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Habitat Modeling of Three Endemic Crayfish Species in the Black River Drainage of Missouri and Arkansas: Factors Affecting Distribution and Abundance

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HABITAT MODELING OF THREE ENDEMIC CRAYFISH SPECIES IN THE BLACK RIVER DRAINAGE OF MISSOURI AND ARKANSAS: FACTORS AFFECTING DISTRIBUTION AND ABUNDANCE

HABITAT MODELING OF THREE ENDEMIC CRAYFISH SPECIES IN THE BLACK RIVER DRAINAGE OF MISSOURI AND ARKANSAS: FACTORS AFFECTING DISTRIBUTION AND ABUNDANCE

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

By

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Oklahoma State University
Bachelor of Science in Agricultural Sciences and Natural Resources, 2010

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ABSTRACT

The Ozark faunal region of Missouri and Arkansas harbors a high level of aquatic biological diversity, especially in regards to endemic crayfish. *Orconectes eupunctus, Orconectes marchandi,* and *Cambarus hubbsi* are three such endemics that are threatened by a limited natural distribution and the invasions of *Orconectes neglectus*. I sought to determine how natural and anthropogenic factors influence these three species across multiple spatial scales. Local and landscape data were used in decision tree analyses (CART) to determine their influence effect on presence/absence and density of the three species. Predictive models were validated using k-fold cross validation. *O. eupunctus* presence was positively associated with factors related to stream size, current velocity, and spring discharge. *Orconectes marchandi* presence was predicted primarily by dolomite geology and water chemistry metrics, both of which may be related to spring flow volume. *Cambarus hubbsi* was associated with factors related to stream size and spring flow volume, with highest densities occurring in deep waters. Models predicting crayfish presence/absence consistently outperformed random models. *Orconectes eupunctus* was the rarest of the three species, occurring at only 9 sites. *Orconectes marchandi* was restricted to the Spring River drainage, and *C. hubbsi* was found in all three drainages. The models were effective in modeling rare crayfish species and the results were consistent with previous observations of the three species. Conservation attention may be necessary to protect groundwater resources and to safeguard against further invasions of *O. neglectus*.

This thesis is approved for
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INTRODUCTION

Loss of biological diversity is an escalating concern as human impacts rise to meet the demand of a growing world population. Freshwater aquatic fauna can be particularly at risk because of the anthropogenic demand, manipulation, and exploitation of freshwater resources. Freshwater mussels, snails, and crayfish comprise the top three most threatened taxonomic groups worldwide (NatureServe 2010) and therefore warrant conservation efforts. In the southeastern United States, aquatic biodiversity is high, particularly with respect to endemic crayfish (Taylor et al., 2007). The Ozark Highlands of Missouri and Arkansas is known for its biodiversity and endemism of fish and crayfish (Pflieger, 1975; Pflieger, 1996). Thirty-six species of crayfish are found in the Ozark Highlands of Missouri and Arkansas, with 18 of those being endemic (Pflieger 1996, Wagner, 2008). Many of these species are poorly studied and therefore difficult to manage. Intensive field research and predictive habitat modeling can better inform managers of the distributions and habitat needs of endemic crayfish. Such research can then be applied in the form of management or mitigation plans that can protect endemic crayfish from present or impending threats. This study employed a modeling approach to determine how natural and anthropogenic factors affect three Ozark crayfish species.

Three endemics, the coldwater crayfish *Orconectes eupunctus,* the Mammoth Spring crayfish *Orconectes marchandi*, and the Hubbs' crayfish *Cambarus hubbsi* are found in north central Arkansas and south central Missouri. *Orconectes eupunctus* is the rarest of these endemics, and is found within this region in restricted portions of the Spring River, Eleven Point River, and Strawberry River drainages in southern Missouri and northern Arkansas (Pflieger, 1996). *Orconectes eupunctus* is currently designated as globally imperiled by Missouri Department of Conservation (MDC) (S2/G2; Missouri Natural Heritage Program 2011), as a species of greatest conservation need by Arkansas Game and Fish Commission (AGFC), and as threatened by the American Fisheries Society Endangered Species Committee (Taylor et al. 2007). Fewer than 10,000 individuals of *O. eupunctus* are estimated to exist (DiStefano et al. 2010).

As with other North American crayfish species, the restricted range of *O. eupunctus* makes it particularly vulnerable to environmental change and invasive species (Lodge et al. 2000). In the Ozarks, the endemic Gap Ringed Crayfish *Orconectes neglectus chaenodactylus* (hereafter *O. neglectus)* appears to have displaced *O. eupunctus*, as well as Hubbs' Crayfish *Cambarus hubbsi*, from a portion of its former range within the Spring River drainage (Magoulick and DiStefano, 2007). However, competition may not be responsible for the displacement of *O. eupunctus* by *O. neglectus* (Larson and Magoulick 2009), and comparative life histories between the two show similar patterns in reproductive timing and juvenile growth (Larson and Magoulick, 2008). There does not appear to be competition for habitat between *O. neglectus* and *O. eupunctus* (Rabalais and Magoulick, 2006), but *O. neglectus* is more resistant to desiccation and low summer flows compared to *O. eupunctus* (Larson, et al. 2009). This desiccation resistance may facilitate the extirpation of *O. eupunctus* by *O. neglectus* (Larson et al. 2009). Further research is needed to explain the apparent displacement of *O. eupunctus* by *O. neglectus* in the Spring River drainage. An invasive, reproducing population of *O. neglectus* was recently discovered in the Eleven Point River drainage, where it threatens the largest known populations of *O. eupunctus* (Imhoff et al. 2012). Invasive crayfish are the greatest threat to native crayfish (Lodge et al. 2000), and invasions by *O. neglectus* in both the Spring River and Eleven Point drainages constitute a substantial threat to *O. eupunctus* and other native crayfish species. *Orconectes eupunctus* therefore warrants conservation attention to determine its critical habitats and potential invasion threats.

The Mammoth Spring Crayfish *Orconectes marchandi* is only found in the Spring River drainage of northeastern Arkansas and southeastern Missouri (Pflieger 1996, Flinders and Magoulick 2005). *Orconectes marchandi* was once listed as endangered by the American Fisheries Society Endangered Species Committee, but was reduced to threatened when its known distribution was increased from three streams to over twenty streams (Flinders and Magoulick 2005, Taylor et al. 2007). The species is currently listed as globally (G2) imperiled by the Missouri Natural Heritage Program (2011). *Orconectes marchandi* is found primarily in small streams in shallow portions with slower flow and larger substrate, or in backwaters (Flinders and Magoulick 2005, Flinders and Magoulick 2007). Intermittent streams have been shown to have higher densities of *O. marchandi* than permanent streams (Flinders and Magoulick 2003). These findings are inconsistent with Pflieger (1996), which described the species as a large river riffle-dweller, but Pflieger (1996) was based on limited data from three localities in the Warm Fork Spring River in Oregon County, Missouri. *Orconectes marchandi* has been shown to be negatively associated with the invasive *O. neglectus* in the Spring River watershed (Flinders and Magoulick 2005), and it might therefore face extirpation threats similar to *O. eupunctus*. The effects of *O. neglectus* on *O. marchandi* have not been studied directly, but the drought tolerance of *O. neglectus* (Larson et al. 2009) could allow it to invade intermittent streams where *O. marchandi* is most abundant (Flinders and Magoulick 2003). Previous research suggests that *O. neglectus* could adversely affect *O. marchandi* (Flinders and Magoulick 2005), though more research is necessary. The narrow range of *O. marchandi* and the threats from invasive crayfish make *O. marchandi* vulnerable to extirpations and reduced prevalence (Lodge et al. 2000), and therefore necessitate conservation efforts.

The Hubbs' Crayfish *Cambarus hubbsi* is endemic to the Ozarks of southern Missouri and northern Arkansas (Pflieger 1997) and is listed as currently stable by the American Fisheries Society Endangered Species Committee (Taylor et al. 2007) and secure (G5) by the Missouri Natural Heritage Commission (2011). *Cambarus hubbsi* is found in the St. Francis River, Eleven Point River, Strawberry River, and Spring River drainages and is collected rarely in the White River drainage (Pflieger 1996). *Cambarus hubbsi* is found in both small and large perennial streams where it buries under large, deep-seated substrate (Pflieger 1996, Larson and Magoulick 2011). *Cambarus hubbsi* is positively associated with *O. eupunctus* and negatively associated with *O. neglectus* in the Spring River drainage (Magoulick and DiStefano 2007). The invasion of *O. neglectus* in the Spring River and Eleven Point drainages likely threatens *C. hubbsi*, though more research is needed to assess the impact of these threats. In contrast to *Orconectes* species, *C. hubbsi* grows slowly and produces comparatively few offspring later in life (Larson and Magoulick 2011). *Cambarus hubbsi* has been proposed as a k-strategist, and might consequently be more sensitive to environmental impacts, invasive species, or both (Larson and Magoulick 2011). Therefore, *C. hubbsi* may require special conservation attention to assess and to protect against any real or perceived threats. Conversely, *C. hubbsi* may not be particularly vulnerable to an invasive crayfish in a different genus, especially given the substantial differences in the life histories between *C. hubbsi* and *O. neglectus*. More research is needed on biotic interactions between *Cambarus* and *Orconectes* species.

The limited data on *O. eupunctus*, *O. marchandi*, and *C. hubbsi*, as well as the invasion threats from *O. neglectus*, merit further research to assess the conservation needs of these three species. Life history data are available for these species (Larson and Magoulick 2008, Flinders and Magoulick 2005, Larson and Magoulick 2011), but a comprehensive analysis on habitat use and anthropogenic threats has not been conducted. Habitat and threat data are needed to determine conservation needs, construct and implement management plans, and evaluate plan effectiveness. This study was therefore designed to address how anthropogenic and natural factors affect *O. eupunctus*, *O. marchandi*, and *C. hubbsi* across multiple spatial scales. These questions were addressed by using a modeling approach based on data collected in the field, as well as gleaned from geographic information systems. Multiple spatial scales were assessed because scale has been shown to be critical factor when utilizing GIS and remote sensed data (Goodchild and Proctor 1997). The models in this study quantified the habitat needs of the three target species, as well as predicted possible occupancy sites within the study area. Based on previous research, *O. eupunctus* and *C. hubbsi* were hypothesized to be associated with larger-order rivers with high volumes of spring flow and colder water temperatures, while *O. marchandi* was expected to be associated with smaller streams with slower flow.

STUDY AREA

The study area consisted of the Strawberry River, Eleven Point River, and Spring River drainages, and ten sites in the lower Black River (Figure 1). These drainages are part of the Ozark faunal region of southern Missouri and Northern Arkansas, which is characterized by chert, limestone, and dolomite geology, with streams typically exhibiting a riffle, run, pool structure (Pflieger 1997). Natural springs are abundant in this region, particularly in the Eleven Point River basin. The study area is overwhelmingly comprised of private land holdings, with the exception being portions of the Eleven Point River in the Mark Twain National Forest in Missouri. The study area was primarily comprised of hardwood forests (oak-hickory), though pastureland was also relatively common. Extensive cropland (non-hay) was common only in the lower Black River Drainage and occasionally along portions of the largest rivers in the study area. No major urban areas exist in this basin (USDA 1999).

METHODS

FIELD COMPONENT

Distribution and density data were obtained by using a quantitative kicknet method within stream segments. A stratified random sampling design was used, where stream segments (*as defined by* Westhoff et al. 2006, DiStefano et al. 2008) consisted of stream sections between confluences with tributaries that cause an increase in stream order. Only streams with year-round flow were included in the sample pool. Geographic information systems (GIS) were used to catalog and stratify (by stream order) all stream segments existing (365 perennial) among the known 224 perennial streams in these drainages, and 102 of these segments were randomly chosen and sampled between 25 May and 3 September, 2010 and between 24 May and 9 September, 2011. Sampling reaches (locations within stream segments to be sampled) were selected randomly, but were also based on accessibility (e.g., landowner permission). Additional randomly selected sites were available to replace those that were unable to be accessed.

Four riffle habitats and 4 run habitats were identified within each sampling reach. Riffles and runs were delineated by qualitatively assessing depth and flow of the stream. These riffle and run habitats constituted independent units and were separated by pools or each other. A quantitative kicknet method was used to determine densities of crayfish in each stream segment. A 1-m^2 polyvinyl chloride (PVC) pipe quadrat frame was used to define the sample space and was placed randomly within each riffle or run. Crayfish were dislodged from a the quadrat “sub-sample” (*sensu* MacKenzie et al. 2006) area by thoroughly kicking and disturbing the substrate directly inside the PVC quadrat frame directly upstream of a 1.5 x 1.0-m seine net (3-mm mesh). Crayfish dislodged from the substrate were washed into the seine net with the aid of the current. If no current existed, crayfish could be effectively collected by directionally kicking towards the seine, thus creating current. Collected crayfish were identified to species, sex, and life stage (adult or juvenile). Three kicknet sub-samples were randomly collected from each riffle or run site, leaving a 1-m “buffer” around any previous sub-sample locations to minimize the potential effect of previous sub-samples. Sampling occurred only in water depths of $\leq$1 m because we were unable to effectively use the kick seine in deeper water.

Physical characteristics of riffle and run habitats were recorded to determine the fine-scale variables both within the stream and immediate riparian zone. Dominant substrate coarseness composition (Bain et al. 1985) was measured in each 1-m^2 subsample using a 5-pointed 0.5 m x 0.5 m rebar cross (Litvan et al. 2010). The substrate cross was haphazardly placed within the quadrat, then substrate at each of the 5 points was noted. These substrate measurements were used to estimate a site mean. Stream depth and mean (0.6 depth) current velocity of each 1-m^2 sub-sample area were determined using a meter stick and Marsh-McBirney® flow meter, measured just upstream of the upstream-most edge of the quadrat to avoid disturbing crayfish inside the frame prior to sampling. The length of the riffle or run and the width at a randomly-chosen point were measured using a surveyors tape. Percent canopy cover was estimated using a clinometer, where degrees of canopy cover are measured from an observer standing in the middle of the stream at randomly chosen points. Water temperature (Celsius), dissolved oxygen (mg/L), conductivity (uS/cm), and pH were measured prior to sampling crayfish. Temperature loggers were deployed at all sites where *O. eupunctus* was detected and at a subset of sites where the species was not detected to determine temperature regimes and changes over a period of one year.

LANDSCAPE COMPONENT

GIS was used to link the *O. eupunctus* distribution and density data to landscape and stream segment scale anthropogenic and natural variables. These variables were selected at multiple spatial scales and based on Westhoff et al. (2011). Landscape scale natural variables included factors related to soils, geology, land cover, and hydrology, while anthropogenic variables included factors related to agriculture, urbanization, mining, hydrology, and water quality (Table 1). Landscape scale natural and anthropogenic variables were obtained from databases at Missouri Resources Assessment Partnership (MoRAP), which categorized stream segments and their associated environmental attributes (Sowa et al, 2005).

A major issue with this form of spatial modeling is the potential impact of variables measured at different levels of resolution being mismatched among themselves and with the biological data (Poff 1997, Goodchild and Proctor 1997, Brewer et al. 2007). Therefore, scale was considered and assessed at varying levels, as predictive models can yield varying results when measured at different levels even within the same watershed (Lammert and Allan 1999). Landscape data were therefore analyzed at two different scales: the stream segment scale and the local catchment scale. The stream segment scale was the immediate drainage of each stream segment (essentially an expanded riparian zone), but none of its tributaries (Figure 16A). The local catchment was the drainage of each stream segment and its direct tributaries, but none of its upstream inputs (Figure 16B). For instance, a fourth order stream segment examined at the local catchment scale would not include the habitat from that stream's first, second, or third order segments and their drainages. Field data were collected in a hierarchical design as well, but preliminary analysis of the data indicated that averaged variables within stream segments yielded more robust decision trees as opposed to individual measurements within segments. This is likely because the variation between seine hauls was great and therefore masked more general characteristics of the stream as a whole. The field data variables were therefore averaged across the whole stream segment prior to final analysis.

MODEL DEVELOPMENT

Decision tree analysis (CART) was used to produce probability-based models of crayfish occurrence and densities within the Eleven Point River, Spring River, Strawberry River, and lower Black River watersheds, collectively. Classification and regression trees are useful in ecological research because they are easily interpreted and can handle both continuous and categorical data, among other attributes (De'ath and Fabricious 2000). Both the presence/absence data and the density data served as the two primary response variables for use in CART, while the natural and anthropogenic variables served as explanatory variables. Both landscape and field data were used to develop the global model, while the landscape data alone were used to determine probability of presence within every stream in the study area. This was accomplished by applying the landscape only classification tree formula to unsampled sites in the study area, for which the same landscape data was available. This predictive modeling was performed with only the data from the spatial scale that resulted in the highest correct classification rate. Species were assumed to inhabit a stream segment if the resulting probability of occurrence was greater than 50 percent. Data measured in the field were excluded from these predictive models because those data were unavailable for unsampled sites. During summer 2012, sites that were predicted to harbor one or all species were cross-referenced with reconnaissance information from the initial survey, and unvisited sites were selected for follow-up sampling. Visited sites not sampled during the initial study were excluded because most were prone to complete drying or had been denied access by the landowners.

Density regression trees were developed using all available field and landscape data. Landscape environmental and anthropogenic variables were chosen from a larger variable set and were reduced by dropping highly correlated variables (≥+/-0.7 Pearson correlation coefficient) and by dropping explanatory variables containing all zeroes. We also used principal components analysis to reduce variables correlated in multidimensional space. This variable selection process was repeated at both spatial scales.

Classification trees were validated using the k-fold cross validation method. The data were split into ten subsets and nine of those subsets were used to create the model which was then validated against the remaining subset. This method is then repeated, leaving out a different subset for each iteration of the model validating process, which leads to an average misclassification rate. The model was limited *a priori* to a minimum node size of five and a maximum of ten splits. The traditional problem associated with growing overly large trees was not an issue for the species examined given the limited number of presence localities in the data.

Data from a preliminary study were used in one instance where the intended stream segment was not sampled and another segment was mistakenly sampled. Data were collected from this segment during a preliminary study (see Figure 1) that utilized the same sampling protocol, though without water chemistry measurements, and this data were used for the unsampled segment. Water quality measurements were retained from the incorrectly sampled stream segment, which was 13 kilometers further downstream.

Orconectes eupunctus presence/absence was analyzed against mean yearly water temperature and coefficient of variation for yearly temperatures from June 2011 to May 2012 using one-way ANOVAs. These analyses were repeated for the months of June 2011 to September 2012, when water temperatures are warmest. Temperature logger data were unable to be incorporated into CART models because of the limited number of loggers, logger failure and loss, and because of temporal issues associated with logger retrieval.

RESULTS

STREAM SEGMENT SCALE

Over 16,500 crayfish were collected across 2,488 seine hauls during the study, with 9, 20, and 14 sites harboring *O. eupunctus, O. marchandi,* and *C. hubbsi*, respectively. Ten crayfish species were collected, with Spothand Crayfish *Orconectes punctimanus* (n=7,148) and Ozark Crayfish *Orconectes ozarkae* (n=5,791) being the most common.

In the global model, *O. eupunctus* presence was predicted by current velocities exceeding 0.54 m/s (Figure 2A). At slower current velocities, *O. eupunctus* presence was predicted by larger-order streams with a rock fragment volume between 20.1 and 40.0 percent (Figure 2A). Breaks and foothills were associated with high densities of *O. eupunctus* (Figure 2C). Stream order (4-6) was the strongest predictor of presence when only landscape data were used, followed by the percentage of open water and rock fragment volume between 20.1 and 40.0 percent (Figure 2B).

Orconectes marchandi presence in the global model was positively associated with dolomite geology and negatively associated with smooth plains (Figure 3). Roads, pH, stream order, and depth further refined the model, but were of less overall importance. Density of *O. marchandi* was greatest where there was high conductivity and low elevations (Figure 4). The global model and the landscape only model were nearly identical in the upper four levels of the tree, with most of the differences being in the split values (Figures 3, 5). Both models classified presence/absence equally well (Table 2).

Cambarus hubbsi presence was best predicted in the global model by stream order, followed in lower splits by dissolved oxygen, drainage channels, and dolomite geology (Figure 6A). The highest densities of *C. hubbsi* were found in deeper water (Figure 7). The landscape only model was similar, with segment order, drainage channels, dolomite geology, and grassland being important within the upper three levels of the tree (Figure 6B).

LOCAL CATCHMENT SCALE

Orconectes eupunctus presence was best predicted by spring flow volume in the global model at local catchment scale, followed by scrub/shrub vegetation and escarpments (Figure 8A). The landscape only model and associated fit statistics was identical to the global model. The highest densities of *O. eupunctus* at this scale were found in swift waters (Figure 8B).

Orconectes marchandi presence was positively associated in the global model with dolomite geology, dry uplands, large stream substrate (pebble or greater), and high conductivity (Figure 9A). *Orconectes marchandi* was most abundant at this scale where there was a high percentage of alluvium and fine-textured sediment (Figure 10). Dolomite geology, dry uplands, and evergreen forest were important in predicting presence in the landscape only model (Figure 9B).

Segment order, slope, dry uplands, spring flow volume, canopy cover, and smooth plains were important predictors of *C. hubbsi* in the global model (Figure 11A). The landscape only model was nearly identical to the global model, with irregular plains replacing canopy cover (Figure 11B). *Cambarus hubbsi* was again most abundant in deeper waters (Figure 12).

CLASSIFICATION ACCURACY

Models at all scales and resolutions (landscape or global) exhibited correct classification (CC) rates ranging from about 91 percent to just over 98 percent, with chance correct classification (CCC) rates ranging from about 83 percent to about 91 percent (Table 2; see Olden et al. 2002 for proper validation procedures). Models performed 4.88 to 11.52 better than random assignment, and stream segment scale models performed better or equal to local catchment models. Species with low prevalence (fewer sites occupied) generally exhibited higher CC rates, but improvements over random assignment were almost always greatest for more prevalent species (Table 2).

WATER TEMPERATURE

Mean yearly water temperature did not significantly differ at sites where *O. eupunctus* was present or absent (p=0.171), nor did the coefficient of variation in water temperature (p=0.194). Mean daily water temperature between June 2011 and September 2011 did not significantly differ at sites where *O. eupunctus* was present or absent (p=0.158), but did significantly differ for the coefficient of variation (p=0.012) during the same period.

PROBABILTY OF OCCURRENCE

Stream segment scale classification tree formulas (landscape only models) were used to create probability of occurrence (POP) maps (Figures 13, 14, 15) since models at that scale performed best (Table 2). *Orconectes eupunctus* was predicted to occur (prob>0.5) at twelve stream segments, eleven of which were sampled during the study (Figure 13). The one unsampled site, a 4th order tributary to the Eleven Point River, was predicted to harbor *O. eupunctus* with a probability of fifty-two percent. This site was visited and sampled with the approach standard to the study, and *O. eupunctus* was not collected. Probability of presence values for *O. eupunctus* ranged from less than one percent to over eighty-seven percent. *Orconectes eupunctus* was predicted to occur primarily in the largest portions of the main stem rivers in the study area.

Orconectes marchandi was predicted to occur at seventy stream segments, with forty-seven of those being unsampled sites (Figure 14). Probability of presence values ranged from less than one percent to over ninety-two percent. A cluster of high presence (>0.75) streams were predicted on the eastern portion of the Spring River, Arkansas, with most of these being direct tributaries to the Spring River (Figure 14). The Spring River itself, however, was not predicted to harbor the species. The models predicted *O. marchandi* to occur in the Strawberry River, Black River, and Eleven Point River drainages, despite these drainages being outside of the species current known distribution. *Orconectes marchandi* was not collected from two predicted sites (POP>0.5) that were sampled during summer 2012 as part of a follow-up survey.

Cambarus hubbsi was predicted to occur at twenty sites, with five of those being unsampled during the study (Figure 15). Probability of presence values ranged from less than one percent to over eighty-six percent. *Cambarus hubbsi* was predicted to occur in both main stem rivers and their tributaries within the study area (Figure 15). The Spring River drainage was predicted to contain more suitable habitat than the other drainages in the study (Figure 15).

DISCUSSION

STREAM SEGMENT SCALE

The habitat requirements for *O. eupunctus* at the stream segment scale reinforce previous knowledge of the species. Past research has indicated that *O. eupunctus* is a large river specialist (Pflieger 1996, Magoulick and DiStefano 2007, Flinders and Magoulick 2005), and extensive field sampling during this study further supported these observations. Field sampling failed to collect *O. eupunctus* in any stream segment lower than fourth order, regardless of their proximity or connectivity to known populations. In the Strawberry River drainage, *O. eupunctus* was found almost exclusively in the fifth order segment of the mainstream Strawberry River. Sampling in the fourth order Strawberry River failed to detect *O. eupunctus*, though later distribution surveys located the species in the fourth order segment, only a few hundred meters upstream of the fifth order segment. The distribution data indicates that *O. eupunctus* disappears from the fourth order Strawberry River only a few kilometers above the confluence with Piney Fork Creek in Sharp County, Arkansas. *Orconectes eupunctus* was collected exclusively from the fourth and fifth order main stem segments in the Spring River system. The Eleven

Point River system did yield collections of *O. eupunctus* in main stem tributaries, though all were fourth order or larger. Given these observations, factors relating to stream size were expected in the models.

The global classification model indicated that after current velocity, stream order was the most important predictor of *O. eupunctus* presence. In the landscape only model, the amount of open water was secondarily important to stream order, and both are likely representative of stream size. Many physical, biological, and chemical gradients exist along a continuum of stream size (Vannote 1980), and determining which of those are important to a particular organism can be problematic. For *O. eupunctus,* stream order is likely important for several reasons. The major rivers in the study area are heavily influenced by large springs, particularly the Eleven Point River and Spring River, each of which receive over 757 million liters of spring flow daily from Greer Spring and Mammoth Spring, respectively (United States Department of Agriculture, 1999, “Mammoth Spring State Park”). High spring flow volume is important to *O. eupunctus* presence and density when analyzed at a larger spatial scale (Figure 8A), and higher order streams in the study area have a greater accumulation of spring flow volume. This species appears to require a high volume of spring flow and has thus evolved in higher order streams where spring flow volume exceeds some biological threshold.

Orconectes eupunctus may also benefit from high spring flow as it relates to stream permanence. *Orconectes eupunctus* is sensitive to drought and stream drying and exhibits a negative association between density and low summer flows (Larson et al. 2009). Additionally, *O. eupunctus* cannot survive more than two days without water, which is relatively intolerant when compared to its invasive competitor *O. neglectus* (Larson et al. 2009). Spring flow volume may therefore play an instrumental role in sheltering *O. eupunctus* from drought and stream drying. Water temperature was thought to be an important factor related to stream order in the study area because of its close association with spring flow, though the data in this study did not support this. Water temperature was measured during field sampling and was included in the models, though one-time measurements are likely a poor surrogate for the overall thermal profile of area streams. Temperature probe data from June 2011 to May 2012 did not indicate that mean yearly temperature or the coefficient of variation significantly differed between sites where *O. eupunctus* was present or absent, nor did mean daily temperatures during the warmest months of the year in 2011 (p=0.158). Only the coefficient of variation for temperature during June through

September 2011 significantly differed between *O. eupunctus* presence and absence sites (p=0.012), and the difference was small and may not be biologically significant. This was a surprising result and it may indicate that *O. eupunctus* is not influenced by water temperature as once thought. Spring flow volume may therefore strongly influence *O. eupunctus* in terms of its contributions to high flow and stream permanence and not by its association with colder waters.

High current velocity was of primary importance for *O. eupunctus* at the stream segment scale and might also be related to stream size and spring flow. Discharge is positively related to velocity for a given cross-sectional area (Q=VA), and the large volume of spring flow in the main stem rivers of the study area may directly account for swifter waters. Swifter waters may also contribute to higher dissolved oxygen content, though DO was included in the models and failed to exhibit importance to *O. eupunctus.*

A rock fragment volume percentage between 20.1 and 40.0 percent was important to *O. eupunctus* in both the global and landscape only model. Rock fragments are defined as rupture resistant particles that are 2 mm or larger in diameter (Donalatos et al. 1995). This was a surprising result and it is currently unknown how rock fragment volume affects *O. eupunctus*, crayfish, or aquatic macroinvertebrates in general. Rock fragment volume could be associated with factors relating to vegetation or hydrology, which may be more directly affecting *O. eupunctus*.

High density of *O. eupunctus* at the stream segment scale was explained by breaks and foothills (Figure 2C). This was an unexpected result, as factors relating to spring flow, current velocity, or substrate size were anticipated. Breaks and foothills may have been important in the model because they may occur along river margins where the surrounding Ozark Mountains give way to the low lying areas that form large river channels and floodplains. The density regression tree at this scale, however, performed rather poorly, explaining only thirty-nine percent of the variation in density (Figure 2C).

Orconectes marchandi was closely associated with dolomite geology. Dolomites, a calcium and magnesium bearing carbonate, are a major constituent of limestone (“Missouri Limestone”). The known distribution of *O. marchandi* is centered in the vicinity of Mammoth Spring, and this area is characterized by Cotter and Jefferson City dolomites (Haley 1993). Therefore, it is not surprising that dolomites are important in explaining the presence of *O. marchandi*. Additionally, the importance of mineral loaded geology likely also explains the importance of segment pH in the global model. The importance of both dolomite geology and a relatively neutral pH (around 7.8) likely indicate that *O. marchandi* prefers well buffered, mineral rich water. The presence of magnesium and calcium cations would also suggest an association with high water conductivity, and this is supported by the density regression tree where high segment conductivity (>456 uS/cm) is the most important predictor of high densities of *O. marchandi* (Figure 4). The importance of dolomite geology may therefore be more significant in terms of its contribution to water chemistry, especially given the high volumes of upwelling spring flow in the area. Additionally, dolomite geology may be important because of its association with springs common to karst geology ("Karst, Springs, and Caves in Missouri"). Smaller springs that feed lower order streams in the study are likely unaccounted for in the landscape data, and dolomite geology may be indirectly representing spring flow. The importance of water chemistry in the models, however, would indicate that mineral contributions from limestone geology are important to *O. marchandi*. Clearly, geology, water chemistry, and spring flow are all likely important components of the habitat requirements of *O. marchandi*.

O. marchandi was negatively associated with road crossings in both the global and landscape models, and negatively associated with population density in the landscape model. These findings may indicate that *O. marchandi* is sensitive to anthropogenic threats, such as sedimentation. Increased sedimentation due to roads and road-stream crossings is well documented (Witmer et al. 2009, Luce and Black 1999, Leslie and Dunne 1984), as is crayfish sensitivity to sediment-bound contaminants (Simon and Morris 2009). Road crossings were of less importance to *O. marchandi* than geology, but still warrant further investigation to assess road-related threats. Anthropogenic threats combined with threats from invasive species like *O. neglectus* could have detrimental additive or synergistic effects on *O. marchandi*. It is important to note, however, that only perennial streams were sampled in this study and that intermittent streams are important habitat for *O. marchandi* (Flinders and Magoulick 2003). These findings are therefore limited in scope and do not fully encompass all habitats utilized by *O. marchandi*.

Cambarus hubbsi was found in all stream orders but disproportionately favored fourth and fifth order streams (Figure 6A, 6B). This was somewhat surprising, as it was expected that all large streams would be positively related to *C. hubbsi* presence. However, only one sixth order stream segment, the Eleven Point River below Fredrick Creek, was sampled and the local habitat at the sampled site may have been coincidentally poor for *C. hubbsi*. It is possible that either our kick seining approach was inadequate for collecting *C. hubbsi* in large rivers or that our specific sample site in the sixth order lacked proper habitat for the species. The former is especially likely, as deeply seated boulder substrate is common in the Eleven Point River, and this type of habitat has been shown to be preferred by *C. hubbsi* (Pflieger 1996, Larson and Magoulick 2011, Flinders and Magoulick 2005). The density regression tree also indicated that *C. hubbsi* was most dense in deeper waters (Figure 7), which supports previous research (Flinders and Magoulick 2007). Pools were not sampled in this study, but a more comprehensive study on this particular species should consider sampling deeper runs and pools. More data on *C. hubbsi* in the largest rivers in its distribution would be useful to explain why our sixth order stream segment was grouped with smaller streams and associated with reduced prevalence. Regardless, the ability of *C. hubbsi* to inhabit streams of any size is well documented (Pflieger 1996, Larson and Magoulick 2011, Larson and Magoulick 2005) and supported by the data in this study. It is important to note, however, that the study area did not encompass the entire distribution of the species, as was the case with both *O. eupunctus* and *O. marchandi. Cambarus hubbsi* is also found in the St. Francis River drainage and the White River drainage (Pflieger 1996), and these drainages were not sampled. *Cambarus hubbsi* has also been collected from intermittent streams (Flinders and Magoulick 2003), which were not sampled in this study.

Dissolved oxygen was important to *C. hubbsi,* which is supported by its preference towards fast flowing systems (Larson and Magoulick 2011, Flinders and Magoulick 2007). The importance of drainage channels in both the global and landscape model was surprising (Figures 6A, 6B). The effect of drainage channels on *C. hubbsi* is unknown and their importance disappears in the local catchment models (Figures 12A, 12B) which classifies the species equally well (Table 2).

LOCAL CATCHMENT SCALE

At a larger scale, *O. eupunctus* presence/absence is primarily predicted by spring flow volume. This reinforces observations at the stream segment scale that *O. eupunctus* is a large river specialist with associations with high spring flow volume, swift currents, and stream permanence and stability. Spring flow volume was expected in *O. eupunctus* models and it is at this larger spatial scale that spring flow is an important factor. Average current velocity, spring flow volume, and segment order all explained density of *O. eupunctus* at this scale. These three variables are consistent with most all previous knowledge of the species and were expected in the models, though they explained only thirty-nine percent of the variation in density (Figure 8B). Temperature again fails to fall out in the model, but is surely related spring flow. Better temperature data are needed to assess whether it is spring flow, temperature or both that is important to the species. Escarpments and scrub and shrub vegetation were included in the model but are likely unimportant, biologically, to *O. eupunctus*. Both split values (percentage of the drainage in question) that predicted *O. eupunctus* presence were well below one percent, which are likely biologically insignificant and should be considered to be overfitting issues.

Orconectes marchandi presence was again strongly associated with dolomite geology, but was secondarily predicted by dry uplands at this scale. Dry uplands are likely more common among lower order streams as opposed to large rivers; this may explain their prevalence in the model as *O. marchandi* has been shown to prefer smaller streams (Flinders and Magoulick 2003). Large substrate and conductivity were important in the global model, and these are likely biologically significant given the strengths of the splits and sample sizes. Conductivity has already been discussed as being important to *O. marchandi*, and substrate diversity has also been shown to be important to the species (Flinders and Magoulick 2007). Density was poorly explained at this level (R-squared = 0.28), but exhibited overall trends in water chemistry and landscape scale factors (Figure 10).

Segment order, slope, and area-weighted spring flow volume were all positive predictors of *C. hubbsi* presence in both the global and landscape models at the local catchment scale. Stream size was again the strongest predictor, showing the same pattern observed already discussed. Slope and spring flow (area-weighted) were novel to the local catchment scale. High gradient has been previously noted as important to *C. hubbsi* (Pflieger 1996), and this also likely explains the importance of current velocity observed by others (Flinders and Magoulick 2007, Pflieger 1996). The importance of spring flow is unsurprising given a high incidence of *C. hubbsi* in larger, spring fed rivers in this study area and given the close association between *C. hubbsi* and *O. eupunctus* (Flinders and Magoulick 2005). Density was again explained poorly (R-squared = 0.30), but depth, canopy, and springs appeared to positively affect density, which accords with previous studies (Flinders and Magoulick 2007).

PROBABLITY OF PRESENCE

The POP maps for all species are a powerful tool to the conservation biologist because they provide a graphical means for determining future sample locations, identifying optimal habitat, and locating and prioritizing conservation efforts. The POP maps for *O. eupunctus* and *O. marchandi* are particularly valuable because they encompass the full known distribution of each species. The POP map for *O. eupunctus* is especially revealing, as the highest POP values closely follow the large main stem rivers of the study area. All sites predicted to harbor *O. eupunctus* were sampled either in the initial study or the follow-up survey, and the results indicate that *O. eupunctus* is found in only nine stream segments. Rarely in ecological studies can a species entire distribution be known, but given the high specialization and rarity of *O. eupunctus* and the intensity of modeling and sampling, it is probable that every presence locality for *O. eupunctus* is now documented. The POP map also correctly predicted *O. eupunctus* to occur in a segment where the initial survey failed to detect it. *Orconectes eupunctus* was predicted to occur in the 4th order Strawberry River main stem (Figure 13), where the species was not collected in the initial survey. However, *O. eupunctus* was located in the extreme lower 4th order Strawberry River segment, just above the confluence of Piney Fork (4th order), during a related genetics and distribution study. The POP map for *O. eupunctus* is therefore especially useful because it confirms the extreme rarity and specialization of the species and further reveals the necessity of conservation efforts.

Though model fit for *O. marchandi* was the highest, the POP map predicts the species to occur outside of its known range. *Orconectes marchandi* was predicted to occur in the Eleven Point River, Strawberry River and lower Black River drainages, all of which are outside of the distribution of the species, which is known to occur in the Spring River drainage only (Pflieger 1996, Flinders and Magoulick 2005). This could suggest that *O. marchandi* is more restricted by geography and evolutionary history than by suitable habitat. It may also suggest that *O. marchandi* could itself become established as a non-native species if introduced into neighboring drainages. *Orconectes marchandi* has been predicted to be capable of invasions into nearby drainages (Larson and Olden 2010), and our predictive modeling may indicate those streams where establishment might be most likely.

Cambarus *hubbsi* was predicted to be absent from the fifth order Strawberry River, which was a surprising result as *C. hubbsi* was abundant in that segment during field sampling and because fifth order segments were shown to indicate presence in the models (Figure 6A, 6B). The POP map for *C. hubbsi* is useful in graphically illustrating the Hubbs' crayfish's ability to inhabit streams of any size, while revealing a preference towards larger streams (Figure 15).

MODEL FIT

Too often in aquatic sciences, a model is evaluated only by its correct classification rate (or misclassification rate) without respect to its chance correct classification rate (Olden et al. 2002). This can lead to misperceptions about model validity. A high correct classification rate is expected purely by chance for both common and rare species (Olden et al. 2002). The probability of occurrence of a species is not a simple coin flip (i.e. present or absent, 0 or 1), but rather it is related to the prevalence of the species in the data set (Olden et al. 2002). It is more likely (i.e. > 50%) for an abundant species to be present in a given stream and vice versa for rare species. Therefore, a seemingly "good" model may have a quite low misclassification rate, but yet perform no better than random, especially for rare or common species. In other words, models for abundant species may fail entirely to predict absence and models for rare species may fail entirely to predict presence, but both models would exhibit a high correct classification rate. The predictive models in both cases would be effectively useless to the conservation biologist despite both exhibiting a low misclassification rate.

Developing predictive models for exceptionally rare or common species can be problematic because there is often little room left for improvement over random assignment (Olden et al. 2002). For example, *O. eupunctus* is quite rare in the data, occurring in only 8.8 percent of sampled streams. Consequently, the model is expected to correctly classify presence/absence about ninety-one percent of the time by chance alone (Table 2). The model can therefore only be improved by nine percent. Is a nine percent improvement over random useful or informative? In this example, a nine percent improvement over random would achieve 100 percent predictive power. A nine percent improvement here would be undoubtedly more useful than a nine percent improvement over a random model with a forty percent misclassification rate. For *O. eupunctus* models, improvement over chance predictions ranged from 4.88 percent to 6.84 percent. Models for all three species showed improvement over random predictions, with improvements ranging from 4.88 percent to 11.52 percent (Table 2). These improvements are substantial given the already high CCC rate. It is also important to consider these improvements in context. Modeling rare species is difficult; low sample sizes, sporadic occurrences, low detection probabilities, logistical constraints, and other issues can greatly complicate research on rare species. Often, field collection fails to yield enough data on rare species to provide meaningful, if any, results. The models in this study made highly efficient use of low sample sizes and provided marked improvements over random predictions. The final models were sensible and the modeling process performed as anticipated. Additionally, the habitat models supported previous research on all three species while providing additional insight concerning habitat associations.

THE EFFECT OF SCALE

One of the central problems in ecology and ecological modeling is determining what spatial (or temporal) scale or scales to examine (Levin 1992). Rarely can a complex ecological system be studied simultaneously across all organizational, temporal, and spatial scales; this forces ecologists to scale up or down their level of interest, which is often done arbitrarily in lieu of logistical constraints and at the expense of detail (Levin 1992). This can be especially true when using remote sensed or other cartographic data, where the representative fraction of a particular metric forces generalization in order to accommodate page size or pixels (Goodchild and Proctor 1997). Therefore, scale must be considered carefully when conducting ecological studies. Fortunately, the purpose of ecological modeling is to fit observations and generalize while suppressing superfluous details. This still, however, leaves the ecologist wondering what scale is appropriate to examine the system of interest. Generally, finer scales of observation will lead to more “unpredictable, unrepeatable individualistic cases” and broader scales of observation will result in “collections of cases whose behavior is regular enough to allow generalizations to be made,” the latter of which is the desired outcome (Levin 1992). Because the environment exists as a continuum, there is no singularly correct scale at which to study a system, though some scales may be better than others (Levin 1992). The ideal solution to these problems is to understand what driving forces are creating the ecological patterns of interest and determine what scale best encompasses those forces (Levin 1992). Better still, is to combine this approach across several scales at once (Levin 1992). Despite these difficulties with scale selection, success is achieved if the end result is a useful ecological model that conforms well to observable patterns and predicts outcomes with reasonable accuracy.

Scale was carefully considered prior to and during this study. Our definition of stream segments was a compromise between stream size, which was thought to be a principle driving force in species presence/absence and density, and logistical and statistical constraints. Stream order is traditionally used to denote stream size and power, though it does so fairly generally, as all inputs from lower order streams relative to the main channel are ignored. These lower order streams certainly do increase water volume, which likely increases stream size and power, particularly when larger order tributaries confluence with still larger rivers. Stream segments could have been defined at a finer scale, that is, between confluences of all streams, regardless of size. This would have resulted in a sampling universe of about 12,700 stream segments. Variation at this spatial scale would likely mask more general phenomena, as well as creating a host of logistical issues. Stream segments were therefore defined in this study at a scale which could be feasibly sampled with a reasonable sample size and still retain a characterization of stream size, which was deemed important. Stream order at this scale was often an important predictor of species presence/absence in the resulting models, indicating that this scale still retained essential information while eliminating cumbersome detail.

The field data were collected in a spatially hierarchical design and was initially examined at both the habitat unit scale (i.e. riffle or run) and the stream segment scale. Models were more robust at the stream segment scale, indicating that scaling up captured the predictor variables responsible for presence/absence better than finer scale models. Scaling up, in this instance, improved model fit and interpretability. In this circumstance, a courser scale likely eliminated variance issues that confounded models at a finer scale.

Models at both the stream segment scale and the local catchment scale performed well, with both scales outperforming chance correct classification rates (Table 2). Stream segment scale models consistently performed better than or equal to models at the local catchment scale, indicating that a finer scale was more appropriate. Stream segment scale models were at most 3 percent better than local catchment models in terms of their differences from chance correct classification rates (Table 2), though for *C. hubbsi* there were no differences between any models. A coarser scale within the landscape data therefore reduced model fit as compared to a finer scale.

The effects of scale in this study reinforce the notion that several scales should be examined in ecological modeling studies. The local variables performed best when analyzed at a coarser scale, and the reverse was true for the landscape variables. Had scale not been considered carefully and analyzed at different resolutions, the models in this study would have likely performed poorer and may not have exhibited improvements over random models. Furthermore, scale should be considered early during experimental design, prior to data collection, as issues regarding scale are difficult to address without proper data collection or execution.

MANAGEMENT IMPLICATIONS

Orconectes eupunctus is a large river specialist, and thus conservation efforts for this species should focus on the protection of the larger order segments of the Eleven Point River, Spring River, and Strawberry River. *Orconectes eupunctus* did not appear to be sensitive to anthropogenic threats, but human impacts could affect the environmental habitat needs of the species. Groundwater resources in the study area, for instance, should be protected from withdrawals and contaminations that reduce the quality or quantity of spring flow, since spring flow volume appears to be an important component of coldwater crayfish habitat. Our extensive field sampling and predictive modeling indicate that *O. eupunctus* is found in only nine stream segments. Physical habitat protection for *O. eupunctus* should therefore prove relatively straightforward, given the limited number of segments. However, the majority of habitat for *O. eupunctus* lies within privately owned property, so collaboration with landowners in the area, particularly those with river frontage, would be necessary.

The largest threats to *O. eupunctus* appear to be the invasions of *O. neglectus* in the Spring River and Eleven Point River drainages. These invasions will be difficult and costly to control by anthropogenic means and with no guarantee of success. To date, no control method has been shown to be effective in removing invasive crayfish entirely, though some methods have a limited potential to reduce invasive crayfish density or slow dispersal (Gherardi et al 2011). Preventing further invasions of *O. neglectus* is the most promising and cost-effective approach in safeguarding *O. eupunctus* against further extirpations. Prevention methods should include regional education and awareness, policy implementation, and continual monitoring.

Special attention should be given to the population of *O. eupunctus* residing in the extreme lower fourth and upper fifth order main stem of the Strawberry River. These segments harbor the only known population of *O. eupunctus* in the Strawberry River drainage. Preliminary results from a related study indicate that this population is genetically divergent from populations in the Spring River and Eleven Point

River drainages (J. Fetzner et al. unpublished data). The isolation of *O. eupunctus* in the Strawberry River makes that population particularly vulnerable to environmental changes, human impacts, or invasive species. An invasion of *O. neglectus* in the Strawberry River, in particular, could extirpate *O. eupunctus* from the entire drainage. Therefore, the population of *O. eupunctus* in the Strawberry River should be treated as an evolutionarily significant unit and protected as such.

Management for *Orconectes marchandi* should also include protection of groundwater resources, as spring flow is closely linked with dolomite geology ("Missouri Limestone"). Road construction should be considered and planned carefully within the distribution of *Orconectes marchandi*, as the species may be sensitive to roads (Figures 3, 5), sedimentation, or both. Further invasions of *O. neglectus* likely constitute the greatest threat to *O. marchandi*, and preventative measures should be taken to limit the spread of *O. neglectus* into streams inhabited by *O. marchandi*. As mentioned above, few management options remain after the establishment of invasive crayfish populations. Care should be taken to ensure *O. marchandi* is not introduced into neighboring drainages, as predictive modeling and trait analysis (Larson and Olden 2010) indicate that this species may be capable of small scale introductions.

Cambarus hubbsi should be managed similarly to *O. eupunctus*, as the two are closely associated with many of the same critical habitat needs, such as stream size and spring flow. Special consideration may be necessary when managing *C. hubbsi* due to life history differences between it and *Orconectes* species. *Cambarus hubbsi* appears to grow slowly, reproduce later in life, and produce few young relative to *Orconectes* species (Larson and Magoulick 2011), and therefore may respond differently to management techniques. *Cambarus hubbsi* may or may not be impacted by invasions of *O. neglectus*, but previous data suggests that the two are negatively associated (Magoulick and DiStefano 2007). Both the field survey and predictive models indicate that *C. hubbsi* is rare in the Strawberry River drainage (Figure 15). *Cambarus hubbsi* is only currently known to exist in the fourth and fifth order segments of the Strawberry River main stem. Hubbs' crayfish in this drainage may therefore require special attention as evolutionarily significant units, as there is likely little to no gene flow between the Strawberry River and adjacent drainages. Currently, however, management for *C. hubbsi* may be unnecessary, as the species is found throughout the Ozarks of northern Arkansas and southern Missouri and appears to be stable (Pflieger 1996, Taylor et al 2007). *Cambarus hubbsi* is also abundant in the St.

Francis River drainage (Pflieger 1996), where *O. neglectus* is not currently known to be present. Still, *Cambarus hubbsi* populations should be periodically monitored to assess whether management is needed to address biotic or abiotic threats.

CONCLUSION

Crayfish, especially narrow-ranged endemics, warrant conservation attention and there is often little information by which to base management decisions. Three such Ozark endemics were modeled in this study using classification and regression trees, and the resulting information, combined with previous studies, will provide biologists with the necessary ability to make informed management decisions. Of particular conservation concern is the imperiled coldwater crayfish *Orconectes eupunctus*, which this study has indicated requires swift currents in the largest rivers in its distribution. The intensity of sampling, along with predictive modeling, would strongly suggest that all streams inhabited by *O. eupunctus* are now known to managers, which will facilitate conservation efforts. *Orconectes marchandi* and *Cambarus hubbsi* were also modeled in this study, which expanded on previous research. Anthropogenic threats were of minor importance in the models overall, indicating that invasion threats from *O. neglectus* are of greatest concern to the target species. Initial hypotheses for *O. eupunctus* and *C. hubbsi* were generally supported by the models, with both exhibiting preferences towards higher order streams. Hypotheses concerning *O. marchandi* were generally unsupported by the models, with water chemistry and spring flow volume being of greater importance than initially anticipated. Classification tree models performed well when compared to random assignment, but density regression trees were generally poor in explaining overall variation in density. Models were furthermore affected by scale, and the multi-scale approach used ensured that models exhibited high prediction rates.

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Figure 1: Map of the study area, consisting of the Strawberry River, Eleven Point River, and Spring River drainages and a portion of the lower Black River. Sampled sites are indicated by points, with hexagonal points indicating *O. eupunctus* presence localities.

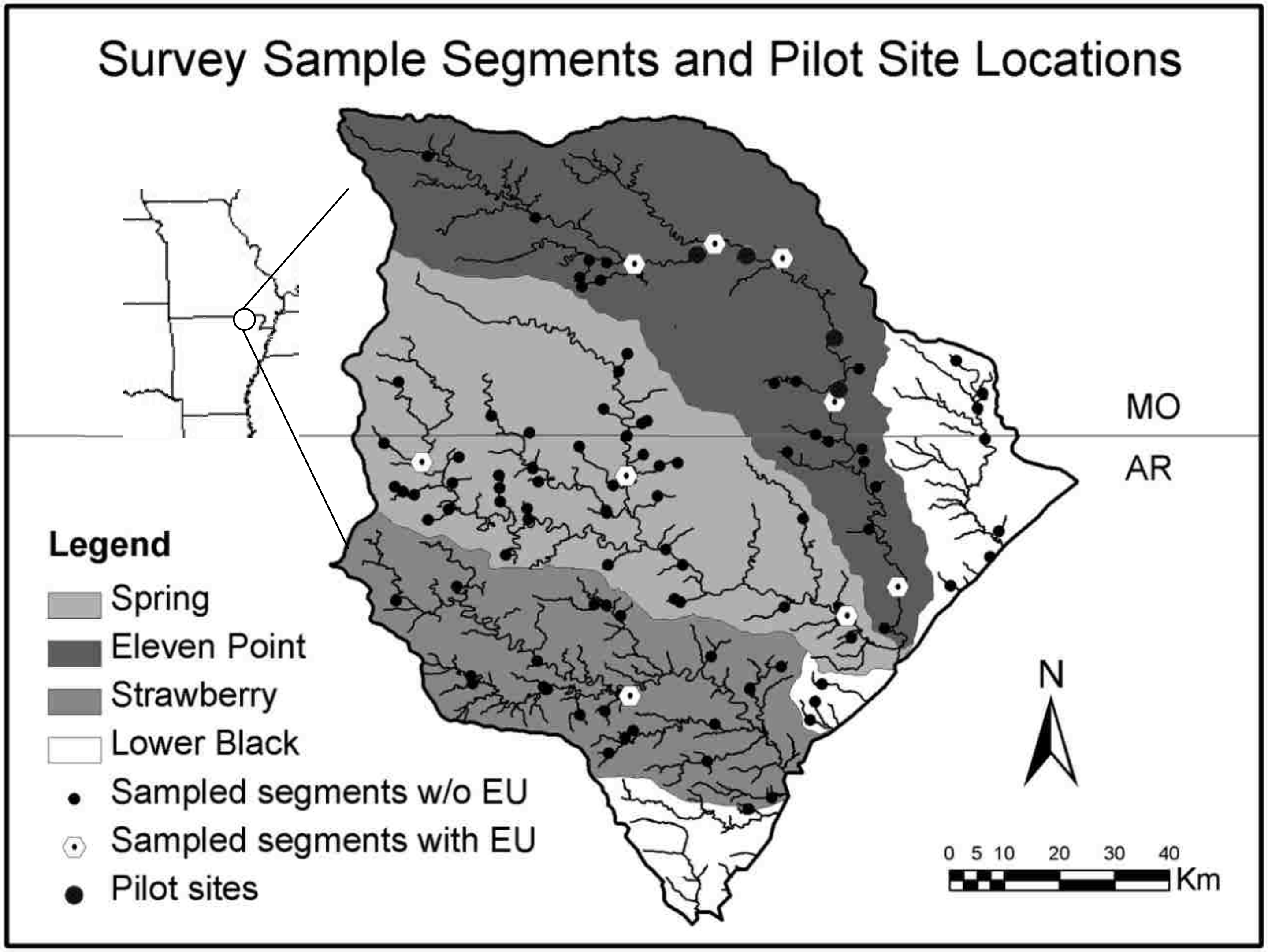
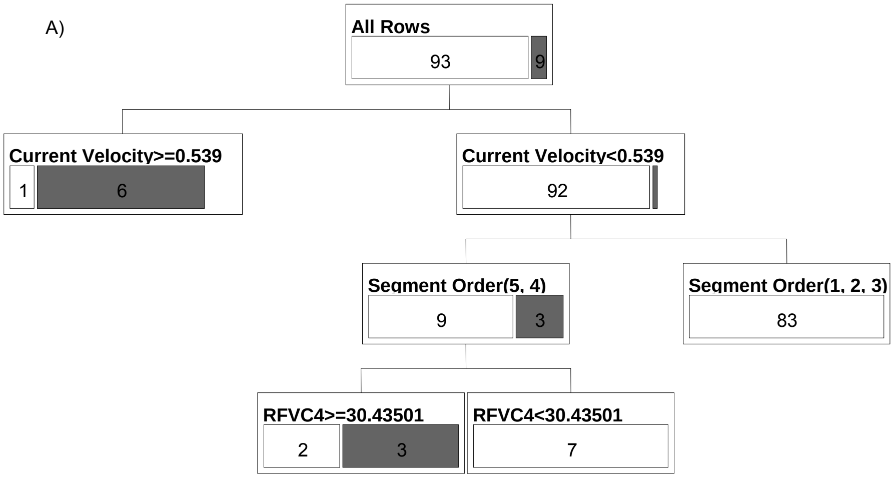
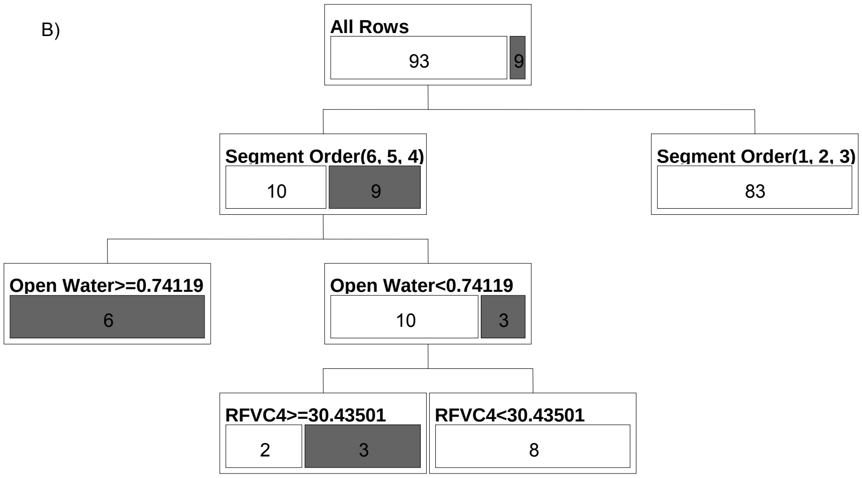

Survey Sample Segments and Pilot Site Locations
MO
AR
N
Legend
Spring
Eleven Point
Strawberry
Lower Black
Sampled segments w/o EU
Sampled segments with EU
Pilot sites
0 5 10 20 30 40
Km

Figure 2: (A) Global classification tree for *O. eupunctus* at the stream segment scale, (B) Classification tree for *O. eupunctus* at the stream segment scale when only landscape data are used, (C) Global density regression tree for *O. eupunctus*. Shaded bands represent presence sites, white bands represent absence cites, and numbers in the bands indicate the band sample size. Refer to Table 1 for more information concerning predictor variables.

A)


All Rows
93
9
Current Velocity>=0.539
1
6
Current Velocity<0.539
92
Segment Order(5, 4)
9
3
Segment Order(1, 2, 3)
83
RFVC4>=30.43501
2
3
RFVC4<30.43501
7

B)


All Rows
93
9
Segment Order(6, 5, 4)
10
9
Segment Order(1, 2, 3)
83
Open Water>=0.74119
6
Open Water<0.74119
10
3
RFVC4>=30.43501
2
3
RFVC4<30.43501
8

C)

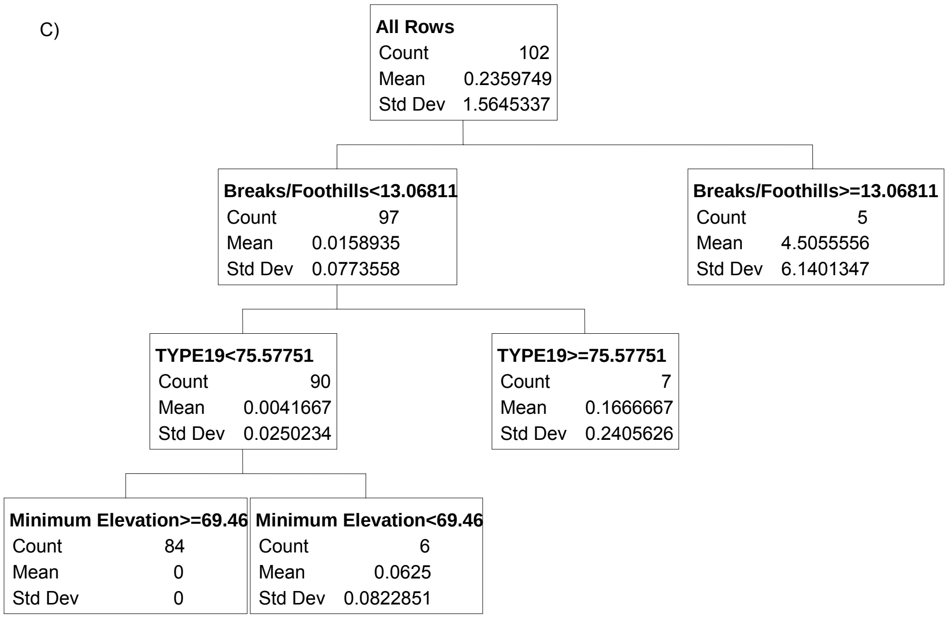

All Rows
Count 102
Mean 0.2359749
Std Dev 1.5645337
Breaks/Foothills<13.06811
Count 97
Mean 0.0158935
Std Dev 0.0773558
Breaks/Foothills>=13.06811
Count 5
Mean 4.5055556
Std Dev 6.1401347
TYPE19<75.57751
Count 90
Mean 0.0041667
Std Dev 0.0250234
TYPE19>=75.57751
Count 7
Mean 0.1666667
Std Dev 0.2405626
Minimum Elevation>=69.46
Count 84
Mean 0
Std Dev 0
Minimum Elevation<69.46
Count 6
Mean 0.0625
Std Dev 0.0822851

Figure 3: Global classification tree for *O. marchandi* at the stream segment scale

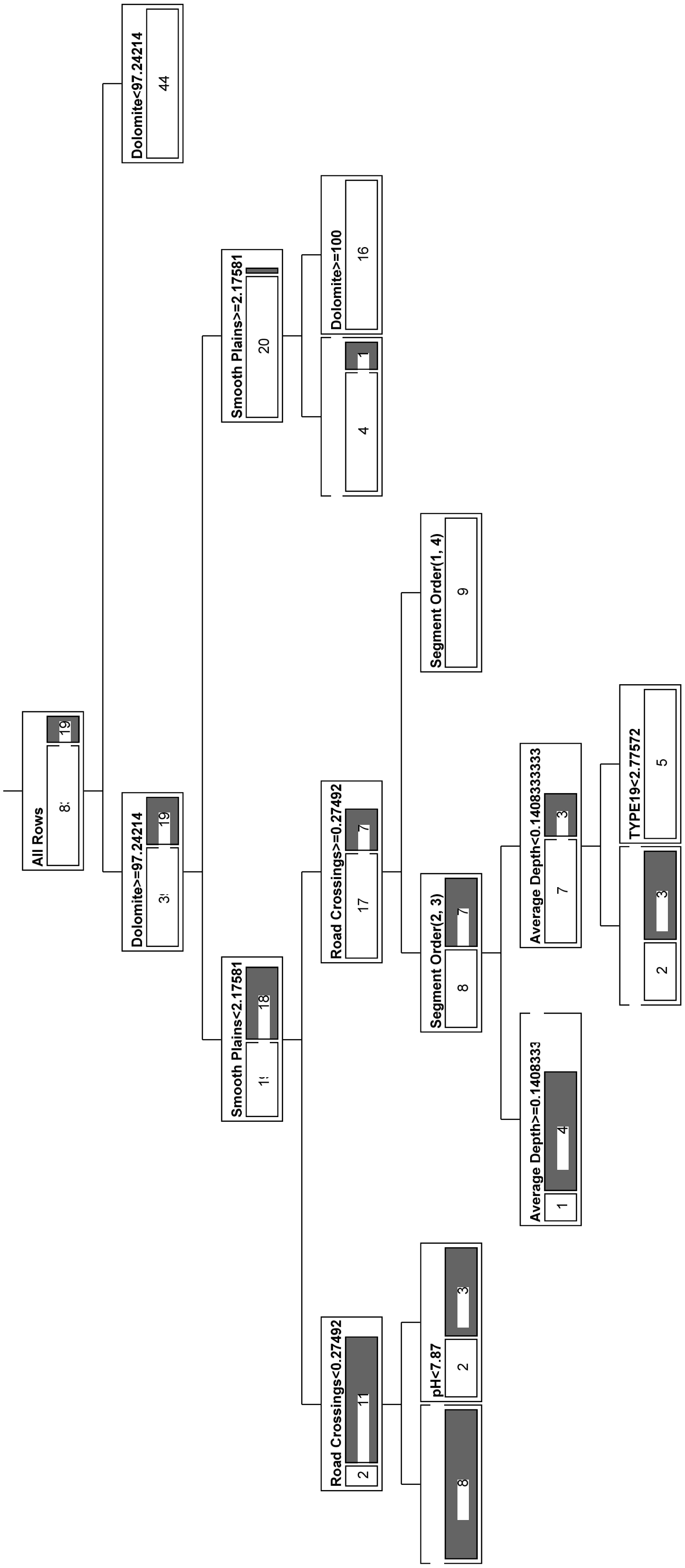

All Rows
19
Dolomite<97.24214
44
Dolomite>=97.24214
19
Smooth Plains>=2.17581
20
Dolomite>=100
16
4
1
Smooth Plains<2.17581
18
Road Crossings>=0.27492
17
7
Segment Order(1, 4)
9
Segment Order(2, 3)
8
7
Average Depth<0.1408333333
7
3
TYPE19<2.77572
5
2
3
Average Depth>=0.1408333
1
4
Road Crossings<0.27492
2
11
pH<7.87
2
3
8

Figure 4: Global density regression tree for *O. marchandi* at the stream segment scale

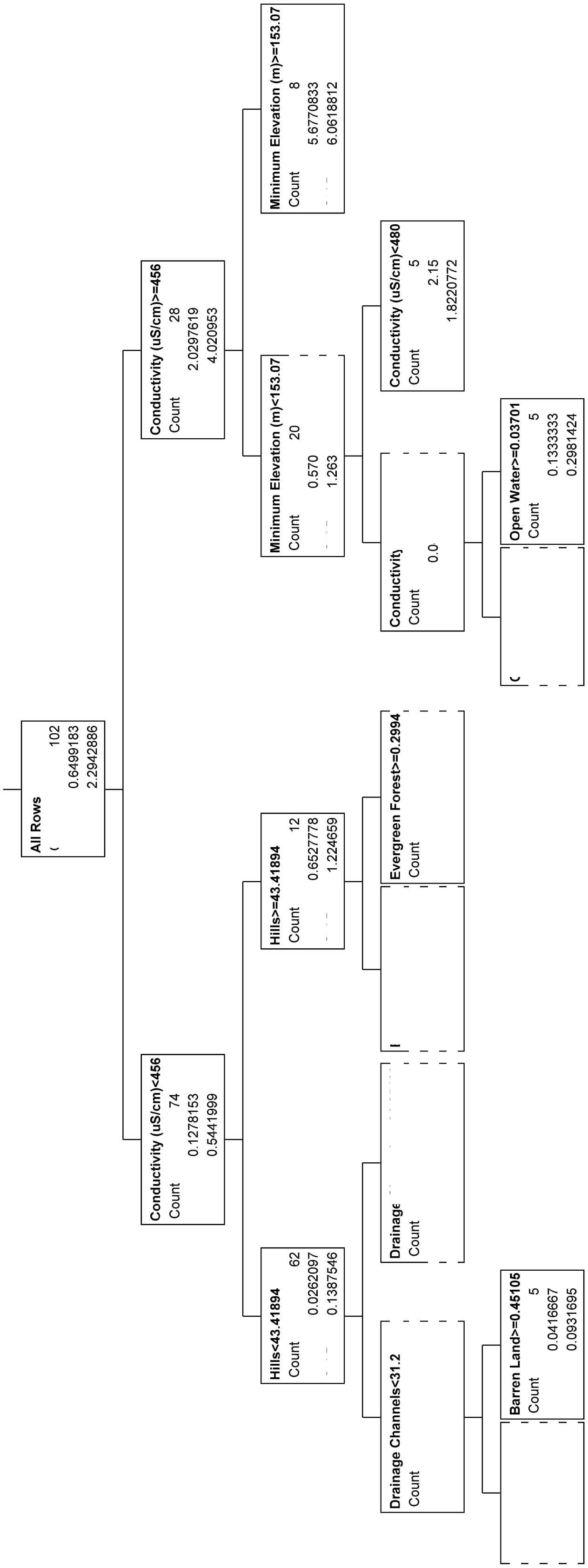

All Rows
102
0.6499183
2.2942886
Conductivity (uS/cm)>=456
Count 28
2.0297619
4.020953
Conductivity (uS/cm)<456
Count 74
0.1278153
0.5441999
Minimum Elevation (m)>=153.07
Count 8
5.6770833
6.0618812
Minimum Elevation (m)<153.07
Count 20
0.570
1.263
Conductivity (uS/cm)<480
Count 5
2.15
1.8220772
Conductivit
Count
0.0
Open Water>=0.03701
Count 5
0.1333333
0.2981424
Hills>=43.41894
Count 12
0.6527778
1.224659
Evergreen Forest>=0.2994
Count
Hills<43.41894
Count 62
0.0262097
0.1387546
Drainage
Count
Drainage Channels<31.2
Count
Barren Land>=0.45105
Count 5
0.0416667
0.0931695

Figure 5: Classification tree for *O. marchandi* at the stream segment scale when only landscape data are used

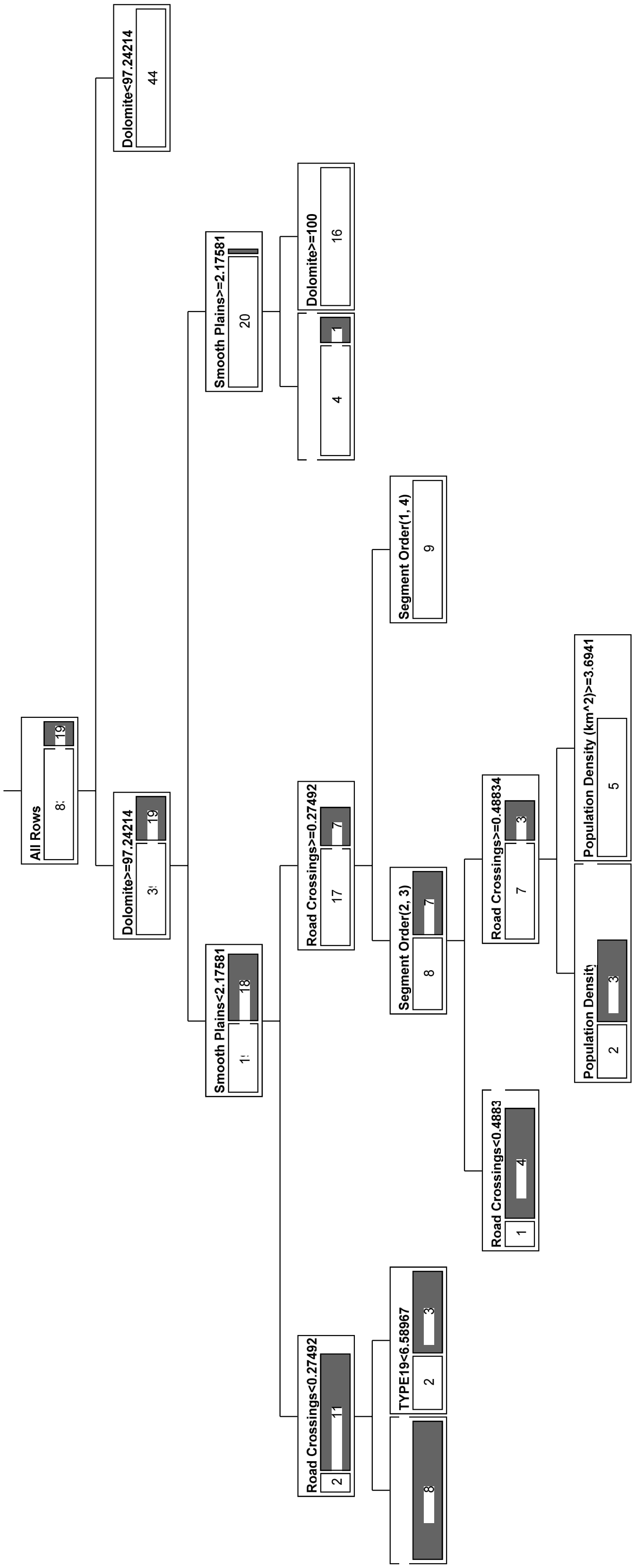

All Rows
8:
19
Dolomite<97.24214
44
Dolomite>=97.24214
3!
19
Smooth Plains>=2.17581
20
Dolomite>=100
16
4
1
Smooth Plains<2.17581
1!
18
Road Crossings>=0.27492
17
7
Segment Order(1, 4)
9
Segment Order(2, 3)
8
7
Road Crossings>=0.48834
7
3
Population Density (km^2)>=3.6941
5
Population Density
2
3
Road Crossings<0.4883
1
4
Road Crossings<0.27492
2
11
TYPE19<6.58967
2
3
8

Figure 6: (A) Global classification tree for *C. hubbsi* at the stream segment scale, (B) Classification tree for *C. hubbsi* at the stream segment scale when only landscape data are used

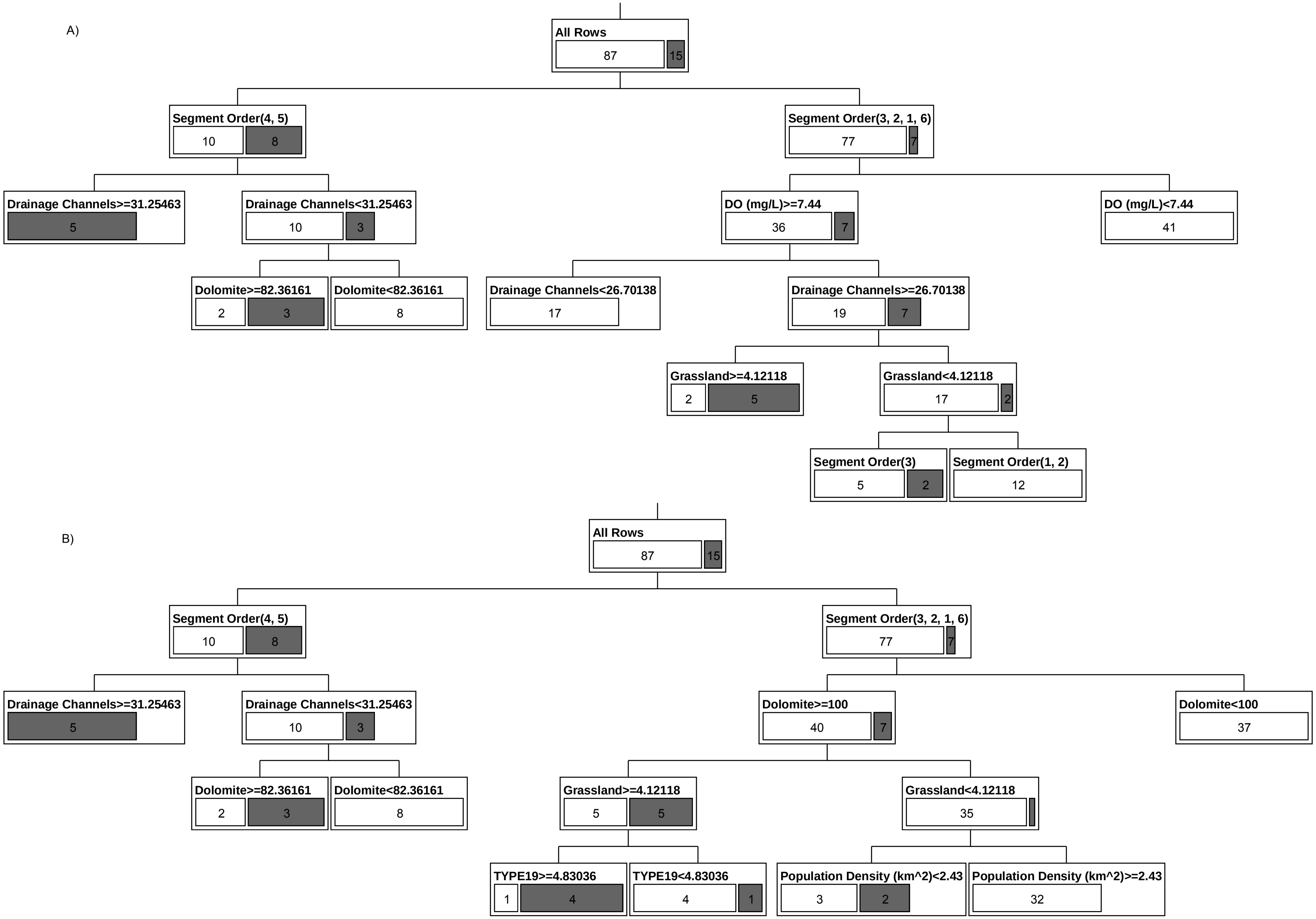

A)
All Rows
87
15
Segment Order(4, 5)
10
8
Segment Order(3, 2, 1, 6)
77
7
Drainage Channels>=31.25463
5
Drainage Channels<31.25463
10
3
DO (mg/L)>=7.44
36
7
DO (mg/L)<7.44
41
Dolomite>=82.36161
2
3
Dolomite<82.36161
8
Drainage Channels<26.70138
17
Drainage Channels>=26.70138
19
7
Grassland>=4.12118
2
5
Grassland<4.12118
17
2
Segment Order(3)
5
2
Segment Order(1, 2)
12
B)
All Rows
87
15
Segment Order(4, 5)
10
8
Segment Order(3, 2, 1, 6)
77
7
Drainage Channels>=31.25463
5
Drainage Channels<31.25463
10
3
Dolomite>=100
40
7
Dolomite<100
37
Dolomite>=82.36161
2
3
Dolomite<82.36161
8
Grassland>=4.12118
5
5
Grassland<4.12118
35
TYPE19>=4.83036
1
4
TYPE19<4.83036
4
1
Population Density (km^2)<2.43
3
2
Population Density (km^2)>=2.43
32

Figure 7: Global density regression tree for *C. hubbsi* at the stream segment scale

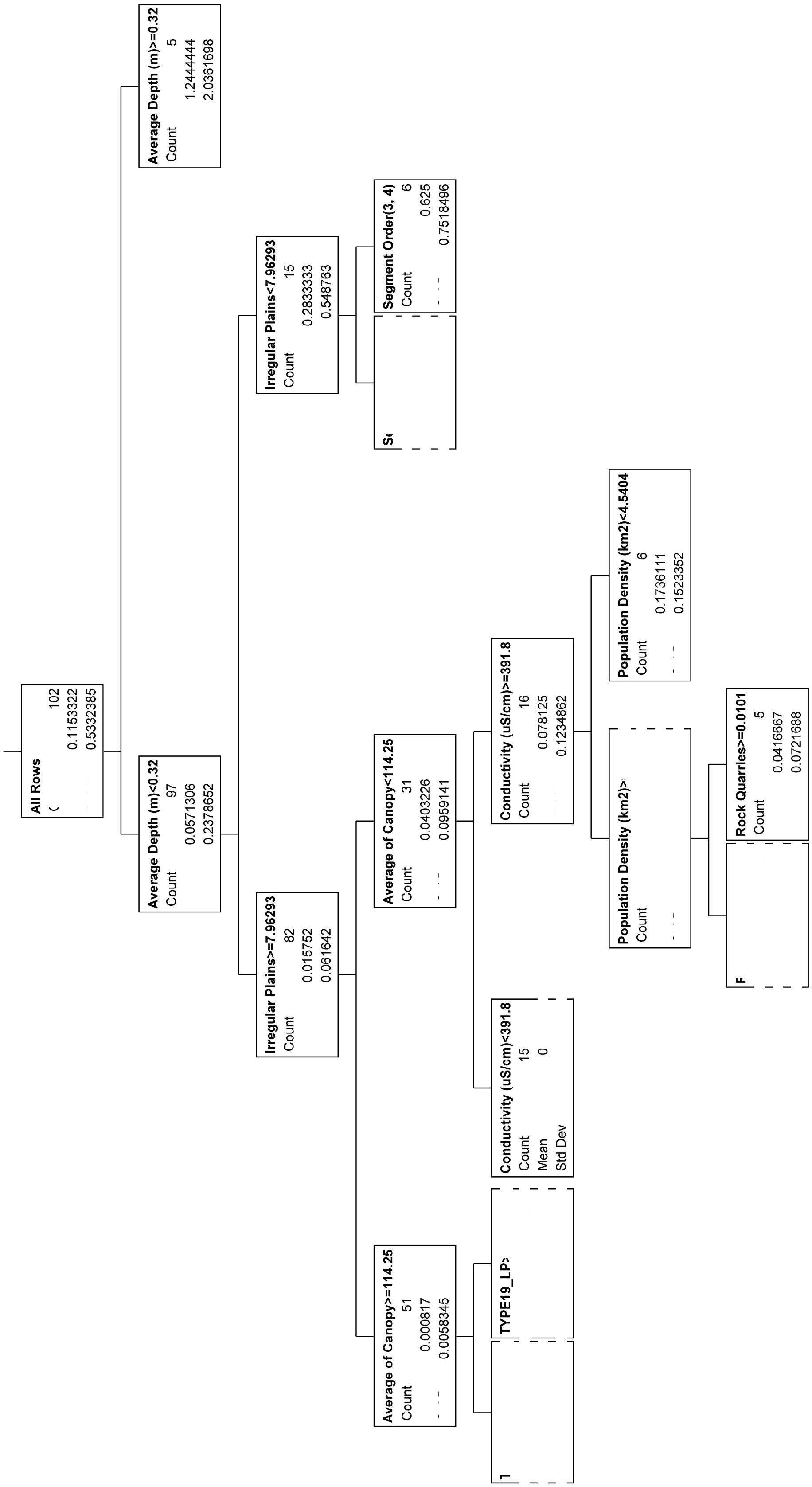
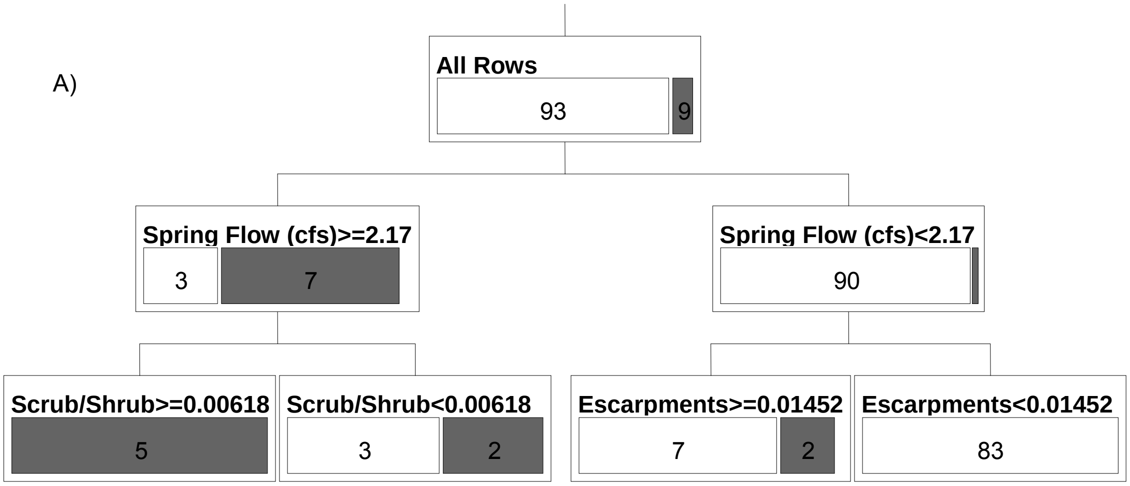

All Rows
102
0.1153322
0.5332385
Average Depth (m)>=0.32
Count 5
1.2444444
2.0361698
Average Depth (m)<0.32
Count 97
0.0571306
0.2378652
Irregular Plains<7.96293
Count 15
0.2833333
0.548763
Segment Order(3, 4)
Count 6
0.625
0.7518496
Irregular Plains>=7.96293
Count 82
0.015752
0.061642
Average of Canopy<114.25
Count 31
0.0403226
0.0959141
Average of Canopy>=114.25
Count 51
0.000817
0.0058345
TYPE19_LP
Conductivity (uS/cm)>=391.8
Count 16
0.078125
0.1234862
Conductivity (uS/cm)<391.8
Count 15
Mean 0
Std Dev
Population Density (km2)<4.5404
Count 6
0.1736111
0.1523352
Population Density (km2)>
Count
Rock Quarries>=0.0101
Count 5
0.0416667
0.0721688

Figure 8: (A) Classification tree for *O. eupunctus* at the local catchment scale for both the global model and the landscape only model, (B) Global density regression tree for *O. eupunctus* at the local catchment scale.

A)


All Rows
93
9
Spring Flow (cfs)>=2.17
3
7
Spring Flow (cfs)<2.17
90
Scrub/Shrub>=0.00618
5
Scrub/Shrub<0.00618
3
2
Escarpments>=0.01452
7
2
Escarpments<0.01452
83

B)

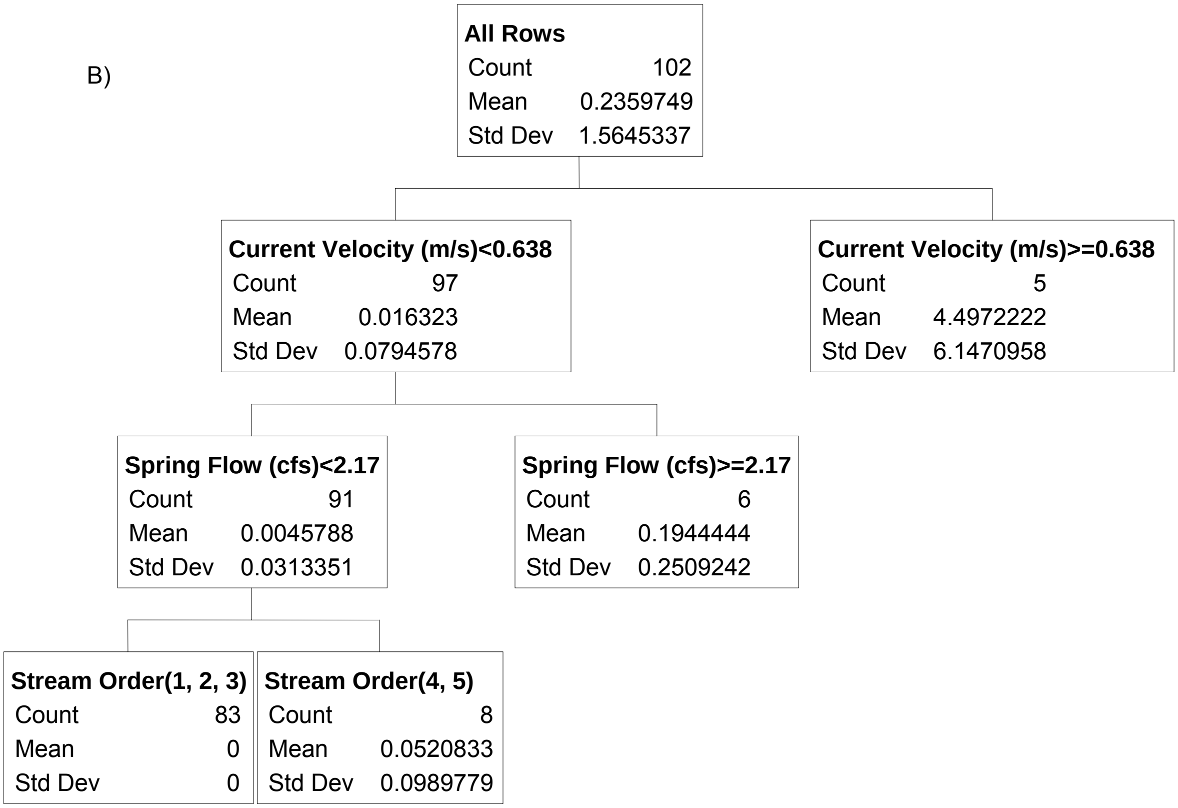
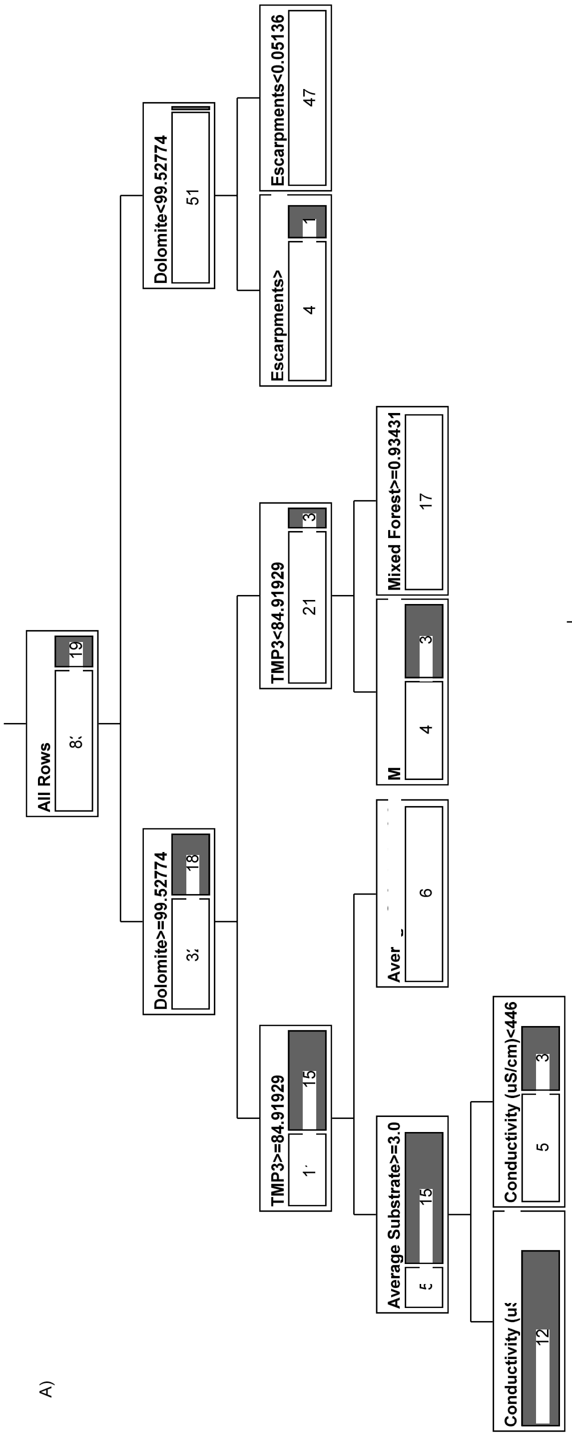

All Rows
Count 102
Mean 0.2359749
Std Dev 1.5645337
Current Velocity (m/s)<0.638
Count 97
Mean 0.016323
Std Dev 0.0794578
Current Velocity (m/s)>=0.638
Count 5
Mean 4.4972222
Std Dev 6.1470958
Spring Flow (cfs)<2.17
Count 91
Mean 0.0045788
Std Dev 0.0313351
Spring Flow (cfs)>=2.17
Count 6
Mean 0.1944444
Std Dev 0.2509242
Stream Order(1, 2, 3)
Count 83
Mean 0
Std Dev 0
Stream Order(4, 5)
Count 8
Mean 0.0520833
Std Dev 0.0989779

Figure 9: (A) Global classification tree for *O. marchandi* at the local catchment scale, (B) Classification tree for *O. marchandi* at the local catchment scale when only landscape data are used

A)


All Rows
19
Dolomite<99.52774
51
Escarpments>
4
1
Escarpments<0.05136
47
Dolomite>=99.52774
18
TMP3<84.91929
21
3
M
4
3
Mixed Forest>=0.93431
17
TMP3>=84.91929
15
Aver
6
Average Substrate>=3.0
15
Conductivity (uS
12
Conductivity (uS/cm)<446
5
3

B)

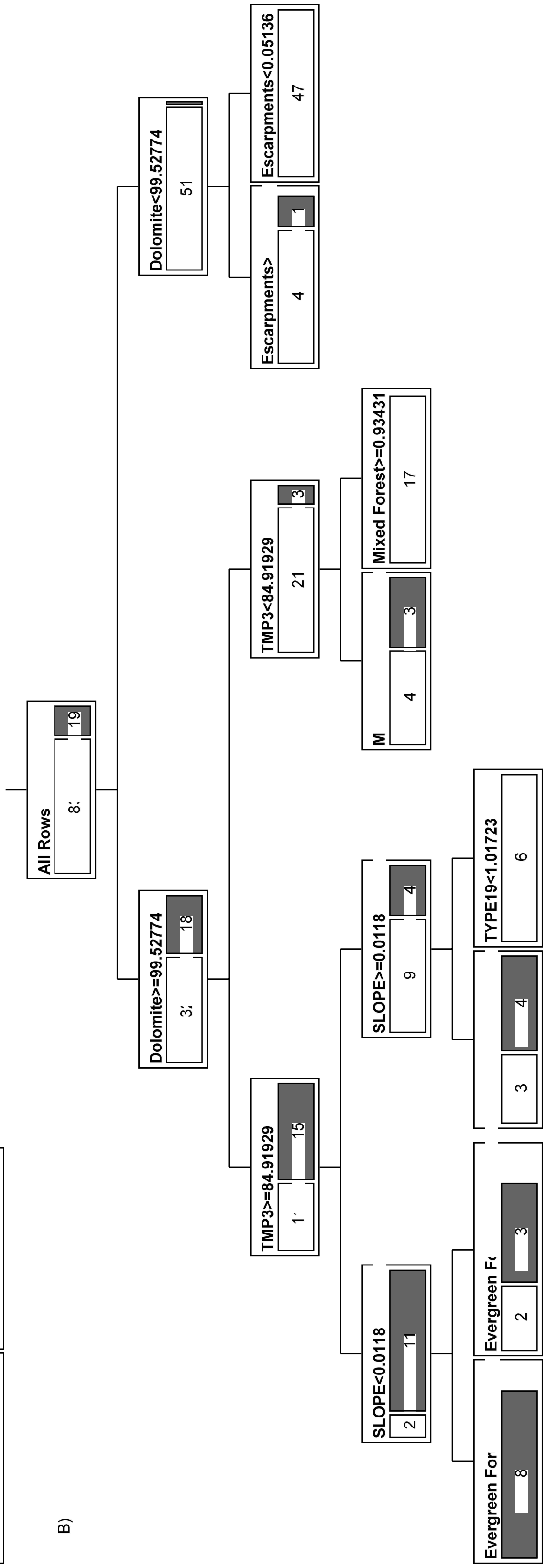

All Rows
19
Dolomite<99.52774
51
Escarpments>
4
1
Escarpments<0.05136
47
Dolomite>=99.52774
18
TMP3<84.91929
21
3
M
4
3
Mixed Forest>=0.93431
17
TMP3>=84.91929
15
SLOPE>=0.0118
9
4
3
4
TYPE19<1.01723
6
SLOPE<0.0118
2
11
Evergreen For
8
Evergreen Fo
2
3

Figure 10: Global density regression tree for *O. marchandi* at the local catchment scale

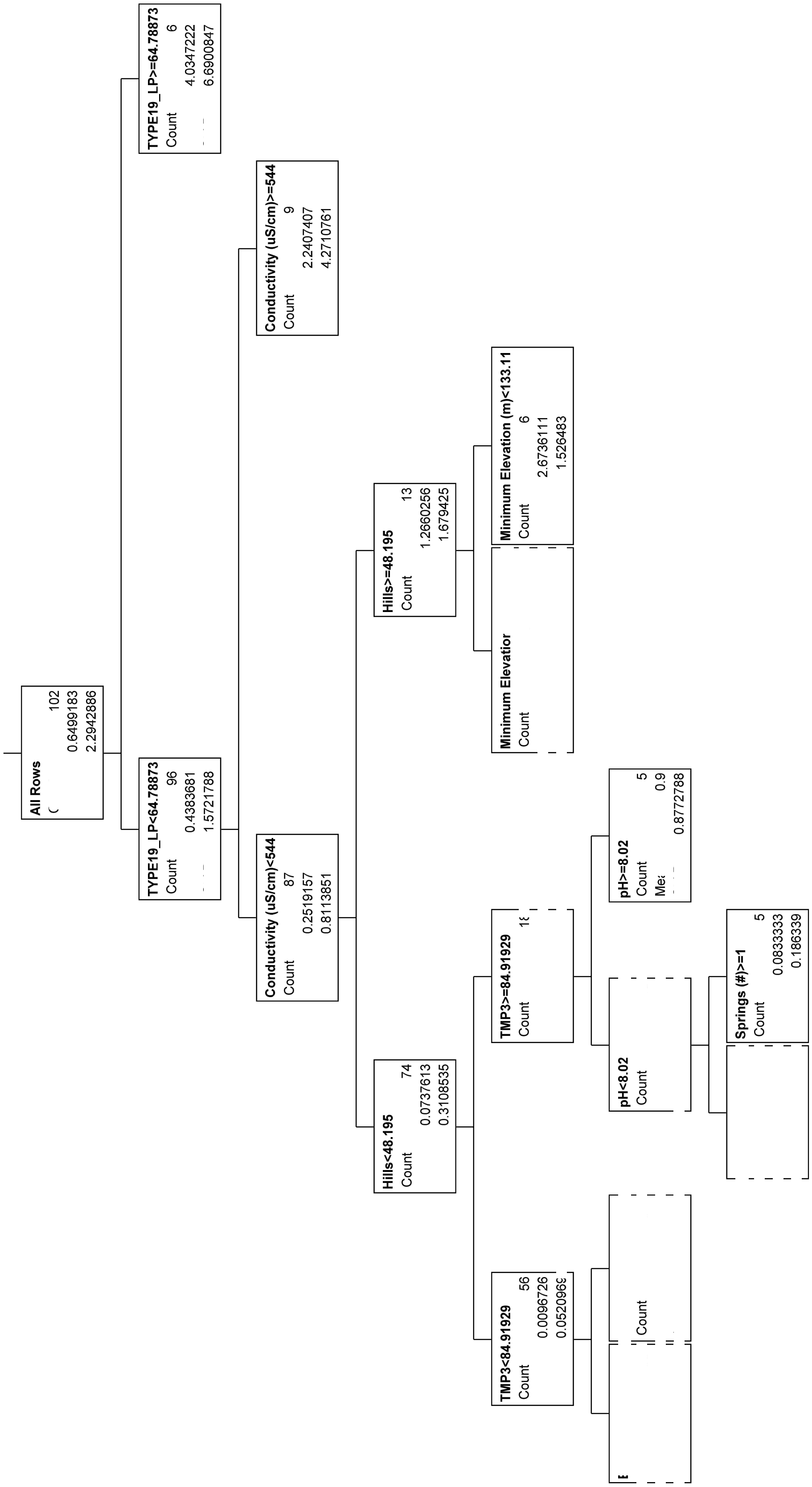
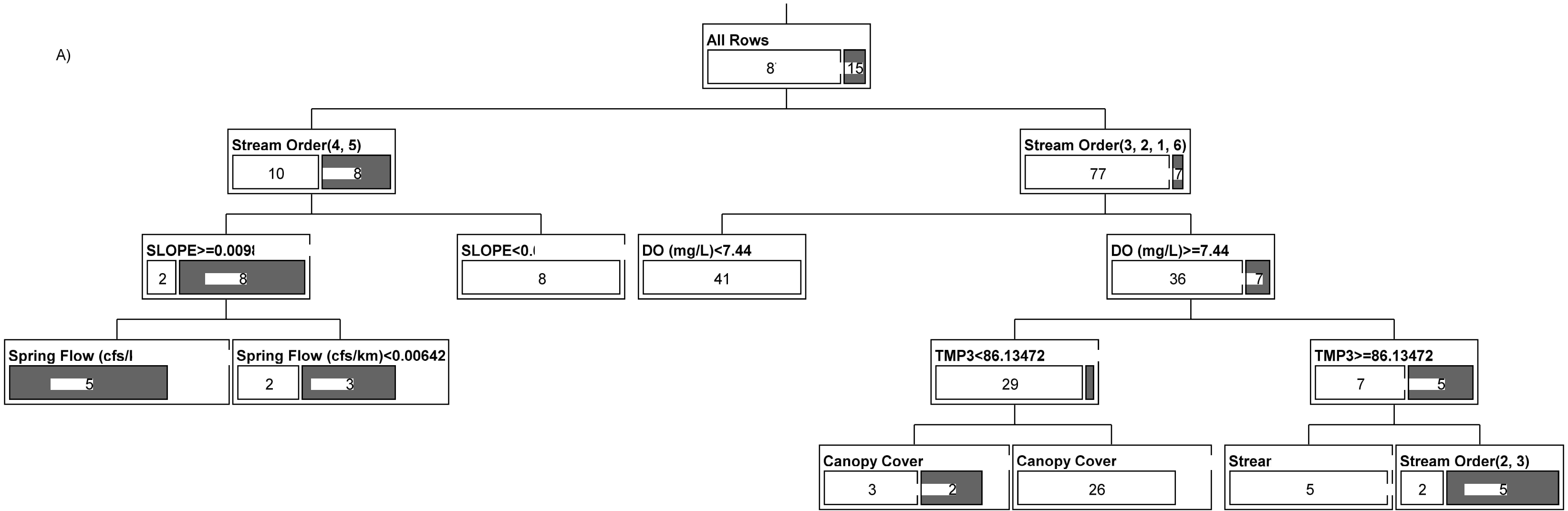

All Rows
102
0.6499183
2.2942886
TYPE19_LP>=64.78873
Count 6
4.0347222
6.6900847
TYPE19_LP<64.78873
Count 96
0.4383681
1.5721788
Conductivity (uS/cm)>=544
Count 9
2.2407407
4.2710761
Conductivity (uS/cm)<544
Count 87
0.2519157
0.8113851
Hills>=48.195
Count 13
1.2660256
1.679425
Minimum Elevation (m)<133.11
Count 6
2.6736111
1.526483
Minimum Elevatior
Count
Hills<48.195
Count 74
0.0737613
0.3108535
TMP3>=84.91929
Count
pH>=8.02
Count 5
0.9
0.8772788
pH<8.02
Count
Springs (#)>=1
Count 5
0.0833333
0.186339
TMP3<84.91929
Count 56
0.0096726
0.0520969
Count

Figure 11: (A) Global classification tree for *C. hubbsi* at the local catchment scale, (B) Classification tree for *C. hubbsi* at the local catchment scale when only landscape data are used

A)


All Rows
8'
15
Stream Order(4, 5)
10
8
Stream Order(3, 2, 1, 6)
77
7
SLOPE>=0.009
2
8
SLOPE<0.
8
DO (mg/L)<7.44
41
DO (mg/L)>=7.44
36
7
Spring Flow (cfs/l
5
Spring Flow (cfs/km)<0.00642
2
3
TMP3<86.13472
29
TMP3>=86.13472
7
5
Canopy Cover
3
2
Canopy Cover
26
Strear
5
Stream Order(2, 3)
2
5

B)

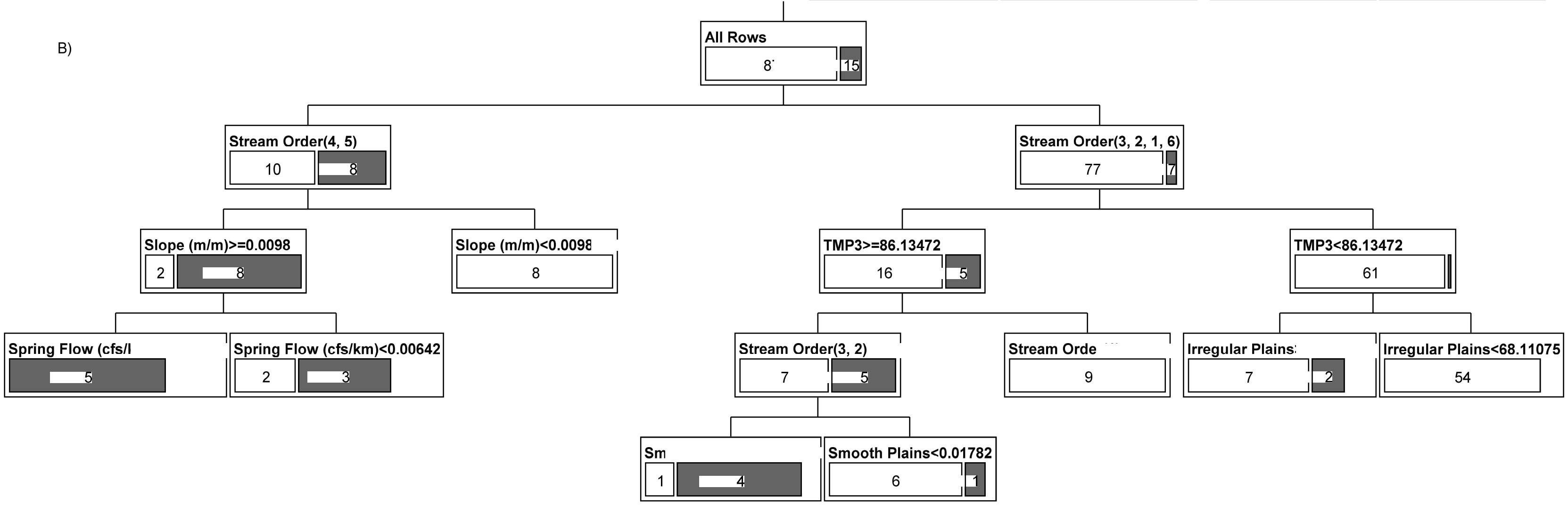

All Rows
8'
15
Stream Order(4, 5)
10
8
Stream Order(3, 2, 1, 6)
77
7
Slope (m/m)>=0.0098
2
8
Slope (m/m)<0.0098
8
TMP3>=86.13472
16
5
TMP3<86.13472
61
Spring Flow (cfs/l
5
Spring Flow (cfs/km)<0.00642
2
3
Stream Order(3, 2)
7
5
Stream Orde
9
Irregular Plains
7
2
Irregular Plains<68.11075
54
Sm
1
4
Smooth Plains<0.01782
6
1

Figure 12: Global density regression tree for *C. hubbsi* at the local catchment scale

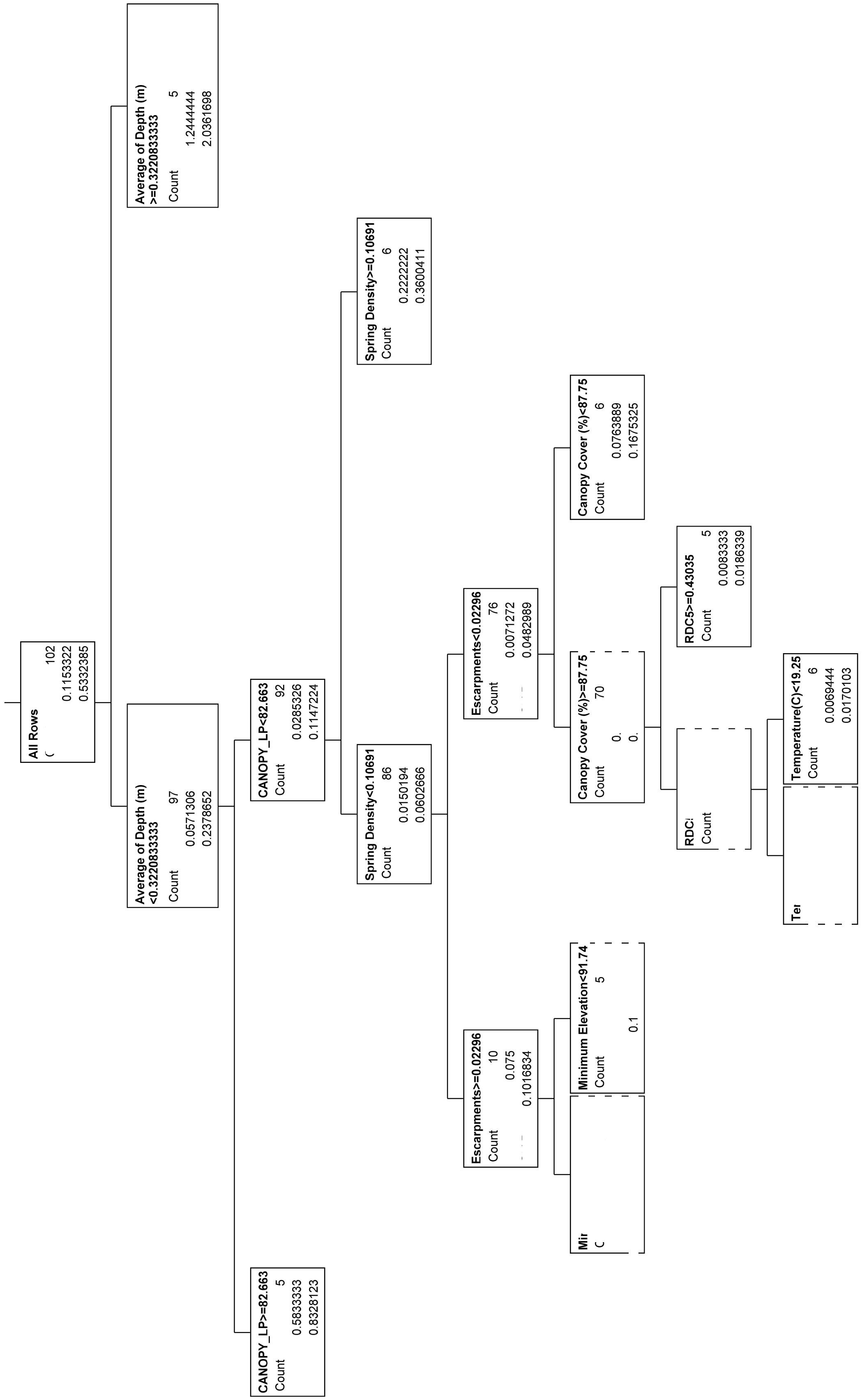

All Rows
(
102
0.1153322
0.5332385
Average of Depth (m) >=0.3220833333
Count 5
1.2444444
2.0361698
Average of Depth (m) <0.3220833333
Count 97
0.0571306
0.2378652
CANOPY_LP<82.663
Count 92
0.0285326
0.1147224
CANOPY_LP>=82.663
Count 5
0.5833333
0.8328123
Spring Density>=0.10691
Count 6
0.2222222
0.3600411
Spring Density<0.10691
Count 86
0.0150194
0.0602666
Escarpments<0.02296
Count 76
0.0071272
0.0482989
Escarpments>=0.02296
Count 10
0.075
0.1016834
Canopy Cover (%)<87.75
Count 6
0.0763889
0.1675325
Canopy Cover (%)>=87.75
Count 70
0.
0.
RDC5>=0.43035
Count 5
0.0083333
0.0186339
RDC
Count
Temperature(C)<19.25
Count 6
0.0069444
0.0170103
Te
Minimum Elevation<91.74
Count 5
0.1
Mi
C

Figure 13: Probability of occurrence map for *Orconectes eupunctus*

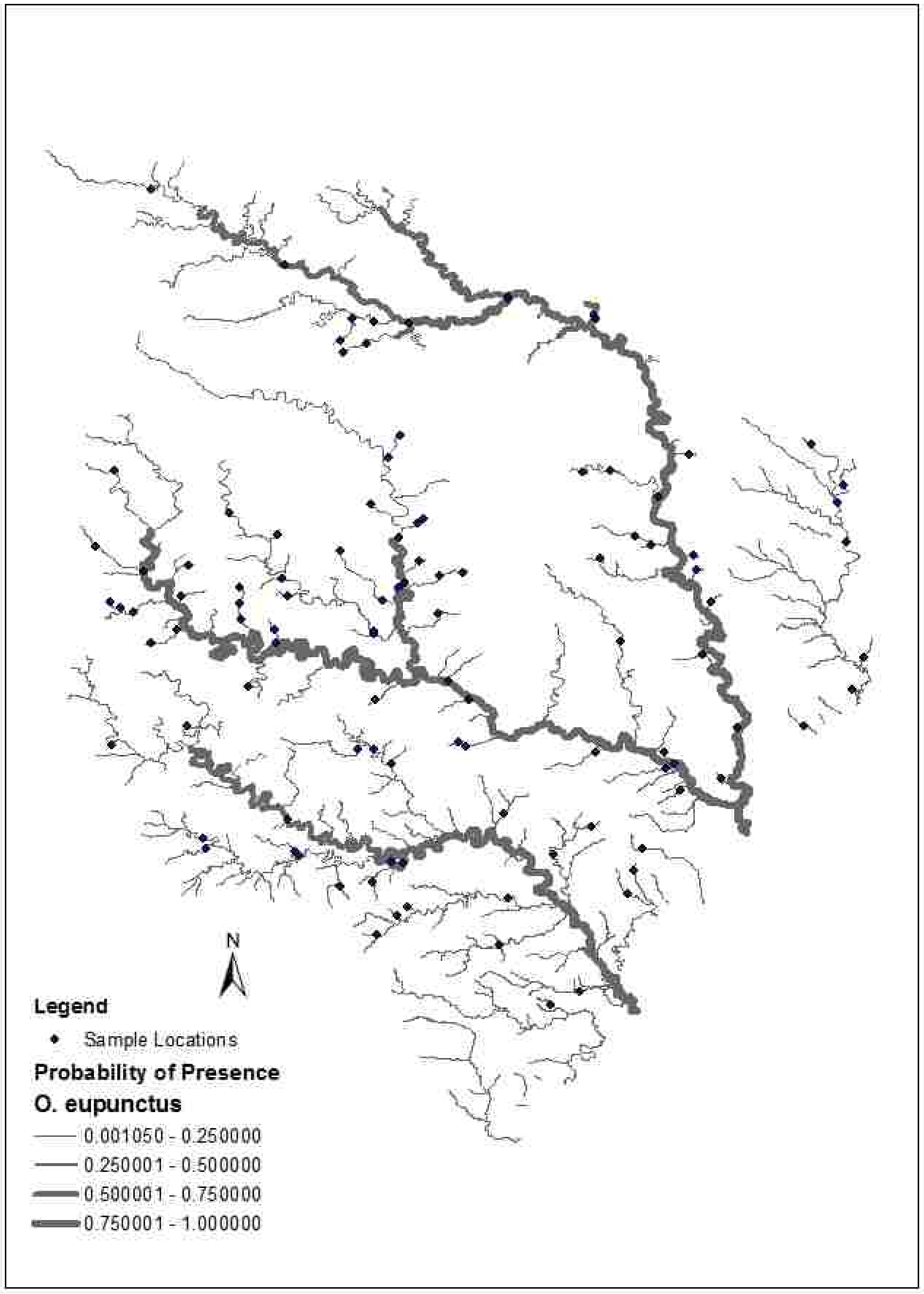

N
Legend
◆ Sample Locations
Probability of Presence
O. eupunctus
0.001050 - 0.250000
0.250001 - 0.500000
0.500001 - 0.750000
0.750001 - 1.000000

Figure 14: Probability of occurrence map for *Orconectes marchandi*

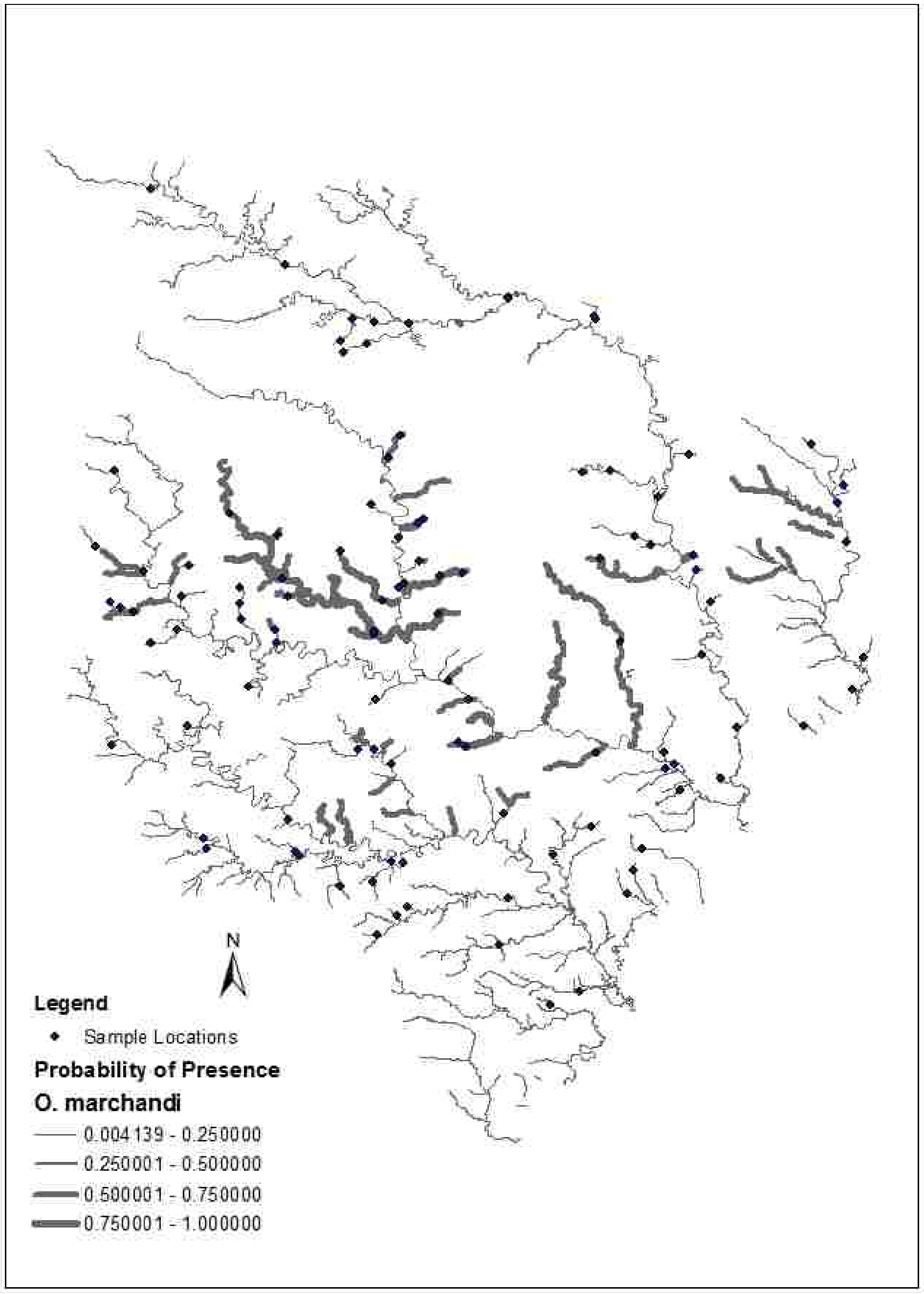

N
Legend
◆ Sample Locations
Probability of Presence
O. marchandi
0.004139 - 0.250000
0.250001 - 0.500000
0.500001 - 0.750000
0.750001 - 1.000000

Figure 15: Probability of occurrence map for *Cambarus hubbsi*

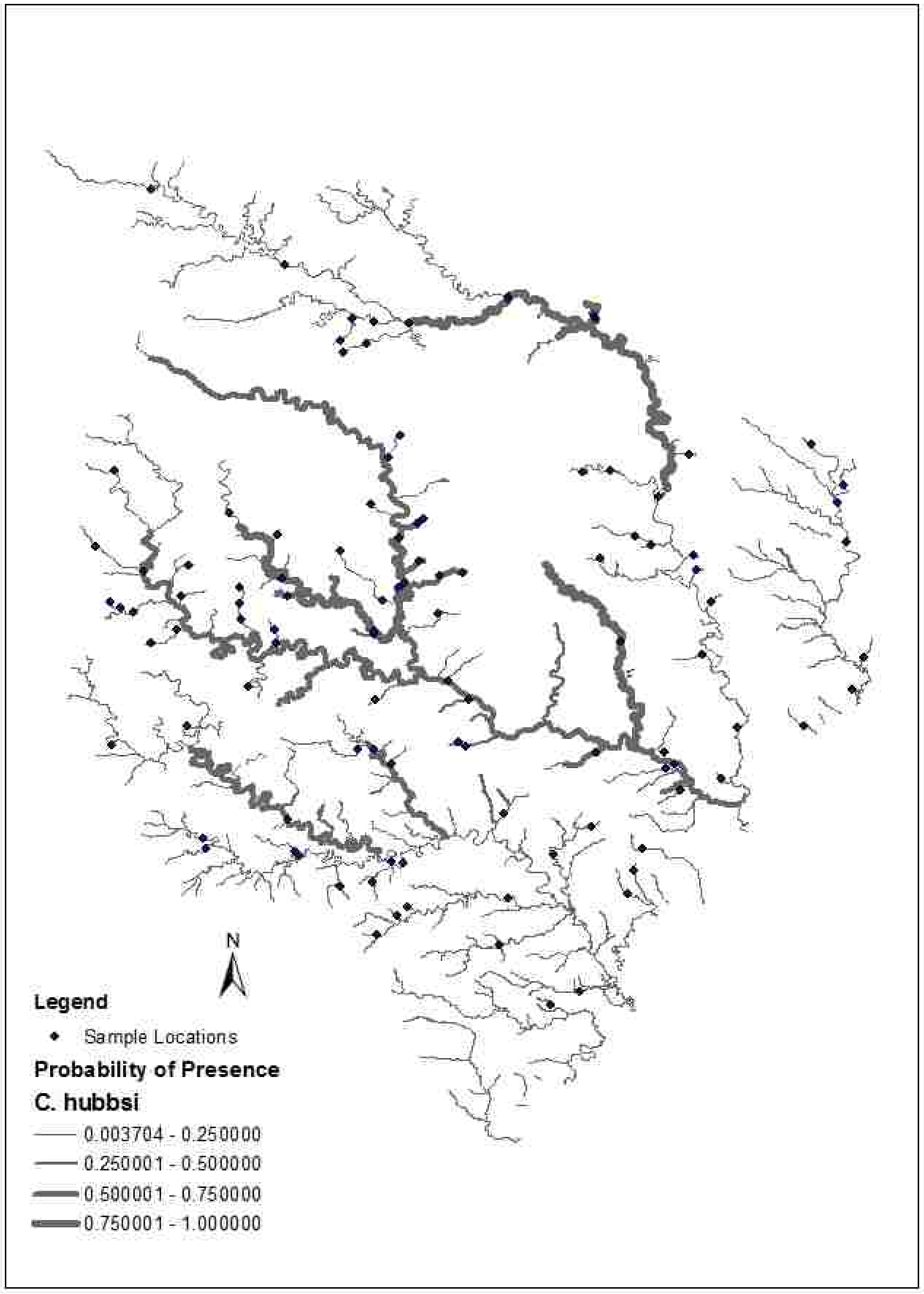
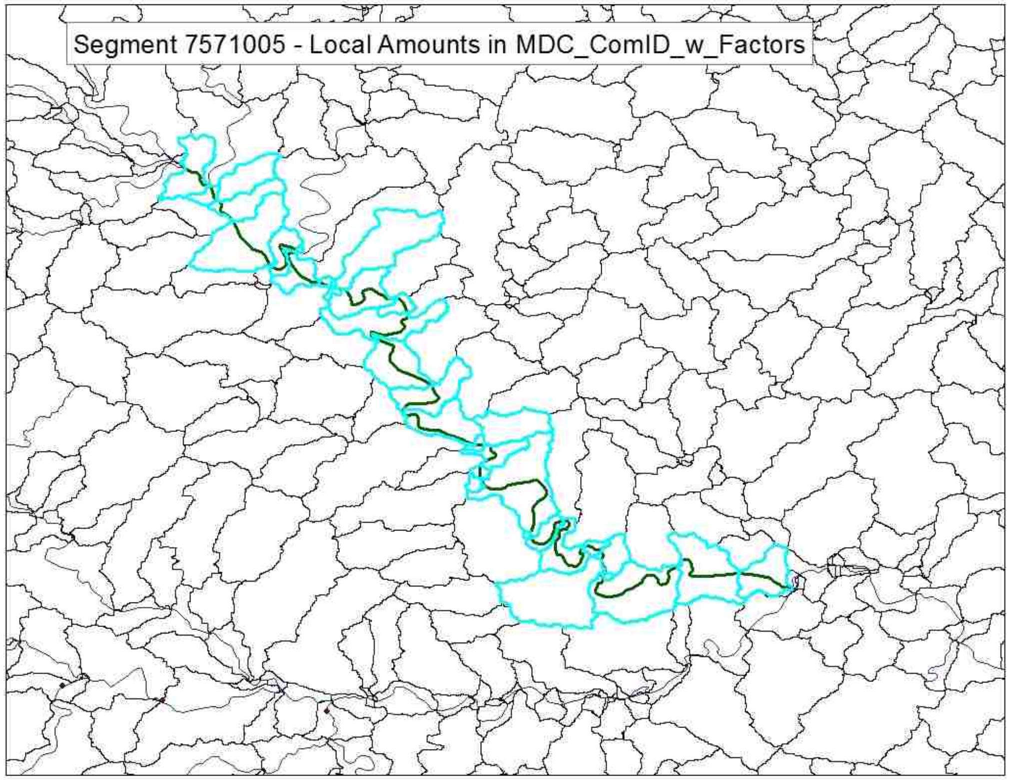
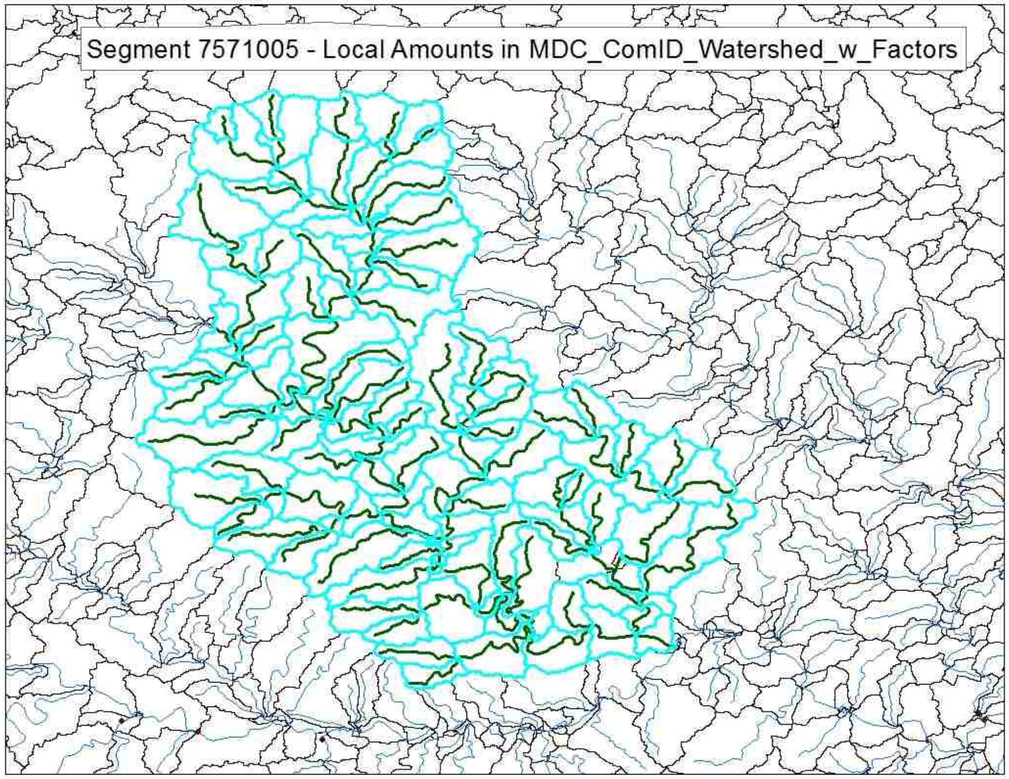

N
Legend
◆ Sample Locations
Probability of Presence
C. hubbsi
0.003704 - 0.250000
0.250001 - 0.500000
0.500001 - 0.750000
0.750001 - 1.000000

Figure 16: (A) Stream segment scale showing included sub-catchments outlined in blue. (B) Local Catchment scale showing included sub-catchments outlined in blue.


Segment 7571005 - Local Amounts in MDC_ComID_w_Factors

A


Segment 7571005 - Local Amounts in MDC_ComID_Watershed_w_Factors

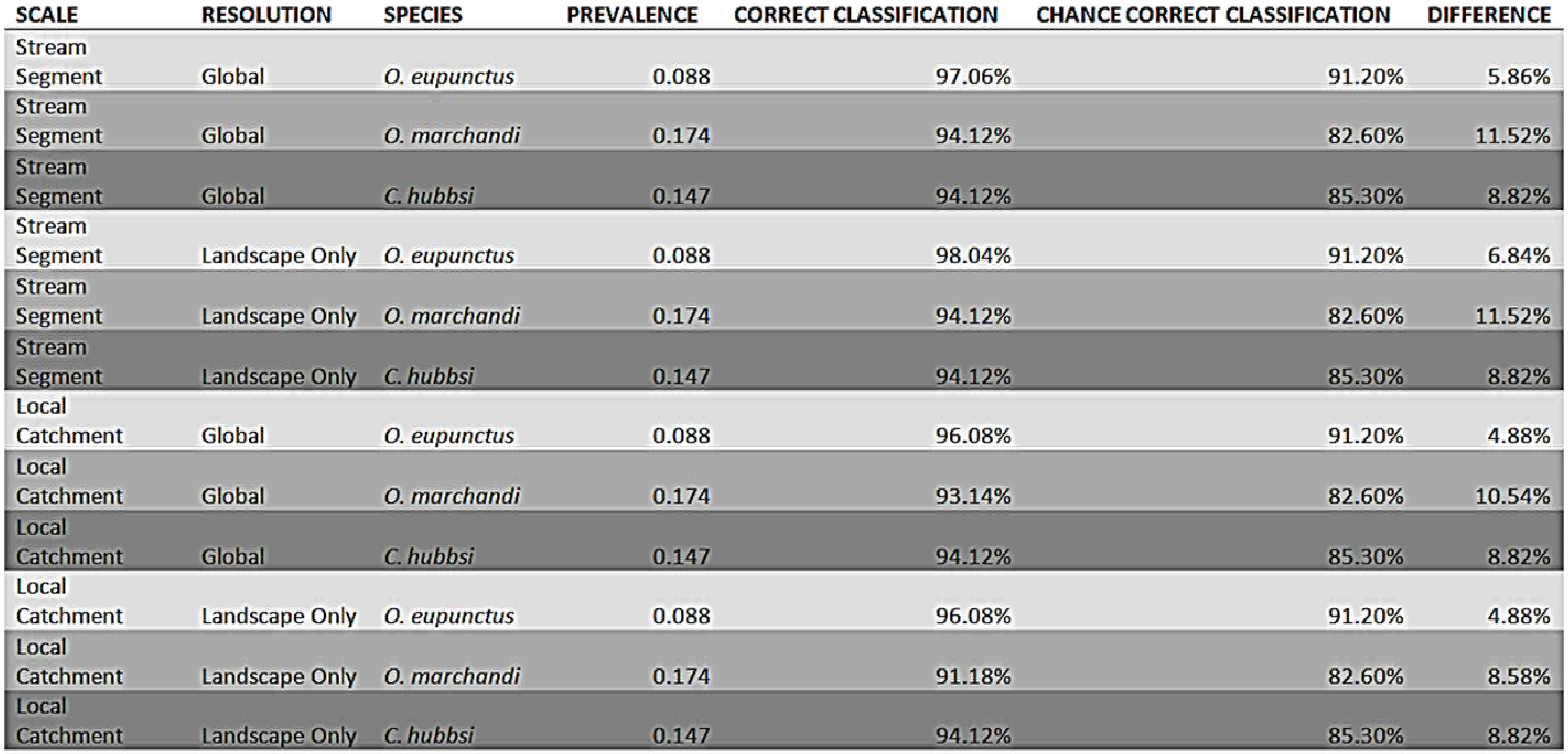
B

Table 1: Variable name key for variables used in tree models. Actual variables used in the models depended on the models.

Variable	**Variable Definition**
Segment Order	Strahler stream order
Average of canopy	The average canopy cover of the site in degrees cover
Average Substrate	The stream-wide average of averaged quadrat particle size classes
Average Depth	The combined average depth (m) of riffles and runs
Current Velocity	The average current velocity (m/s)
Temperature	Temperature (C) reading prior to sampling
pH	pH reading prior to sampling
DO	Dissolved oxygen (mg/L) reading prior to sampling
Conductivity	Conductivity (uS/cm) reading prior to sampling
Airports	The density of airports (number/km2) in the local catchment
CANOPY_LP	The average percent canopy cover within the local catchment
Alluvium	The local catchment percent of geology type 1 (alluvium)
Clastic	The local catchment percent of geology type 2 (clastic)
Dolomite	The local catchment percent of geology type 3 (dolomite)
Gravel	The local catchment percent of geology type 5 (gravel)
Limestone	The local catchment percent of geology type 7 (limestone)
Sand	The local catchment percent of geology type 8 (sand)
Sandstone	The local catchment percent of geology type 9 (sandstone)
Shale	The local catchment percent of geology type 10 (shale)
Water	The local catchment percent of geology type 11 (water)
Impervious Surface	The average percent impervious surface within the local catchment
Smooth Plains	The local catchment percent of landform type 2 (Smooth Plains)
Irregular Plains	The local catchment percent of landform type 3 (Irregular Plains)
Escarpments	The local catchment percent of landform type 4 (Escarpments)
Hills	The local catchment percent of landform type 6 (Hills)
Breaks/Foothills	The local catchment percent of landform type 7 (Breaks/Foothills)
Drainage Channels	The local catchment percent of landform type 10 (Drainage Channels)
Lead Mines	The density of lead mines (number/km2) in the local catchment
Springs	The number of springs in the local catchment
Spring Density	The density of springs (number/km2) in the local catchment
Spring Flow	The amount of spring flow (cfs) in the local catchment
SPFLW_LPK	The amount of spring flow per unit area (cfs/km) within the local catchment
Open Water	The percentage of local catchment in open water
Developed, open	The percentage of local catchment in developed, open space
Barren Land	The percentage of local catchment in barren land
Evergreen Forest	The percentage of local catchment in evergreen forest
Mixed Forest	The percentage of local catchment in mixed forest
Scrub/shrub	The percentage of local catchment in scrub/shrub

Grassland	The percentage of local catchment in grassland
Pasture/hay	The percentage of local catchment in pasture/hay
Cropland	The percentage of local catchment in cropland
Woody Wetlands	The percentage of local catchment in woody wetlands
Herbaceous wetland	The percentage of local catchment in emergent herbaceous wetland
RDC4	The percentage of the local catchment comprised of depth to bedrock class 4 (62 - 91 cm)
RDC5	The percentage of the local catchment comprised of depth to bedrock class 5 (92 - 122 cm)
RFVC4	The percentage of the local catchment comprised of rock fragment volume class 4 (20.1 - 40 %)
RFVC5	The percentage of the local catchment comprised of rock fragment volume class 5 (40.1 - 60 %)
ROCKQ_LPK	The density of rock quarries (number per km2)
RRLEN	The length of railroads (meters) within the local catchment
TYPE19	The local catchment percent of surficial lithology type 19 (Alluvium and fine-textured coastal zone sediment)
TMP1	The local catchment percent of topographic moisture potential 1 (Wetlands)
TMP2	The local catchment percent of topographic moisture potential 2 (Mesic Uplands)
TMP3	The local catchment percent of topographic moisture potential 3 (Dry Uplands)
Maximum Elevation	Maximum elevation (smoothed) in meters
Minimum elevation	Minimum elevation (smoothed) in meters
SLOPE	Slope of flowline (m/m)
Population Density	Population density/km2 in the local catchment
Road Crossings	Density of road crossings (number/km2) within the local catchment
Road Length	Density of road length (m/km2) in the local catchment
Dams	Density of dams (number/km2) in the local catchment
Groundwater Recharge	Mean annual natural groundwater recharge (mm)

Table 2: Correct classification rates for crayfish models as compared to chance correct classification rates.



SCALE	RESOLUTION	SPECIES	PREVALENCE	CORRECT CLASSIFICATION	CHANCE CORRECT CLASSIFICATION	DIFFERENCE
Stream Segment	Global	*O. eupunctus*	0.088	97.06%	91.20%	5.86%
Stream Segment	Global	*O. marchandi*	0.174	94.12%	82.60%	11.52%
Stream Segment	Global	*C. hubbsi*	0.147	94.12%	85.30%	8.82%
Stream Segment	Landscape Only	*O. eupunctus*	0.088	98.04%	91.20%	6.84%
Stream Segment	Landscape Only	*O. marchandi*	0.174	94.12%	82.60%	11.52%
Stream Segment	Landscape Only	*C. hubbsi*	0.147	94.12%	85.30%	8.82%
Local Catchment	Global	*O. eupunctus*	0.088	96.08%	91.20%	4.88%
Local Catchment	Global	*O. marchandi*	0.174	93.14%	82.60%	10.54%
Local Catchment	Global	*C. hubbsi*	0.147	94.12%	85.30%	8.82%
Local Catchment	Landscape Only	*O. eupunctus*	0.088	96.08%	91.20%	4.88%
Local Catchment	Landscape Only	*O. marchandi*	0.174	91.18%	82.60%	8.58%
Local Catchment	Landscape Only	*C. hubbsi*	0.147	94.12%	85.30%	8.82%