The Environmental Factors Regulating the Distribution of Crayfish in the Upper Green River Basin Kentucky, USA

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THE ENVIRONMENTAL FACTORS REGULATING THE DISTRIBUTION OF CRAYFISH IN THE UPPER GREEN RIVER BASIN KENTUCKY, U.S.A.

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Master of Science

By
Eva Mutindi Ngulo

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Dedication
In memory of my dad you continue to be a major influence in who I am today.

To my mum,
You are my inspiration, thank you for being there for me. Your love and support has strongly contributed to my academic growth.

To Edward and Ian,
For your support, love and encouragement, despite the distance.
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Despite the importance of crayfish in aquatic systems there are major issues threatening their conservation, including invasive species, habitat alterations, and species with small distributions or have limited geographical ranges. There is limited information regarding native range, habitat requirements, life histories and biological interactions between crayfish species. In order to examine the relationship between lotic crayfish assemblages and environmental variables at both the watershed and reach scales, data were collected from 46 stream segments in the Upper Green River Basin of Kentucky, U.S.A. An independent sample t-test compared crayfish densities between segments with gravel - small cobble, and large cobble - small boulder substrates and revealed non-significant differences for both all and large (>15 mm) carapace length individuals. Correspondence analyses were conducted separately for gravel–cobble and cobble–small boulder segments and large boulder segments and some species showed strong associations with each other. In the gravel–cobble/cobble–small boulder segments a series of one-way ANOVA’s showed significant effects of subbasin location on both crayfish density and species richness whereas in the large boulder segment there were no significant differences. An exploratory canonical correspondence analysis in the forward
selection procedure was performed to reduce the number of environmental variables in
the gravel–cobble/cobble–small boulder segments and large boulder segments. The
second CCA performed between crayfish species and environmental variables showed
relationships with several environmental variables in the gravel–cobble/cobble–small
boulder segments. Significant variables elucidated were summer mean temperature,
depth, and total phosphate. The second CCA in the large boulder segment, however,
failed to find strong relationships between the crayfish and environmental variables.
Further testing using multiple linear regression stepwise forward selection analysis
demonstrated that crayfish were responding to total phosphorous, % riffle, %run, gravel,
cobble, boulder, total phosphorous and ammonia in the gravel–cobble/cobble–small
boulder segments. The results indicated that stream size gradient and not % land use were
linearly related to both diversity (richness) and density
INTRODUCTION

The structure of an ecological community is influenced by biotic and abiotic factors. Biotic factors include competition, predation, parasitism, and the potential negative impacts of exotic species. Abiotic factors include anthropogenic disturbance, chemical and physical parameters, and habitat availability. The partitioning of local-scale habitat by species is a key concern to ecologists (Arscott et al., 2003) because resulting patterns allow better understanding of the structure and dynamics of a community (Boyero, 2003). Aquatic ecologists, and conservationists concerned with restoring, protecting and conserving aquatic habitats are faced with the problem of determining which suite of potential factors are the most critical (Naimann and Turner, 2000).

Crayfishes are a diverse and important component of freshwater aquatic and semi-aquatic ecosystems globally. Crayfishes are ecologically important members of freshwater communities because they process organic material and help in the transformation and flow of energy throughout aquatic systems (Hobbs III, 1991). Some crayfish species are widely distributed while others are highly restricted in their ranges. Species with small distributions are more susceptible to changes in habitat or non-native species introduction (Jones and Bergey, 2007). Knowledge of how crayfish communities use lotic habitats is important to managers and ecologists because exotic species, habitat degradation and other threats can affect native crayfish (Taylor et al., 1996). Little is known, however, about how human alteration of aquatic environments affects crayfish assemblages (Schofield, 2001).

The importance of crayfish in structuring stream communities has been documented by many researchers (Gelwick and Mathews, 1992; Pringle et al., 1993;
Charlebois and Lamberti, 1996; Flecker, 1996). Habitat partitioning by crayfish is generally attributed to several physical factors, namely flow regimes, substrate types, vegetation and canopy cover (Distefano et al., 2003; Flinders, 2000). However, there have been few studies in determining the crayfish assemblage in relation to both habitat selection and physicochemical parameters.

Interactions between crayfish may have significant consequences for their distribution and abundance via competition for resources and predator-free space. Crayfish will move to new sites when food availability is low, predation risk is high, and acceptable sites are abundant (Ruetz III and Stephens, 2000). Habitat partitioning is observed among potential interspecific competitors (Olson and Young, 2000). Competition for limited food resources is a major driving force within assemblages of similar species (McArthur and Levins, 1967). For many organisms changes in foraging ability and predation risk force convergence in habitat use among different species (Olson and Young, 2000). When species compete, the inferior competitors are driven to extinction, forced to coevolve a habitat shift, or limited to a preferred niche (Gause, 1934; Hutchinson, 1958; Schoener, 1974).

Crayfish are generally associated with large substrates (Flint, 1975; Shimizu and Goldman, 1981; Davies, 1989; Mitchell and Smock, 1991). Studies about organisms occupying different available habitats of freshwater communities are well established (DiStefano et al., 2003; Rabeni et al., 2002). In contrast, crayfish studies of habitat partitioning and habitat use among multiple species of a community have been largely overlooked (DiStefano et al., 2003) and few studies have examined crayfish microhabitat
preferences or relationships with environmental variables (Daniels, 1998; Flinders, 2000; DiStefano et al., 2003).

Agricultural practices have altered stream ecosystems globally by increasing sediment and nutrient loads, increasing stream temperature and altering channel morphology, hydrological regime and composition and abundance of riparian vegetation (McCarthy, 1985; Berkman and Rabeni, 1987; Swales, 1988; Schlosser, 1991; Poff and Allan, 1995; Waters, 1995; Wohl and Caline, 1996; Allan et al., 1997; Wang et al., 1997; Harding et al., 1998; Schleiger, 2000). Impacts and disturbance to riverine systems affect not only water quality, but also the condition and distribution of riverine habitat (Stewart et al., 2000). Physicochemical parameters that determine structure and composition of a community such as water quality, energy source, substrate, channel morphology, flow and thermal regimes are largely determined by watershed scale factors including soil type, bedrock type and depth, watershed topography, land cover and climate.

Crayfish assemblage patterns may be the result of lack of suitable habitat in certain areas that the species require and are thus not able to disperse broadly. Studies that examine sensitivity of crayfish to environmental impairments are lacking and may be the key to their conservation. Considering the diversity of crayfish species, it is logical to suspect that many are vulnerable to the same suite of human-induced factors that affect aquatic organisms (Butler, 2003).

Most studies have shown that agriculture-dominated watersheds can influence both reach scale and watershed factors of aquatic habitats (Richard et al., 1996). Taylor et al. (1996) argued that crayfish are threatened by stream channelization, sedimentation, habitat destruction caused by dams, water pollution, erosion, siltation, in stream gravel
degrading, introduction of nonnative crayfish and other exotics. Most organisms are
faced with a patchwork of habitats and environmental conditions that vary temporally and
spatially across a landscape (Fortino et al., 2006). The spatial arrangement of land uses
within a watershed can influence stream habitat (Stewart et al., 2000). Anthropogenic
disturbance of land cover from various human activities is an extensive factor influencing
aquatic habitats (Sawyer et al., 2004), and the effects of changing land uses on
ecosystems manifest at several spatial and temporal scales (Goossenlink et al., 1990).

Differences in physicochemical parameters of aquatic habitats have been
identified as factors responsible for observed differences in crayfish communities
(Flinders, 2000). Physical habitat is important in crayfish distribution because natural
feature habitats can result to community structuring at both the reach and watershed
scales (Flinders and Magoulick, 2005). Land use practices can result to habitat
degradation and thus fewer areas available as shelter to crayfish due to siltation,
sedimentation, logging, development, agricultural and urban runoff (DiStefano et al.,
2003).

The primary objective of this study was to assess the influence of watershed- and
reach-scale environmental variables of the crayfish assemblage in the Upper Green River
Basin, U.S.A. Several questions were addressed: (1) are assemblages distinctive to
individual subbasins?, (2) does land-use patterns influence assemblages?, and (3) are
there other environmental parameters that exert an influence on assemblages?
METHODS

Sampling area

All fieldwork was conducted in 46 100-m stream segments distributed among six small subbasins of the Upper Green River Basin (UGRB) located in the Interior Plateau Level-III Ecoregion of central Kentucky, U.S.A. (Woods et al., 2002; Fig. 1). Segments encompassed 3rd- to 6th-order reaches and drainage area ranged from 11.0 – 749.3 km². The number of segments per subbasin varied, as Little Russell Creek (n=1), Lynn Camp Creek (n=2), Big Brush Creek (n=8), Big Pitman Creek (n=11), Little Barren River (n=10), and Russell Creek (n=15). Most stream segments are positioned within the Eastern Highland Rim Level-IV Ecoregion and underlain by limestone, sandstone, and shale. The remaining segments are located in the Western Pennyroyal Karst Plain Level-IV Ecoregion. This karst region is underlain by Mississipian-age St. Genevieve and St. Louis limestones (McDowell et al., 1988). Springs, sinkholes, and groundwater input are common.

The Green River comprises most of the west-central portion of Kentucky and drains approximately 24,000 km² of the Highland Rim and Shawnee Hills in Kentucky and nearly 1000 km² in northern Tennessee. The Green River Basin varies in topography from the upland, rolling plateau in the upper reaches to the lowland, broad floodplain near the mouth. The two major physiographic regions are the Western Coalfield region, which comprises the western portion of the basin, and the Mississippian Plateau region, which makes up the majority of the basin. The Western Coalfield is characterized by broad, alluvial bottoms of major rivers and hilly uplands, exposed rocks of sandstone, siltstone, and shale with beds of limestone, dolomite, and coal. The Mississippian Plateau
region is divided into the Mammoth Cave Plateau to the north and the Pennyroyal Plateau to the south. Subterranean streams and sinkholes are common (Kentucky Department of Natural Resources and Environmental Protection, 1972; Burr and Warren, 1986).

**Environmental variables**

Environmental variables were partitioned into watershed- and reach-scale spatial categories. Land use was quantified at the watershed scale using 8-, 11-, and 14-digit Hydrologic Unit Code (HUC) watersheds produced by the U.S. Geological Survey (USGS). Karst inferred drainages and karst basins were obtained as GIS layers through the Kentucky Division of Water. Surface drainages upstream of particular sites were selected using the National Hydrography Dataset (NHD) and NHD Toolkit published by the U.S. Environmental Protection Agency (EPA). Selections of partial HUC watersheds (e.g., upstream from a particular sampling site) were made using 30-m USGS Digital Elevation Models (DEMs) and combining those partial sections with appropriate HUC watersheds. Where necessary, sections of contributing karst drainage basins were added to surface flow watersheds for accuracy in evaluating contributing area and runoff sources. Buffer polygons were created using ArcGIS with the NHD as the stream layer. The Kentucky Land Cover Data Set used for the landuse data source was published by the USGS in 1999, a joint project of the U.S.G.S. and E.P.A., and was derived from Landsat Thematic Mapper (TM) data obtained ca. 1992 with a spatial resolution of 30 m. Land use classes assigned were those of the National Land Cover Dataset (NLCD) Land Cover Classification System (Rev. 07/99). To derive landuse summaries, polygon features were converted to raster format using Spatial Analyst, and landuse and polygon
(watershed or buffer) rasters were combined, adding an area calculation to the derived attribute table, and summing areas for each landuse class. GIS work was performed using ArcGIS 8.1, with NHD work using ArcView 3.2.

Total watershed area above each sampling point was determined using an online tool to obtain drainage area for Kentucky streams (U.S.G.S., 2004). In-stream parameters were quantified from May – October 2002 and 2003 by a two-tiered approach. Tier one parameters were measured monthly with a Hydrolab Series 4a multiprobe sonde (Loveland, CO, U.S.A.) (2002) and Hydrolab Quanta (Loveland, CO, U.S.A.) (2003) as temperature (°C), pH (S.U.), turbidity (NTU), dissolved oxygen (mg/L), total dissolved solids (mg/L), and conductivity (μs/cm). Tier two parameters were analyzed bimonthly according to Standard Methods (AHPA, 1998) as nitrate (mg/L), ammonia (mg/L), orthophosphate (mg/L), total phosphorous (mg/L), sulfate (mg/L), chloride (mg/L), and total suspended solids (mg/L). Environmental parameters that fell below detection limits (DL) were treated as DL/2 prior to statistical analyses (Helsel, 1990; EPA, 1998).

At each segment, width (m), depth (cm), and current velocity (m/s) were quantified during baseflow conditions, the latter with a Marsh-McBirney Flo-Mate Model 2000 Portable Flowmeter (Frederick, MD, U.S.A.). The proportion of riffle, run, and pool were estimated visually, and proportions of individual substrates (e.g., cobble) were estimated visually according to the Wentworth Scale (Cummins, 1962).

Crayfish sampling

Each segment was characterized either as gravel - cobble or cobble - small boulder based on the dominant coarse substrates present. In addition, each cobble - small
boulder segment was also characterized by the presence of several larger boulders but gravel-cobble segments lacked any boulder habitat. Sampling occurred only in riffle or run habitat. Crayfish were collected with a quantitative kick-net method by disturbing the substrate of a 1 mm² area directly upstream of a 0.5-mm mesh seine (Flinders and Magoullick, 2003). Ten replicate samples were taken from the gravel-cobble segments. In the cobble-small boulder segments, 10 replicate samples were taken each from the smaller substrates and from larger boulders. If crayfish could not be field-identified to species they were preserved in 95% ethyl alcohol and returned to the laboratory. After sampling was completed, both depth and current velocity were measured immediately upstream of the sampling quadrat.

Carapace length for all individuals was measured with a caliper to the nearest mm. All individuals were subsequently classified either as small (carapace ≤ 15mm) or large (carapace length > 15mm) (Flinders and Magoullick, 2003). Species identifications in the laboratory were based on Hobbs (1989), Schuster and Lawson (2000) and Guenter and Taylor (2004).

Statistical analyses

Data were divided initially into environmental and biological categories. Proportion environmental variables (e.g., riffle, land-use) were double-transformed as arcsine of the square root and all remaining environmental variables, except pH, were \(\log_{10}(1+x)\)-transformed. Biological data were simplified into three abundance matrices and species richness per segment. The abundance matrix was based on the mean density value per stream segment and data were \(\log_{10}(1+x)\), where \(x =\) density, prior to analyses.
Crayfish density was partitioned into total density and both density of large (>15 mm carapace length) and small (<15 mm carapace length) individuals.

Both univariate and multivariate statistical analyses were performed to assess the relationship between crayfish assemblages and environmental variables. Preliminary independent sample t-tests were used to compare crayfish densities between segments with gravel–cobble and cobble–small boulder substrates. This test was used to assess if there were density differences between the above-mentioned substrates and if non-significant, then all stream segments could be pooled together for subsequent analyses. Both density (p = 0.274) and richness (p = 0.934) were not significantly different and data were pooled.

The effect of subbasin location on crayfish species richness and density were tested with one-way ANOVA’s. The subbasin location ANOVA was performed to assess if there were geographic-specific differences between only the four largest subbasins (Big Brush Creek, Big Pitman Creek, Little Barren River, and Russell Creek). Both Little Russell Creek and Lynn Camp Creek were not used in this analysis because only one and two segments, respectively, were included in each subbasin. Alpha was set at p < 0.05 and if significant differences were found a Tukey HSD test was performed on pair-wise comparisons. The ANOVA’s were run identically on both the gravel–cobble/cobble–small boulder substrates and large boulder substrates. The main difference between the two sets of analyses was sample size. The former included all gravel - cobble + cobble - small boulder segments (n = 46) compared to the latter segments with large boulders (n = 20).
Multivariate analyses

Correspondence analysis (CA) was performed in order to investigate species (or taxon) assemblage relationships to site characteristics without the influence of environmental variables. A CA is a multivariate ordination technique that uses reciprocal averaging in an iterative process to discover the underlying environmental gradient behind species abundances at sites. Its main assumption is that species abundance distributions are unimodal and reflect an approximately normal distribution in response to the environmental gradient (Gotelli and Ellison, 2004). Associations between the distributions of crayfish with environmental parameters were tested with canonical correspondence analysis (CCA) and stepwise multiple regressions. A CCA is a direct gradient eigenanalysis used to relate a predictor variable to response variables. The analyses started with 28 environmental variables, which were subsequently reduced in number to meet the assumptions of CCA (ter Braak, 1986). Pearson correlation coefficients were used to detect collinearity among environmental variables. Redundant variables were identified and removed. A maximum correlation coefficient (r > 0.80) and (p < 0.05) was used for unacceptable collinearity. The CCA and multiple regressions were run identically on both the gravel–cobble/cobble—small boulder and large boulder substrates. A CCA with forward selection was employed to further reduce environmental variables from twenty-one to ten where p ≤ 0.05. A second CCA using only those variables retained during the forward selection procedure was performed to determine species - environmental relationships. All Monte Carlo permutations used 999 interations. Multiple linear regressions assessing the relationships between species abundance and the environmental variables used in the second CCA were performed, but only if either CCA was significant for both the first and all axes.
CANOCO (Version 4.5 for Windows, Agricultural Mathematics Group Wagenningen, The Netherlands) was used for CA and CCA while SYSTAT® 11 (SPSS, Inc, Chicago, IL, USA), and SPSS (Version 12 for Windows, SPSS, Inc) was applied for the independent sample t-tests, ANOVA, multiple regressions, and Pearson correlations.
RESULTS

*Environmental variables*

Stream habitat features varied between the stream segments. Mean stream widths ranged from 0.6 - 26.0 m and maximum summer temperatures ranged from 17.5 - 27.6 °C (Table 1). Channel morphology and substrate composition varied substantially with sites having an abundance of fast-flowing riffles and gravel-cobble substrate to sites with smooth flow (runs) and large amounts of large boulder substrate.

Stream reaches exhibited a wide range of water chemistry characteristics (Table 1). Dissolved oxygen values ranged from 7.6 to 11.2 mg L\(^{-1}\), conductivity ranged from 169 to 450 µs, and turbidity varied from 16 to 98 nephelometric turbidity units (NTU). Orthophosphates varied from <0.01 to 0.5 mg L\(^{-1}\), total phosphorous ranged from <0.01 to 0.7 mg L\(^{-1}\), sulfate ranged from 4.4 to 77.3 mg L\(^{-1}\), ammonia varied from 0.01 to 0.4 mg L\(^{-1}\), nitrate ranged from 0.4 to 9.8 mg L\(^{-1}\), and chloride varied from 2.6 to 27.0 mg L\(^{-1}\).

*Crayfish assemblages*

A total of 738 crayfish individuals belonging to 8 species were collected. Five species of *Cambarus* were collected followed by two species of *Orconectes* and the monotypic *Barbicambarus cornutus*. Russell Creek (n=278) produced the most individuals followed by Big Pitman Creek (n=206), Little Barren River (n=177), Big Brush Creek (n=55), Little Russell Creek (n=16), and Lynn Camp Creek (n=6). The most abundant species in the gravel-cobble/cobble-small boulder segments were *O. rusticus*, comprising 68% of the total individuals obtained. The only other commonly collected species was *O. putnami* (27%), while *C. graysoni*, *C. dewasee*, *C. rustiformis*,
C. ortmanni, C. cumberlandensis each contributed < 3 % (Figure 4). Orconectes rusticus was the most widely distributed species, found in 36 of 46 sites, followed by O. putnami (28 sites). Cambarus was less widely distributed and relatively few individuals were collected per site. Barbicambarus cornutus was obtained only from one site (Table 2).

Three species of Cambarus were collected in the large boulder segments followed by two species of Orconectes, and B. cornutus. The most abundant species was O. rusticus, comprising 72 % of the total individuals obtained. The only other commonly collected species was O. putnami 25 %, while C. graysoni, C. rustiformis, C. cumberlandensis and B. cornutus each contributed < 3 % (Figure 5). Orconectes rusticus was the most widely distributed species, found in all twenty sites, followed by O. putnami (14 sites). Cambarus was less widely distributed and relatively few individuals were collected per site.

There were significant differences in total and large crayfish abundance with respect to subbasin location in the gravel–cobble/cobble–small boulder segments (Tables 3-4). There was only one significant pairwise difference with respect subbasin (Big Pitman Creek).

Densities of Orconectes rusticus (both all and large) exhibited significant differences with respect to subbasin location. There were only two significant pairwise differences with respect to subbasin location (Big Pitman Creek and Little Barren River) for large O. rusticus but only one (Big Pitman Creek) when all individuals were included (Table 4). Densities of O. putnami (large and all), Cambarus graysoni (large) C. ortmanni (large) C. dewasee (large) did not differ significantly between subbasin
locations (Table 5). In the large boulder segments there was no significant difference in crayfish abundance (Table 6) and species richness (Table 7) by subbasin location.

The CA for the gravel - cobble + cobble - small boulder segments revealed that *Cambarus rustiformis* (large), *Orconectes rusticus* (large and all), *O. putnami* (large and all), *C. ortmanni*, and *Barbicambarus cornutus* were tightly aligned along Axis 1 yet appeared to form a series of assemblage replacement along Axis 2 (Figure 6). In contrast, *C. cumberlandensis, C. dewasee, and C. graysoni* formed a group distinctive from all remaining species along Axis 1. The CA in the large boulder segments showed that *C. cumberlandensis* was partitioned from the remaining species along Axis 2 (Figure 7).

*Orconectes rusticus* (large and all), *O. putnami* (large and all), *B. cornutus* and *C. graysoni* were tightly clustered along Axis 1. Similar to the gravel – cobble – cobble – small boulder segments, there was a gradual pattern of replacement along Axis 2.

**Species environmental relationships**

The CCA for the gravel - cobble + cobble - small boulder segments with forward selection reduced the number of environmental variables from 21 to 10. The CCA was significant for the first axis (p = 0.04) and the first four axes combined (p = 0.006) (Table 8). The first three axes cumulatively explained 66% of the variation in crayfish assemblage data. The CCA biplot depicted relationships between several environmental variables (e.g., temperature, depth) with crayfish abundance patterns (Figure 8). CCA Axis 1 was depicted a gradient of percent boulder and gravel substrates, percent run habitat, and temperature. *Orconectes putnami* and *Cambarus ortmanni* were associated with stream segments with high _ percent boulder habitat, while *C. rustiformis* and *C.
*cumberlandensis* were tied to segments with high _ percent run and gravel habitats and low_ summer temperature. *Cambarus dewasee* and *C. graysoni* form a group portioned along Axis 2, and associated with high_ percent cobble habitat, low_ velocity, and _ depth.

The CCA for large boulder segment showed no associations between the environmental variables and crayfish species richness. The CCA was not significant on the first axis (p = 0.952) and all four axis (p = 0.898).

Multiple regression analysis with both gravel - cobble + cobble - small boulder and large boulder segments produced several significant linear models between individual species abundance patterns and environmental variables. In the gravel - cobble + cobble - small boulder segment the total density of large crayfish was linearly related to total phosphorus and percent gravel habitats (p = 0.029, r² = 0.51) while density (all) was linearly related to percent boulder and riffle habitats (p = 0.015, r² = 0.52; Table 9). Individually, the abundance of *O. putnami* (large) was linearly related to percent run habitat and summer mean temperature (p = 0.024, r² = 0.35) while *O. putnami* (all) was linearly related only to percent run (p = 0.034, r² = 0.33). *Orconectes rusticus* (large) was linearly related to percent gravel habitat (p = 0.039, r² = 0.26), *O. rusticus* (all) was linearly related to percent riffle and boulder habitats (p = 0.005, r² = 0.67), and *C. graysoni* (large) was linearly related to percent cobble habitat and ammonia (p = 0.001, r² = 0.446).

For the large boulder segments crayfish density (all) was linearly related to total phosphorous, ammonia, percent riffle habitat and depth (p < 0.001, r² = 0.72) while density of the large individuals was linearly related to only to depth (p = 0.025, r² = 0.24;
Table 10). Individually, *Orconectes putnami* (large) was linearly related to percent riffle habitat and summer mean temperature ($p = 0.036, r^2 = 0.45$) and *Orconectes putnami* (all) was linearly related to temperature and percent boulder habitat ($p = 0.019, r^2 = 0.44$). The only remaining species with a significant linear relationship was *O. rusticus* (large and all), as ammonia, run, cobble, depth, and gravel ($p = 0.003, r^2 = 0.69$) and ($p = 0.001, r^2 = 0.75$), respectively.

DISCUSSION

Crayfish assemblage in Kentucky’s Upper Green River Basin showed some patterns of partitioning between individual subbasins. The correspondence analysis in the gravel-cobble/cobble-small boulder segment and in the large boulder segments showed that some crayfish species were strongly associated with each other and some showed no association with other crayfish species or weak associations.

The ANOVA results for the gravel-cobble and cobble-small boulder substrates demonstrated that total crayfish density (all and large) and individually for *O. rusticus* (all and large) were influenced by subbasin location (Big Pitman Creek). The presence of *O. rusticus* (all and large) in these subbasins suggests that Big Pitman Creek and Little Barren River are suitable habitats for this species. Watershed location has been shown to influence stream fish assemblage (Wang et al., 2003). The influence of basins on the distribution of aquatic species provides opportunities for supporting distinct faunal groups (e.g., fishes and mussel; Burr and Warren, 1986; Cicerello et al., 1991).

When river basins transverse one or more physiographic regions, which commonly occurs in Kentucky, a paradigm can occur. A river’s fauna can be more
related within a region than within its own system if the basin crosses several regions like Kentucky’s Upper Green River Basin (Burr and Warren, 1986). Factors influencing this pattern can be attributed to topography and geology, which can in turn dictate features of a stream (e.g., land use, in-stream habitat flow regimes, and temperature). Strande (1999) points out; however, that habitat quality dictates species persistence both within a broader ecological region and within individual systems.

Fortino et al. (2006), in a study of streams in the New River system of North Carolina, showed that the dominant crayfish species changed with increasing stream size. As stream size increases fish appear and become an important part of the predator assemblage as the whole community of larger streams are likely structured by fish predation (Englund and Krupa, 2000; Creed, 1994; Fortino et al., 2006). Momot et al. (1978) suggested that environmental and geographic factors might affect population density, growth and different populations within same species.

Researchers have shown that regional crayfish extinctions can occur as a result of competitive exclusion by other crayfish or differential predation by fish and other predators (Flinders and Magoullick, 2003, 2005). Furthermore, competitive exclusion of other crayfish by *O. rusticus* increases risk of predation. Hill and Lodge (1994) found that *O. rusticus* has been dominant over other native species and succeeded in excluding them from shelters, which result in higher predation rate of the native crayfish species. In Upper Green River Basin *O. rusticus* may be the more successful competitor than other crayfish species, which has resulted in high densities of the species in the subbasin. Since only *O. rusticus* was relatively abundant it was most likely to exhibit changes in distribution and abundance (Daniels et al., 1998). Researchers have suggested that
aggressive interactions and competitive hierarchies among species can lead to
competitive exclusion and crayfish populations consisting of one or two dominant species
(Flinders, 2000).

In the large boulder segment densities of crayfish and species richness did not
differ significantly between subbasins. However, the observed spatial variation in Upper
Green River Basin may be a function of predation, competition and other abiotic factors
associated with each site. These differences in crayfish distribution may be the result of
adaptations of the habitat conditions found in these streams (Flinders and Magoulick,
2005).

The CCA results in the gravel - cobble and cobble - small boulder segments
suggest that the reach-scale variables were important in the structuring of crayfish
assemblage. Crayfish distribution and abundance in the gravel - cobble and cobble-small
boulder substrate differed with species and appeared to be a function of stream size. In
the large boulder segments the CCA results revealed that there was no significant
relationship between the crayfish species richness and environmental variables. The lack
of demonstrated relationship may no be spurious, but there are some important
considerations.

The lack of clear relationships may be due to the habitats sampled were only runs
whereas in the gravel - cobble and cobble - small boulder segments sampling consisted of
both run and riffle habitats. Macrohabitat partitioning of crayfish based on physical and
chemical conditions of the stream may influence their selection of different habitats and
show no habitat associations in analyses (Flinders, 2000). Therefore, species -
environment relationships may not be as strong as expected. Since only O. rusticus and
*O. putnami* were relatively abundant in the large boulder segments they are most likely to interact repeatedly and to exhibit changes in distribution and abundance in the regression analysis.

The regression analysis revealed that *O. putnami* (large and all) in the gravel-cobble and cobble-small boulder segments were significantly related to percent run habitat. Crayfish may partition within larger habitat types (riffles, pools and runs) (Flinders, 2000). Differential habitat use and selection by crayfish may play a role in influencing crayfish assemblages (Daniels et al., 1998; Flinders and Magoulick, 2007). Macrohabitats within streams can be spatially heterogeneous in terms of substrate, resource availability and velocity (Flinders and Magoulick, 2003). Flinders (2000) suggested that crayfish species select habitats with reduced flow because run habitats reduce accidental drift and may increase foraging efficiency. In addition, these habitats are quite deep and provide refuge from harsh conditions while providing cover from avian, aquatic, and terrestrial predators.

Researchers have shown that some crayfish species tolerate a broad range of temperatures, but tolerances are typically species-specific (Flinders and Magoulick, 2003). *Orconectes putnami* (large) densities in the gravel-cobble and cobble-small boulder segments was significantly related to summer mean temperature. Studies have shown that water temperature was important in determining crayfish abundance in Japan (Usio et al., 2007). Spanjer and Cipollini (2006), in a study of 22 caves, showed that temperature was important for the growth and survival of crayfish and was a contributing factor influencing their distribution. If water temperature is too cold than crayfish growth may be reduced and hinder a species ability to compete for resources (i.e, food and
Previous studies suggested that larger crayfish are more aggressive and choose habitats with favorable temperature (Peck, 1985). Fluctuations in water levels as a result of increased temperatures can modify connections between macrohabitats and influence distribution of crayfish (Flinders, 2000, Jones and Bergey, 2007). The degree of shade cover along the banks can modify the water temperature (Wang et al., 2003). Shade cover along waterways reduces the amount of sunlight directly hitting the water, which allows the water to be cooler. Drainage from unshaded agricultural fields or paved urban areas has warmer temperature than drainage from wooded areas because of exposure to sunlight (Michaud, 1994).

Species-specific substrate preferences have been demonstrated for several species (Bovbjerg, 1970; Klosterman and Goldman, 1981; Mitchell and Smock, 1991). Researchers have shown that availability of substrate was a strong factor affecting the population size of crayfish (Hill and Lodge, 1994). This study revealed statistically significant associations between several crayfish species and substrate composition. *Orconectes rusticus* (large) and crayfish abundance (large) were significantly related to percent gravel substrates, while *O. rusticus* (all) and *C. graysoni* (large) were significantly related to percent boulder and cobble substrates. Most studies have shown that crayfish distribution is influenced by substrate composition (Bovbjerg, 1970; Blake and Hart, 1994; Lodge and Hill, 1994; DiStefano et al., 2003; Jones and Bergey, 2007).

Capelli and Magnuson (1983) concluded from their extensive survey of Wisconsin lakes that substrate was the most important variable accounting for crayfish abundance. Substrate is an important factor for shelter and growth of crayfish. Crayfish choose larger or more complex substrates because they provide shelter from predators.
(Stein and Magnuson, 1976; Stein, 1977; Blake and Hart, 1994; Kreshner and Lodge, 1995). In some lotic systems, coarse substrates such as cobble and boulders play important roles in determining crayfish distribution (Usio and Bergey, 2007). Boulders may serve as a refuge against fish, bird, mammal and conspecific predators (Usio and Bergey, 2007).

Some crayfish can be found in stream areas with high currents because many crayfish cannot survive here and thus may take advantage of this underutilized microhabitat. *Orconectes rusticus* (all) abundance and total crayfish abundance in the gravel - cobble and cobble - small boulder segments were significantly related to the percent riffle habitat. Riffles were areas of shallow, faster flowing water consisting of gravel - cobble and cobble - small boulder substrate. Because of the composition of the community occupying riffles, they may function as feeding habitats for many species of crayfish as well as provide predator avoidance opportunities (DiStefano et al, 2003).

Depth is among the most important factor influencing crayfish habitat selection (Flinders, 2000). The use of shallow water habitats to avoid predation has been well documented in crayfish communities (Flinders, 2000; Distefano et al., 2003). The bigger fish - deeper habitat hypothesis suggests that depth selection by fish increases with increasing fish size. It is hypothesized that larger fish select deeper habitats to avoid predation by aquatic predators. Fish that are predators of small crayfish are likely more to be abundant in deeper than shallow habitats so risk of predation by fish may be reduced by crayfish selecting shallower habitats (Flinders, 2000). In the large boulder segment crayfish abundance (large and all) and *O. rusticus* (large and all) were significantly related to depth.
Previous studies have reported that *C. ortmanni*, *C. graysoni*, *C. dewasee*, *O. rusticus* and *O. putnami* occupy similar habitats in the Upper Green River Basin (Lawson, 2003). The co-existence of synoptic species can result in competitive interactions (Garvey et al., 1994) although there was no evidence of limiting resources. Some researchers have suggested that competitive exclusion as an explanation for observed patterns in crayfish distributions (Capelli, 1982). Crayfish are aggressive organisms and compete for limited food resources, shelter and preferred habitat (Rabalais and Magoulick, 2006). Crayfish assemblage patterns may be the result of lack of suitable habitat in certain areas that the species require and are thus not able to disperse broadly (Stein, 1977).

My findings showed that crayfish abundance patterns do not differ significantly between watershed-scale land use types. Land use conditions consistently explained less of the variance in reach conditions than the watershed area. Watersheds dominated by agriculture showed strong correlations where coverage exceeded 50 % (Wang et al., 2003). Sponjer et al. (2000) found that land use within a 200m riparian zone predicted macroinvertebrate assemblages better than watershed scale land use.

Crayfish in the Upper Green River Basin showed specific associations with environmental variables and may have developed species specific tolerances and adaptations to environmental conditions associated with stream size which allow exploitation of all lotic habitats and may explain observed watershed distribution. Stream size and the reach-scale environmental variables consistently explained more variation than watershed-scale percent % land use. Similar results were documented previously by both Flinders and Magoulick (2005) and Fortino et al. (2006), where specific tolerance
and adaptation to environmental conditions associated with stream size influenced crayfish distribution. Variation in distribution of species throughout the basin could be due to abiotic and biotic factors associated with stream size (Flinders and Magoulick, 2005).

Biotic factors, such as interspecific competition and differential predation by fish predators, can play a significant role in determining crayfish habitat use (Flinders and Magoulick, 2005). It is likely that an interaction of biotic factors and crayfish environmental tolerances are responsible for determining the distribution and structure of crayfish communities in the Upper Green River Basin. Competition and body size may dictate habitat use and partitioning among crayfish species and this may be important in understanding crayfish distributions (Stein 1977; Garvey et al. 1994). Fish predation can be an important factor affecting crayfish distribution and abundance (Englund, 1999; Englund and Krupa, 2000; Fotino et al 2006). Interactions between crayfish may have significant consequences for their distribution and abundance via competition for predator-free space.

A feasible hypothesis explaining crayfish distribution in Upper Green River Basin is different competitive hierarchies and predator avoidance under different environmental conditions. Crayfish assemblage patterns may be the result of lack of suitable habitat in certain areas that the species require and are thus not able to disperse broadly (Flinders and Magoulick, 2003). The presence of a species in a particular habitat is determined by its ability to tolerate local environmental conditions as well as coexist with potential competition and predators found there (Fortino et al., 2006). Some researchers have suggested that changes in abiotic factors, presence of competitors and predators across
watershed influence crayfish distributions (Flinders and Magoullick, 2005; Fortino et al., 2006).

Few previous studies have explored how environmental variables operating at different spatial scales are related to crayfish assemblages. From a comparison of our findings with those from limited number of similar studies we conclude that reach-scale environmental variables had a stronger effect on the crayfish assemblages than the watershed land use variables. It was clear the watershed-scale variables (stream size) had indirect effects on crayfish through their direct influence on reach-scale environmental variables.

The results of this study provide previously unknown information about the crayfish assemblage in Kentucky’s Upper Green River Basin. Knowledge of species distribution is essential for conservation and management. The reach scale has been particularly useful for assessing medium- and long-term effects of anthropogenic activities in streams (DiStefano et al., 2006). Previous crayfish distribution studies have incorporated measurements of potentially important environmental variables (DiStefano et al., 2006). Data relating crayfish species richness and abundance to specific environmental variables at different spatial scales should prove useful to future observation efforts. Such efforts should facilitate conservation of crayfishes by providing biologists with the ability to predict locations harboring these species or locations that may be suitable for conservation efforts (DiStefano et al., 2006). I was somewhat successful in identifying variables that may facilitate future searches and conservation planning and management for crayfish species in Kentucky’s Upper Green River Basin.
Much effort is and will continue to be required in order to recover disturbed lotic habitats and to conserve and preserve existing natural ecosystems.

Future research should incorporate the temporal scale. Sampling of this study occurred in the summer months and did not take into account differential seasonal variation in crayfish assemblages. Seasonal variation can influence environmental factors, which affect crayfish assemblage in streams (DiStefano et al., 2003; Flinders and Magoulick, 2003; Jones and Bergey, 2007). Additional research is needed to gain a better understanding of species distribution in terms of interspecies and intraspecific competition and predation closer examination of these variables may improve our understanding of crayfish assemblage and habitat requirements.
FIG 1.—Map depicting the locations of subbasins in the upper green river basin
FIG 2.—Map depicting the landuse activities in the Upper Green River Basin.
FIG 3.–Mean (± SE) crayfish density (no./m$^2$) in gravel-cobble and cobble-small boulder substrates.
FIG 4.—Mean (±SE) density (no./m²) of crayfish species in gravel – cobble + cobble – small boulder segment O. rus (*Orconectes rusticus*), O.put (*Orconectes putnami*), C. gra (*Cambarus graysoni*), C. ort (*Cambarus ortmanni*), C. dew (*Cambarus dewasee*), C. rusti (*Cambarus rustiformis*), C. cum (*Cambarus cumberlandensis*), and B. corn (*Barbicambarus cornutus*).
FIG 5.—Mean (±SE) density (no./m²) of crayfish species in large boulder segment O. rus (Orconectes rusticus), O.put (Orconectes putnami), C.ort (Cambarus ortmanni), C. gra (Cambarus graysoni), C. cum (Cambarus cumberlandensis), C. rusti (Cambarus rustiformis) and B. corn (Barbicambarus cornutus).
FIG 6.—Associations of crayfish species-size class by stream in the gravel – cobble + cobble – small boulder segments. Open circles represent stream sites and closed circles represent species-size classes.
FIG 7.— Associations of crayfish species-size class by stream in the large boulder segments. Open circles represent stream sites and closed circles represent species.
FIG 8.– CCA biplot environmental variables with crayfish abundance in the upper green river subbasins in the gravel – cobble + cobble – small boulder segments. Circles represent crayfish species size classes and arrows indicate environmental variable gradients.
TABLE 1. Mean, standard error (SE) and range for watershed and reach variables that were correlated with crayfish variables in the correlation matrix of the first canonical correspondence analysis (CCA).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Mean ± SE</th>
<th>Range</th>
<th>Crayfish Diversity</th>
<th>Crayfish Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watershed and reach scale</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WSAREA</td>
<td>Watershed area km²</td>
<td>165 ± 30</td>
<td>11 - 749</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEL</td>
<td>Velocity</td>
<td>0.28 ± 0.03</td>
<td>0.00 - 0.73</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Watershed land use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGRICU</td>
<td>% agricultural land use</td>
<td>28.10 ± 2.46</td>
<td>4.88 - 57.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOREST</td>
<td>% forest land use</td>
<td>69.50 ± 2.89</td>
<td>31.5 - 94.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>URBAN</td>
<td>% urban land use</td>
<td>1.03 ± 0.29</td>
<td>0.04 - 8.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reach channel morphology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEP</td>
<td>Depth</td>
<td>9.90 ± 0.81</td>
<td>1.00 - 24.8</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>WID</td>
<td>Width</td>
<td>7.93 ± 0.89</td>
<td>0.60 - 26.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP_POOL</td>
<td>Pool</td>
<td>0.19 ± 0.03</td>
<td>0.00 - 0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>Discharge</td>
<td>0.39 ± 0.08</td>
<td>0.00 - 2.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP_RIFFLE</td>
<td>Riffle</td>
<td>0.28 ± 0.03</td>
<td>0.05 - 0.75</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PROP_RUN</td>
<td>Run</td>
<td>0.53 ± 0.04</td>
<td>0.05 - 0.95</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Reach substrate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP_BD</td>
<td>Bedrock</td>
<td>0.35 ± 0.06</td>
<td>0.00 - 0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP_BO</td>
<td>Boulder</td>
<td>0.02 ± 0.01</td>
<td>0.00 - 0.15</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PROP_CB</td>
<td>Cobble</td>
<td>0.20 ± 0.02</td>
<td>0.00 - 0.60</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PROP_GV</td>
<td>Gravel</td>
<td>0.20 ± 0.02</td>
<td>0.00 - 0.60</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PROP_CBGV</td>
<td>Gravel - Cobble</td>
<td>0.41 ± 0.04</td>
<td>0.04 - 0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP_FN</td>
<td>Fine sand</td>
<td>0.22 ± 0.03</td>
<td>0.00 - 0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reach water quality and temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEMP</td>
<td>Temperature</td>
<td>21.9 ± 0.32</td>
<td>17.5 - 27.7</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PH</td>
<td>pH</td>
<td>7.73 ± 0.04</td>
<td>7.17 - 8.23</td>
<td></td>
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</tr>
<tr>
<td>COND</td>
<td>Conductivity</td>
<td>315 ± 11</td>
<td>164 - 445</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TURB</td>
<td>Turbidity</td>
<td>38.4 ± 2.57</td>
<td>16.0 - 98.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
<td>8.97 ± 0.11</td>
<td>7.60 - 11.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-PO4</td>
<td>Orthophosphate</td>
<td>0.16 ± 0.03</td>
<td>0.02 - 0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOT_P</td>
<td>Total phosphorous</td>
<td>0.16 ± 0.02</td>
<td>0.02 - 0.68</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SUL</td>
<td>Sulfate</td>
<td>23.30 ± 2.44</td>
<td>3.77 - 77.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH4N</td>
<td>Ammonia</td>
<td>0.05 ± 0.01</td>
<td>0.01 - 0.37</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>NO3N</td>
<td>Nitrate</td>
<td>3.62 ± 0.32</td>
<td>0.41 - 9.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>Chloride</td>
<td>11.3 ± 1.02</td>
<td>2.55 - 29.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: An "X" indicates the environmental variables that were retained by the stepwise forward selection of the second CCA for each crayfish variable.
TABLE 2. Presence/absence of crayfish species in each individual subbasin of the Upper Green River Basin. “X” indicates presence in the gravel - cobble and cobble - small boulder substrate and “ Y” indicates presence in the large boulder substrates.

<table>
<thead>
<tr>
<th>Taxonomic Name</th>
<th>Common Name</th>
<th>Lynn Camp Creek</th>
<th>Little Russell Creek</th>
<th>Big Brush Creek</th>
<th>Big Pitman Creek</th>
<th>Little Barren River</th>
<th>Russel Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbicambarus cornutus</td>
<td>Bottle brush crayfish</td>
<td></td>
<td></td>
<td>Y</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cambarus cumberlandensis</td>
<td>Cumberland crayfish</td>
<td></td>
<td></td>
<td>Y</td>
<td></td>
<td></td>
<td>X,Y</td>
</tr>
<tr>
<td>Cambarus deweesae</td>
<td>Valley Flame crayfish</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cambarus graysoni</td>
<td>Two-spot crayfish</td>
<td>X,Y</td>
<td></td>
<td>X</td>
<td></td>
<td>X,Y</td>
<td>Y</td>
</tr>
<tr>
<td>Cambarus ortmanni</td>
<td>Burrowing crayfish</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X,Y</td>
</tr>
<tr>
<td>Cambarus rustiformis</td>
<td>Depression crayfish</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X,Y</td>
</tr>
<tr>
<td>Orconectes rusticus</td>
<td>Rusty crayfish</td>
<td>X</td>
<td>X</td>
<td>X,Y</td>
<td>X,Y</td>
<td>X,Y</td>
<td>X,Y</td>
</tr>
<tr>
<td>Orconectes putnami</td>
<td>Phallic crayfish</td>
<td>X</td>
<td>X</td>
<td>X,Y</td>
<td>X,Y</td>
<td>X,Y</td>
<td>X,Y</td>
</tr>
</tbody>
</table>
TABLE 3. Summary of ANOVA results showing effects of subbasin on crayfish abundance (both all and large) for gravel-cobble + cobble on -small boulder substrate.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance (all)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>3</td>
<td>7.787</td>
<td>3.98</td>
<td>0.016</td>
</tr>
<tr>
<td>Abundance (large)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>3</td>
<td>6.761</td>
<td>3.14</td>
<td>0.039</td>
</tr>
</tbody>
</table>
TABLE 4. Post hoc tukey test showing pairwise mean differences for gravel cobble and cobble small.

<table>
<thead>
<tr>
<th>Source</th>
<th>Subbasin location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Orconectes rusticus</em> (large)</td>
<td>1.323</td>
</tr>
<tr>
<td><em>Orconectes rusticus</em> (all)</td>
<td>1.192</td>
</tr>
<tr>
<td>Crayfish abundance (all)</td>
<td>1.368</td>
</tr>
<tr>
<td>Crayfish abundance (large)</td>
<td>1.237</td>
</tr>
</tbody>
</table>
TABLE 5. ANOVA P-values testing effects of subbasin location on species richness for gravel - cobble + cobble - small boulder substrate.

<table>
<thead>
<tr>
<th>Source</th>
<th>Orconectes putnami</th>
<th>Orconectes rusticus</th>
<th>Cambarus graysoni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance (all)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>0.503</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Abundance (large)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>0.627</td>
<td>0.015</td>
<td>0.833</td>
</tr>
</tbody>
</table>
TABLE 6. Summary of ANOVA results showing effects of subbasin location on crayfish abundance (both all and large) for large boulder substrate.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance (all)</td>
<td>3</td>
<td>2.088</td>
<td>1.61</td>
<td>0.353</td>
</tr>
<tr>
<td>Subbasin location</td>
<td>3</td>
<td>2.088</td>
<td>1.61</td>
<td>0.353</td>
</tr>
<tr>
<td>Abundance (large)</td>
<td>3</td>
<td>1.391</td>
<td>0.73</td>
<td>0.547</td>
</tr>
<tr>
<td>Subbasin location</td>
<td>3</td>
<td>1.391</td>
<td>0.73</td>
<td>0.547</td>
</tr>
</tbody>
</table>
TABLE 7. ANOVA P-values testing effects of subbasin location on species richness for large boulder substrate.

<table>
<thead>
<tr>
<th>Source</th>
<th>Orconectes putnami</th>
<th>Orconectes rusticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance (all)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>0.832</td>
<td>0.114</td>
</tr>
<tr>
<td>Abundance (large)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subbasin location</td>
<td>0.434</td>
<td>0.242</td>
</tr>
</tbody>
</table>
TABLE 8. Summary of CCA eigenvalues and cumulative percentage of species data explained on the first four canonical axes gravel - cobble + cobble - small boulder segment.

<table>
<thead>
<tr>
<th>CCA</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
<th>Total inertia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>0.18</td>
<td>0.01</td>
<td>0.02</td>
<td>1.07</td>
</tr>
<tr>
<td>Cumulative % variance of species data explained</td>
<td>16.4</td>
<td>23.6</td>
<td>25.9</td>
<td></td>
</tr>
<tr>
<td>Cumulative % variance of species-environment relation</td>
<td>56.6</td>
<td>81.7</td>
<td>89.4</td>
<td></td>
</tr>
</tbody>
</table>

Test of significance

Axis1: $F = 7.713$ $p = 0.04$

All canonical axes: $F = 2.225$ $p = 0.006$
TABLE 9. Multiple regression of species richness and crayfish density in the gravel – cobble + cobble – small boulder segments against the environmental variables from CCA forward selection. P-values and multiple $r^2$ values are indicated.

<table>
<thead>
<tr>
<th>Species</th>
<th>Independent variable</th>
<th>Model significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crayfish density (all)</td>
<td>Boulder</td>
<td>0.522 0.015</td>
</tr>
<tr>
<td></td>
<td>Riffle</td>
<td></td>
</tr>
<tr>
<td>Crayfish density (large only)</td>
<td>Total phosphorous</td>
<td>0.505 0.029</td>
</tr>
<tr>
<td></td>
<td>Gravel</td>
<td></td>
</tr>
<tr>
<td>Orconectes putnami</td>
<td>Run</td>
<td>0.332 0.034</td>
</tr>
<tr>
<td>Abundance (all)</td>
<td>Temperature</td>
<td>0.35 0.024</td>
</tr>
<tr>
<td></td>
<td>Run</td>
<td></td>
</tr>
<tr>
<td>Orconectes rusticus</td>
<td>Riffle</td>
<td>0.667 0.004</td>
</tr>
<tr>
<td>Abundance (all)</td>
<td>Boulder</td>
<td></td>
</tr>
<tr>
<td>Abundance (large only)</td>
<td>Gravel</td>
<td>0.262 0.039</td>
</tr>
<tr>
<td>Cambarus graysoni</td>
<td>Cobble</td>
<td>0.446 0.001</td>
</tr>
<tr>
<td>Abundance (large only)</td>
<td>Ammonia</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 10. Multiple regression of species richness and crayfish density in the large boulder segments against the environmental variables from CCA forward selection. P-values and multiple $r^2$ values are indicated.

<table>
<thead>
<tr>
<th>Species</th>
<th>Independent variable</th>
<th>$r^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crayfish density (all)</td>
<td>Total phosphorous</td>
<td>0.721</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ammonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rifle</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orconectes putnami Abundance (all)</td>
<td>Temperature Boulder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orconectes putnami Abundance (large only)</td>
<td>Temperature Rifle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orconectes rusticus Abundance (all)</td>
<td>Ammonia Run Cobble Gravel Depth</td>
<td>0.747</td>
<td>0.001</td>
</tr>
<tr>
<td>Orconectes rusticus Abundance (large only)</td>
<td>Ammonia Run Cobble Gravel Depth</td>
<td>0.688</td>
<td>0.003</td>
</tr>
</tbody>
</table>
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