighting yields the estimate of ψ as 0.60 (i.e., $0.8*0.67 + 0.2*0.33$).

Appendix E. Front and back of a sample datasheet for the sample unit.

Monitoring Sample Unit Datasheet

Sample Unit:	Date:	Personnel:
County:	Landowner's Name:	Phone Number:
Start and End Time:	Driving Directions:	

Dominant Land Use type	Forested	Row Crop	Pasture	Urban
Riparian Zone Intact (≥ 10 m)	Yes	No		
Stream Disturbance	Cattle access Vehicle tracks Gravel mining Other _____ None			
Temperature _____ °C	Dissolved oxygen _____		pH _____	

Discharge

Distance to LBD (m)	Depth (cm)	Velocity (m/sec)	Distance to LBD (m)	Depth (cm)	Velocity (m/sec)

Notes:

Occupancy Results (indicate 1 if species was present in survey , 0 if absent)

Species	Survey 1	Survey 2	Survey 3	Survey 4
HY				
PE				
QU				

Back.

Sample Unit Coordinate Datasheet		
Sample Unit:		Date:
Instructions: Coordinates to be taken halfway across stream at the downstream edge of the survey habitat type HF ₁ = downstream edge of downstream most high-flow survey unit LF ₁ = downstream edge of downstream most low-flow survey unit ... F ₆ = Upstream edge of upstream most low-flow survey unit		
Survey Unit	UTM X	UTM Y
HF ₁		
LF ₁		
HF ₂		
LF ₂		
HF ₃		
LF ₃		
HF ₄		
LF ₄		
HF ₅		
LF ₅		
HF ₆		

Appendix F. Front and back of a sample datasheet for surveys within a sample unit.

ST. FRANCIS RIVER DRAINAGE MONITORING DATASHEET PAGE ____ OF ____						
SAMPLE UNIT:				SURVEY UNIT:		
DATE:				SURVEY #: 1 2 3 4		
<i>O. hylas</i> : HY <i>O. peruncus</i> : PE <i>O. quadruncus</i> : QU <i>O. luteus</i> : LU <i>O. punctimanus</i> : PU <i>C. hubbsi</i> : HU <i>O. virilis</i> = VI <i>O. harrisoni</i> = HA <i>C. diogenes</i> = DI UNKNOWN = UK						
SAMPLE	SPECIES	# MALES(1)	# MALES(2)	# FEMALES	# YOY	HABITAT
High-flow S ₁						DEPTH
						VELOCITY
						SUBSTRATE
High-flow S ₂						DEPTH
						VELOCITY
						SUBSTRATE
High-flow S ₃						DEPTH
						VELOCITY
						SUBSTRATE

ST. FRANCIS RIVER DRAINAGE MONITORING DATASHEET PAGE ____ OF ____						
SAMPLE UNIT:			SURVEY UNIT:			
DATE:			SURVEY #: 1 2 3 4			
<i>O. hylas</i> : HY <i>O. peruncus</i> : PE <i>O. quadruncus</i> : QU <i>O. luteus</i> : LU <i>O. punctimanus</i> : PU <i>C. hubbsi</i> : HU <i>O. virilis</i> = VI <i>O. harrisoni</i> = HA <i>C. diogenes</i> = DI UNKNOWN = UK						
SAMPLE	SPECIES	# MALES(1)	# MALES(2)	# FEMALES	# YOY	HABITAT
Low-flow S ₄						DEPTH
						VELOCITY
						SUBSTRATE
Low-flow S ₅						DEPTH
						VELOCITY
						SUBSTRATE
Low-flow S ₆						DEPTH
						VELOCITY
						SUBSTRATE

VITA

Jacob Westhoff, son of Thomas and Patricia Westhoff, was born on November 16, 1981 in Mexico, Missouri. Jacob developed an appreciation for the natural world through numerous hikes, canoe trips, hunting and fishing adventures, and visits to parks. He graduated from Montgomery County R-II High School in May, 2000 and enrolled in the Fisheries and Wildlife program at the University of Missouri (MU). While pursuing his degree at MU, Jacob worked part-time for the Missouri Department of Conservation as a resource science aide. In December of 2005, he graduated *cum laude* from MU with a B.S. in Forestry and a B.S. in Fisheries and Wildlife. Jacob attended Tennessee Technological University in Cookeville, TN where he received a M. S. in Biology. Jacob returned to MU and became a doctoral candidate in Fisheries and Wildlife.