Use of a riverscape-scale model of fundamental physical habitat requirements for freshwater mussels to quantify mussel declines in a mining-contaminated stream: the Big River, Old Lead Belt, Southeast Missouri.

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Project Purpose: The research described in this report was conducted as part of the Natural Resource Damage Assessment and Restoration process in the Big River. Our purpose was to compare habitat features and landscape factors that may be important for the establishment and persistence of mussel concentrations between the Big River and the adjacent Bourbeuse and Meramec rivers, thereby testing their appropriateness as reference systems for establishing baseline expectations of mussel populations in the absence of mining impacts for the Big River. Based on these comparisons and a published model dileneating suitable habitat for freshwater mussels, we establish expected baseline conditions related to suitable freshwater mussel habitat in the Big River to assist injury determination for mining-related impacts in the Southeast Missouri Lead Mining District Natural Resource Damage Assessment case.

Summary of Author Responsibilities and Results Presented in this Report: Quantification of the area of the main channel in the heavy metal contaminated portion of the Big River that is suitable and predicted to be occupied by high-richness freshwater mussel aggregations through the use of a continuous, spatially explicit habitat model for freshwater mussels and corresponding use of predicted suitable reaches in uncontaminated reference streams (Bourbeuse and Meramec Rivers). This work took place in collaboration with U.S. Fish and Wildlife Service (Service) biologists and includes an estimate of acres, stream miles, and percent of total channel suitable for mussels due to factors other than the physical channel variables represented in the model

Introduction:

Freshwater Unionid mussels are among the most imperiled taxa worldwide and reach their highest diversity in North America, particularly in the Southeastern United States. Mussel diversity and abundance in rivers within the Ozark physiographic region of Missouri rival Southeastern drainages in both mussel diversity and abundance, particularly the Meramec River (Figure 1; Roberts and Bruenderman 2000; Hinck et al. 2012), which represents a diversity hotspot (Hinck et al. 2012). However, the mussel fauna within the Meramec River Watershed is potentially threatened by a variety of both cryptic and documented impacts, including urbanization in the downstream areas around St. Louis, agriculture, recreation (e.g., heavy use by floating parties), water pollution (Key 2019), and, finally, heavy metal contamination from a history of mining in one of its major tributaries, the Big River (Allert et al. 2013; Besser et al. 2009; Roberts et al. 2010).

To quantify the impacts (e.g., loss of species or declines in abundance) related to a specific threat (e.g., heavy metal contamination) on biota in a multiple-threat situation like in the Meramec River Watershed, it is important to establish reference conditions and baseline expectations regarding the distribution of the affected animals in the absence of that threat, preferably in a spatially explicit manner. Without pre-impact data, this reference condition can be estimated via identification of suitable habitat that would otherwise be occupied in the absence of that specific threat; via comparison with a physically similar "reference" area affected similarly by other factors (e.g., agriculture) but otherwise unaffected by the threat under investigation (e.g., heavy metal contamination); or a combination thereof. As a first step in estimating the potential distribution of the affected animals under investigation, one may eliminate those reaches that are unsuitable for their establishment and persistence, or outside of the range of conditions where mussels could establish (Evans et al. 2013; Li 2014). Habitat models are widely used tools for the conservation and management of imperiled species and can be used to delineate fundamentally suitable areas (Chase and Leibold 2003; Guisan and Thuiller 2005). Although niche concepts are typically assigned to individual species (as described originally by Hutchinson 1957), freshwater mussels as a taxonomic group offer a unique advantage for application of the niche concept and these tools. Generally, riverine freshwater mussels tend to have clustered distributions and aggregate in multi-species concentrations (i.e., beds), which suggests a common physical habitat limitation for multiple species. This is likely associated with geomorphological and hydrological characteristics of riverine habitats, which have been successfully linked to mussel distributions (Strayer and Ralley 1993; Layzer and Madison 1995).

A spatially-explicit and longitudinally (upstream to downstream) continuous model for fundamentally suitable habitat for mussel beds was recently developed for the Meramec River Watershed (Key and Lindner 2017; Bouska et al. 2018; Key 2019; Key et al. 2021). This model (hereafter, termed as a fundamental habitat model) was developed for and funded by the Missouri Department of Conservation to assist their statewide mussel conservation program (Model spatial layers for GIS and results are peer reviewed and publically available online; Key and Lindner 2017). The fundamental habitat model uses in-channel characteristics and known mussel bed locations to predict occupiable habitat for mussel beds in the absence of threats or other biological limitations that are not included in the model. In essence, the habitat model delineates specific reaches that fall within the range of conditions that could support mussels in the absence of other limitations (Key and Lindner 2017). This model provides a continuous, in-channel representation of physical habitat characteristics and predicted fundamentally suitable mussel habitat for 530 river kilometers (km) within the Meramec River Watershed, including the Meramec River's two main tributaries, the Big and Bourbeuse rivers (Key 2019; Key et al. *In Review*). In addition to publically available and peer-reviewed data layers (Key and Lindner 2017), the manuscript describing the fundamental habitat model development in full is attached with this report to provide a complete reference to the model methodology, results, and validation.

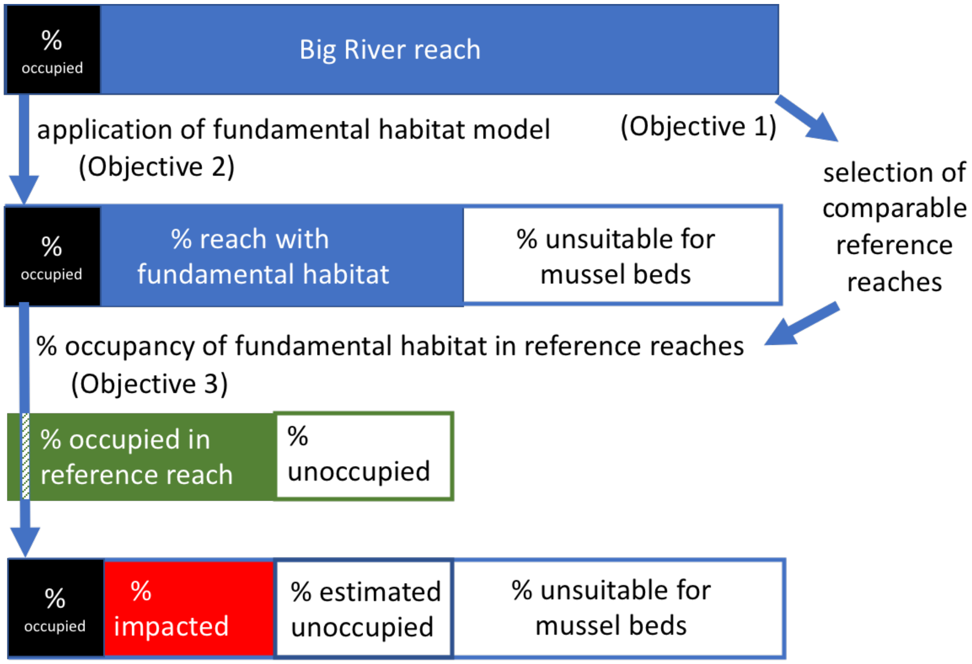
The Big River, a principle tributary of the Meramec River, was impacted by mining for heavy metals, predominantly lead (Pb), which led to a significant decline in mussel relative abundance and diversity along a significant length (Meneau 1997; Pavlowsky et al. 2010; Albers et al. 2016; Roberts et al. 2016). Quantifying the magnitude of mussel declines associated with a limiting factor such as lead is challenging for resource managers due to a lack of pre-impact data. However, the development of the fundamental habitat model eliminates river reaches with geomorphological characteristics, in particular, outside of the tolerance limits of mussel beds. This provides a unique opportunity, in quantifying the impact of lead on freshwater mussels in the Big River, to focus only on those reaches of the river that have the minimum physical habitat characteristcs capable of supporting high-richness mussel beds. Further, identification and estimation of occupancy of similar reaches in size and extent in the whole of the Meramec River Watershed (in Key and Lindner 2017; Key et al 2021) allows for an estimate of mussel occupancy of fundamentally suitable habitat in the presence of other widespread, non-point threats in the basin (e.g., agriculture) *without* the influence of limiting factors such as lead contamination. Together, these steps (Diagram 1) represent an estimation of reference conditions for the Big River that thereby supports calculation of mussel declines associated with heavy metal contamination as the focal limiting factor under investigation.

Our goals are to identify logically and scientifically defensible reference reaches to establish a baseline estimate of occupancy of fundamental habitat for the Big River mussel community in the absence of mining impacts and to use the fundamental model of habitat suitability for mussel beds in the Meramec River Watershed (Key and Lindner 2017; Key et al. 2021) to quantify mussel declines in the Big River. The objectives undertaken to achieve this goal include:

1. Evaluate the Bourbeuse and Meramec rivers as two alternative, appropriate reference or baseline rivers in comparison to the Big River by contrasting:
    a. Physical habitat characteristics identified as important for mussels
    b. Nitrogen/Ammonia factors that may be threatening mussel communities (as comprehensive comparison of a likely widespread threat).
    c. Occupancy of habitat configurations by mussel beds
2. Identify and quantify the distribution of fundamental habitat for mussels in the Big River through application of the Key et al. (2021) fundamental habitat model (Key and Lindner 2017).

3. Estimate loss of potentially occupied suitable habitat in the Big River due to factors other than physical habitat based on the relative occupancy of fundamentally suitable freshwater mussel habitat in reference river reaches (selected via completion of Objective 1 and Key and Lindner 2017).

Diagram 1: Conceptual model of the river reach elimination process used to estimate the percent suitable, yet unoccupied habitat in Big River due to factors other than physical habitat, with associated research objectives:



Study Area: The study is focused in the Meramec River Watershed (10,308 km^2), located in the northeastern portion of the Ozark physiographic region within Missouri (Figure 1). Mussel species within this drainage are included in the upper Mississippi freshwater mussel province (Haag 2012). The Meramec River also includes two major tributaries, the Big River (2,473 km^2) and the Bourbeuse River (2,183 km^2). Upstream of the Big River confluence, the Meramec River is 6% urbanized, 72% wooded/scrubland, and 21% agriculture, while 66% of the Meramec River's watershed bedrock geology is dolostone, 22% sandstone, and 9% shale (Homer et al. 2012; MSDIS 2011). The remaining 3% of the Meramec River Watershed's geology is comprised of diorite, granite, limestone, and rhyolite. When excluding the landscape characteristics of the Big and Bourbeuse watersheds from the Meramec River Watershed, the Meramec River is 5% urbanized, 76% wooded/scrubland, and 18% agriculture (1% other), while 68% and 30% of the Meramec River Watershed bedrock geology is dolostone and sandstone, respectively. The remaining 2% of the Meramec River Watershed's geology is comprised of limestone, rhyolite, and shale. The Big River Watershed is 7% urbanized, 75% wooded/scrubland, and 17% agriculture (1% other). The Big River Watershed is 87% dolostone, 6% sandstone, 4% limestone, and 2% rhyolite, with the remaining bedrock geology comprised of diorite, granite, and shale. The Bourbeuse River Watershed is 7% urbanized, 59% wooded/scrubland, and 33% agriculture (1% other), with a bedrock geology of 38% dolostone, 23% sandstone, and 39% shale. Altogether, the three river systems share not only similar mussel faunas, but similar land-use and underlying geology.

History of Heavy Metal Contamination in the Big River: Several agencies, in association with the Service and the Missouri Department of Natural Resources (MoDNR), have been studying the toxic effects to organisms from releases of heavy metals to the Big River as part of the Southeast Missouri Lead Mining District Natural Resources Damage Assessment (NRDA) process (Allert et al. 2013; Besser et al. 2009; Roberts et al. 2010; Roberts et al. 2016). The Big River Watershed drains an area with a long history of lead and zinc mining called "the Old Lead Belt," which once provided the highest production of lead in the United States (U.S. Geological Survey 1998). Mining activities in the Old Lead Belt have largely ceased, but their legacy remains, including approximately 227 million metric tons of fine-grained dolomitic tailings that are now divided among 6 large piles adjacent to the Big River and its tributaries. Consequently, releases of mine wastes have contaminated river sediments and floodplains with lead (Pb), cadmium (Cd), and zinc (Zn) in more than 144 km of the Big River and its tributaries (Meneau 1997; MoDNR 2007; Pavlowsky et al. 2010).

Heavy metal contaminated sediments negatively affect mussel populations in the Big River downstream of mining areas (Besser et al. 2009; Roberts et al. 2010; Roberts et al. 2016). While other streams in the basin contain relatively healthy mussel assemblages (Roberts and Brunderman 2000; Hinck et al. 2012), the Big River and its 37 native mussel species have reduced population metrics in over 100 km, associated with heavy metal contamination (Roberts et al. 2016). A 2008 assessment of heavy metal contamination and freshwater mussel populations in the Big River (Roberts et al. 2010) showed sediment contaminated with heavy metals at levels greater than the consensus-based Probable Effects Concentration (PEC) for Pb at 128 mg/kg extending more than 150 km downstream of mining areas (MacDonald et al. 2000). Sediment concentrations of Zn and Cd exceeded their respective consensus-based PECs of 458 mg/kg and 4.99 mg/kg for shorter distances (approximately 40 km). Depressed mussel species richness, densities, and abundance compared to reference sites were found over a 157km reach of the river, from km 170.5 - 13.7. Stream reaches nearest to the mining inputs from km 170.5 – 133.1 demonstrated the greatest impacts to the mussel assemblage (Roberts et al. 2010), and toxicity of sediments to mussels was documented in laboratory tests (Besser et al. 2009). Mussel communities in the downstream 16.5 km of the Big River were similar to reference sites in terms of mussel abundance and species richness, and sediments in this reach did not consistently contain heavy metals at concentrations exceeding the PECs (Roberts et al. 2010).

Methodology:

Objective 1. Establishment of reference reaches for estimation of baseline occupancy of fundamental habitat: Multiple analyses were used to evaluate the Bourbeuse and Meramec rivers, which share a watershed and similar mussel fauna and land use factors with the Big River, as suitable references for the Big River. First, analyses compare the physical habitat characteristics from the Meramec River fundamental habitat model among the Big, Bourbeuse, and Meramec rivers. The fundamental habitat model consisted of 10 binary and continuous spatial datasets of physical habitat characteristics used to predict fundamental habitat for 195 km of the Big River, 127 km of the Bourbeuse River, and 177 km of the Meramec River (Figure 1; A1). The extent of the analyses began at the confluence of the Big and Meramec rivers, limiting analysis for the Meramec River to 118 km. The lower 60 km of the Meramec is frequently affected by backwater from the Mississippi River when flooding and has characteristics more akin to a lowland river rather than higher gradient Ozark rivers. The habitat variables include: spatial representations of channel stability, bluff adjacency to the main channel, distributions of gravel- and pool-reaches, stream-power indices, and water availability during low-flow conditions (Table 1). Refer to the included manuscript for a full description of the Meramec River fundamental habitat model (Key et al. 2021).

Analyses compared the quantitative distributions of the habitat characteristics in the Big, Bourbeuse, and Meramec rivers using descriptive analytical techniques and probabilistic kernel density functions. Kernel density methods fit smoothing functions to the data to facilitate comparisons between the data curves, effectively creating probability distributions. This analysis was completed using R 3.5.0 (R Core Team 2013), the *sm* (2.2-5.6; Bowman and Azzalini 2018) package. These functions test for equality between density curves, using statistical bootstrap resampling with 100,000 permutations for convergence to evaluate the statistically significant differences among the habitat variables among rivers. Furthermore, these physical habitat layers were transformed to represent the total length of each physical habitat class comprising the rivers (Figure A2). This transformation has two advantages: 1) it condenses the total sample size of the datasets from >40,000 total data-points to several hundred total data-points to more clearly ascertain meaningful, rather than sample-size driven, statistical differences between the rivers; and 2) it places the channel representation into a context favorable for estimation of mussel declines over a given length of river. After comparing the physical habitat differences of the Big River to the Meramec and Bourbeuse rivers, the river most similar to the Big River was selected as the baseline reference for subsequent analyses.

Second, the location and quantity of species rich mussel beds was compared between the selected reference river and the Big River to determine whether mussel beds were present in similar habitat among systems. High-richness mussel beds were identified using information received from the Missouri Department of Conservation, defined as ≥ the 50th quantile from quantile regression analysis investigating the relationship between mussel bed species richness and river size; beds greater than the 50th quantile were considered ‘high richness’ beds given the stream size in which they were found (Key et al. 2021). Use of the quantile regression rather than a ‘cutoff’ richness value allowed the elimination of the effects of river size on species richness, which was positive for this system (Key 2019; Key et al. 2021). Analysis of variance (ANOVA) and chi-square (X^2) statistical tests of significance were performed on the mussel beds and the habitat characteristics of occupied reaches.

Third, analyses evaluated whether total ammonia and nitrogen (N) concentrations were different between the Big River and the Bourbeuse River. The Meramec River was excluded from this portion of the analysis to streamline workload; the justification was, after completion of the first and second objectives, sufficient evidence indicated that the Bourbeuse River was the closest in physical characteristics with the Big River, though all three adjacent rivers overlap significantly in physical characteristics (see Results). Nitrogen is a common contaminant that affects freshwater mussels (Wang et al. 2007a; Wang et al. 2007b). Nitrogen-related impacts is as an alternative cause for mussel declines in the Big River system due to agricultural activities in its watershed. Similar concentrations of ammonia and nitrogen in the Bourbeuse River compared to the Big River (in combination with the analyses above) would further support the suitability of the Bourbeuse River as a reference system, while greater concentrations in the Bourbeuse River would make its use as a reference for other impacts (e.g., lead contamination) conservative.

Effluent discharge information from wastewater treatment plants was used to compare the two rivers (MoDNR 2007). The facilities were stratified by their discharge capacity and total loads of ammonia (as N in kg/day and concentrations in mg/L) were compared (Figure A3). Freshwater mussels are particularly sensitive to ammonia exposure (Wang et al. 2007a, Wang et al. 2007b). Ammonia is also a common pollutant from municipal and agricultural wastewater discharges and is routinely monitored by regulatory authorities through the federal Clean Water Act National Pollution Discharge Elimination System.

Therefore, as a routinely monitored pollutant with demonstrated high levels of toxicity to mussels (Wang et al. 2007a, Wang et al. 2007b), ammonia is an important indicator of water quality threats unrelated to heavy metal mining. Unfortunately, ammonia input from agricultural activities in these watersheds were not available for analysis; however, the Bourbeuse River has a higher percentage of agriculture dominating the watershed (33% versus 17% in the Big River), suggesting estimates of occupancy of fundamentally suitable habitat based on the Bourbeuse River would again be conservative for the Big River if agricultural activities affect occupancy.

Habitat summary and quantification of declines: Using the fundamental habitat model results (Key and Lindner 2017; Key et al. 2021), suitable and unsuitable reaches for mussel occupancy were delineated for 195 km of the Big River, 127 km of the Bourbeuse River, and 118 km of the Meramec River. Next, using the river with channel characteristics most similar to the Big River, the percent occupancy of fundamentally suitable reaches by high-richness mussel beds was calculated and used to estimate mussel declines in the Big River (see Diagram; MDC mussel data; Key 2019; and Key et al. 2021). For this, we use high-richness mussel beds defined as ≥ 50th quantile from a quantile regression analysis describing the relationship between river size (i.e., watershed area) and estimated species richness for all surveyed mussel beds. This allows us to focus only on mussel beds that represented the highest richness in comparison to other concentrations of mussels at similar river sizes (Key et al. 2021). This percent occupancy of fundamental habitat for high-richness mussel beds in the reference river serves as a conservative estimate of occupancy expected in the Big River to determine estimates of mussel bed loss. The estimate is conservative as it represents only estimated loss of high-richness mussel beds and does not account for mussel beds of various densities with species richness below the 50th quantile - or scattered animals that may or may not be in other habitats, where they can persist for long periods. We estimate mussel declines by scaling the percent predicted occupancy of fundamentally suitable habitat identified in the Big River to the percent observed occupancy of suitable habitat in the selected reference river. This scaling was calculated as, $(1- (\%BIG_{OCC}/\%REF_{OCC}))*100$, where $\%BIG_{OCC}$ and $\%REF_{OCC}$ are the percent of fundamental habitat occupied by high-richness mussel beds over the entire length of Big River and the selected reference river, respectively. Using this modeling approach, the percent of mussel decline is then represented as a conservative, model- an observation-based estimate of the % length (km) and area (km^2) of the total river delineated unoccupied by high-richness mussel beds (see Diagram).

Results:

Determination of reference river reaches: In determining which system in the Meramec River Watershed best served as a reference for the Big River, we conducted analyses to answer four key questions: 1)

Which of the rivers unaffected by heavy metal contamination (the Bourbeuse or the Meramec River) was most similar to the Big River in available habitat? 2) For the most similar system to the Big River, do high-richness mussel beds occupy habitat in a similar manner? 3) Which of the rivers are most similar to the Big River in fundamental habitat quality and amount? 4) Does the most similar system to the Big River have fewer overall human impacts, indicating it may lead to overestimation of impacts when used as a reference?

Channel characteristics between the Big and Bourbeuse rivers are descriptively similar in 5 of the 6 binary classified spatial variables important in the fundamental habitat model, excluding bluff adjacency (Question 1 above; Figure 3). Meramec River reaches, in contrast, belong to a different population of physical channel characteristics in at least 4 of the 6 variables in the fundamental habitat model (channel stability, distance to gravel bar, stream power, and water availability). Distributions of channel characteristics are also similar between the Big and Bourbeuse rivers, with mirrored shape profiles in all 3 of the spatial datasets, again excluding the bluff adjacency metric (Figure 4). The Meramec River is of a different population in 2 of the 4 spatial variables. The interquartile ranges of the channel characteristics for the three rivers overlap (Figure A4). Bootstrap equality tests of paired comparisons of length distributions from channel characteristics of the three rivers show the Big and Bourbeuse rivers fall within the same equality band nearly 100% of the time (Figures A5 – A10). ANOVA tests for equality from the bootstrap permutations show that the Bourbeuse and Big rivers are of the same population in 92% of tests, while the Meramec River is from a different population than the Big and Bourbeuse rivers in 54% of tests (Table 2). The Bourbeuse River was therefore identified as the best available reference to the Big River based on its similarity in available physical habitat, in particular, features important in the fundamental habitat model.

To further test this conclusion, we determined if high-richness mussel beds remaining in the Big River used habitat variables included in the fundamental habitat model in a similar manner as mussels in the Bourbeuse River (Question 2). The Big River has six mussel beds that are considered high-richness, while the Bourbeuse River has 33 high-richness beds (Key 2019; Figure A11). Mussel beds occupy physical channel characteristics in the Big and Bourbeuse rivers in a similar manner, for both the continuous and binary spatial datasets for physical channel characteristics in the fundamental habitat model (Figure A12; A13). In other words, no statistically significant difference was observed between the two rivers in either available habitat or occupied habitat that would indicate that the Bourbeuse River is an inappropriate reference for comparison with the Big River (Table 3).

Interquartile ranges of habitat suitability scores (a measure of fundamental habitat quality, Question 3 above) from the suitability model spanning the full length of the Meramec, Big, and Bourbeuse rivers overlap with each other (Figure A14). The Bourbeuse River interquartile range is contained completely within the Big River interquartile range. The median (0.45) and mean (0.4) values for the habitat suitability scores for all three rivers are nearly identical. From the continuous habitat suitability scores for the Meramec, Big, and Bourbeuse rivers, the Big and Bourbeuse rivers have more similar distributions to each other relative to the Meramec River (Figure A15). Finally, overall, the Big River has shorter reaches of unsuitable habitat (Figure 6a). Paired comparisons of unsuitable habitat show that the distributions for the Meramec and Bourbeuse rivers and the Big and Bourbeuse rivers are not statistically different (Figure A16a; A16c). The Big River has statistically significant differences (p-value 0.01) in unsuitable habitat lengths when compared to the Meramec River (Figure A16b). Paired comparisons show that the Meramec and Bourbeuse rivers do not have statistically significant differences in suitable habitat (p-value 0.2; Figure A16d). The distribution of reach lengths of suitable habitat on the Big River are skewed towards greater lengths relative to the distributions of the Bourbeuse (p-value 0.001) and Meramec (p-value 0.003) rivers (Figure 6b; Figure A16e; A16f).

The Big River, according to the fundamental habitat model, contained a greater amount of habitat occupiable by freshwater mussels in the absence of threats; however, it was most similar to the Bourbeuse River in land use, geology, and hydrology. To answer our final question (Question 4 above), we evaluated whether ammonia concentrations from wastewater treatment plants may be higher in the Big River than in the Bourbeuse River. Comparing all wastewater treatment facilities, the Bourbeuse River has an average load of ammonia as N of 1.05 kg/day, and the Big River has an average of 0.56 kg/day. Further analysis shows facilities with an average daily discharge >100,000 gal/day have an average daily load of N = 3.83 kg in the Bourbeuse River Watershed and 1.63 kg in the Big River (Figure 5a). The average concentration of ammonia as N discharging from all facilities is 10.31 mg/L across the Bourbeuse River Watershed and 3.85 mg/L across the Big River Watershed. The average concentration of ammonia as N discharging from facilities with daily discharge >100,000 gal/day is 1.94 mg/L across the Bourbeuse River Watershed and 1.87 mg/L across the Big River Watershed (Figure 5b) U.S. Environmental Protection Agency (2013) recommended Aquatic Life Chronic Criteria is 1.9 mg/L ammonia as N at pH 7.0 and 20 degrees C.

Based on results summarized above, we selected the Bourbeuse River as the closest and most conservative available reference for estimating expected mussel bed occupancy in the Big River, specifically because of the following lines of evidence: 1) The Bourbeuse River was similar to the Big River in hydrological and geomorphological characteristics known to be important for the presence of mussel beds; 2) Mussel beds in the Big and Bourbeuse rivers used available habitat in a similar manner; 3) Based on the fundamental habitat model, habitat scores and amount were similar or higher in the Big River than the other two river systems; and 4) General human impacts that could affect mussels, but unrelated to mining, were similar or less in the Big River than the other two river systems.

Estimation of Suitable Reach Lengths and Mussel Declines in the Big River using the Bourbeuse River as a Reference: The fundamental habitat model for mussel beds, when applied, delineated river reaches in the Meramec River Watershed as either fundamentally suitable for the presence of high-richness mussel beds or unsuitable (for spatial delineation of these habitats, see Key and Lindner 2017). The length of the Bourbeuse River channel included in the fundamental habitat model is 127 km, of which 72 km (57%) is considered fundamentally suitable for mussel beds. The total area of river modeled for the Bourbeuse River is 5.63 km^2, of which 3 km^2 (53%) was classified fundamentally suitable and 2.63 km^2 unsuitable. Of the 195 km of river channel modeled on the Big River, 130 km (67%) was classified as fundamentally suitable mussel habitat with the remainder unsuitable. The total area of channel modeled was 7.82 km^2, with fundamentally suitable habitat comprising 64% (5.03 km^2) of the reach. Average reach length of suitable habitat in the Big River was 1.3 km and 0.8 km in the Bourbeuse River.

Of the total length (72 km) of suitable habitat in the Bourbeuse River, 32% (23 km) of that length is composed of discrete reaches that are occupied by high-richness mussel beds. For the Big River, only 9% (12 km) of the total length (130 km) of suitable habitat is occupied by high-richness mussel beds (Key 2019; Roberts et al. 2016). Applying a 32% occupancy rate of suitable habitat from the Bourbeuse River as a reference, this equates to a 72% reduction in the occupancy of suitable habitat by high-richness beds in the Big River (Roberts et al. 2016; Key 2019). In other words, of the 130 km of fundamentally suitable habitat in the Big River, 42 km (32%) contains high-richness beds, while, in actuality, only 12 km (9%) of the suitable reaches contain high-richness mussel beds (Roberts et al. 2016; Key 2019). This equates to an estimated loss of at least 30 river km of species-rich mussel beds in the Big River and a 72% loss of high-richness mussel beds in the system. Other fundamentally suitable reaches in the Big River might also be predicted to contain freshwater mussels; however, this conservative estimate is focused only on the loss of the highest-richness mussel beds in the system (Key 2019).

According to studies by Roberts et al. (2016), marked declines in mussel densities and species richness have been observed within the Big River directly downstream of historic mining operations (downstream of river km 170.5 at Leadwood, MO). Mussel densities negatively corresponded to sediment lead concentrations exceeding the PEC, resulting in an estimated 70-75% decline in mussels within contaminated reaches of the Big River. If we apply reference conditions to just those areas of the Big River most contaminated with lead, in other words, downstream from Leadwood, MO at river km 170.5 to 16.5, we obtain a slightly different estimate of impact. For that length of the Big River, the fundamental habitat model delineated 104 km (3.94 km^2) of fundamentally suitable habitat, of which 12% is occupied by high richness mussel beds. Using the 32% occupancy rate of the Bourbeuse River as baseline, we estimate 33 river km or an area of 1.26 km^2 of the Big River between river km 170.5-16.5 may be occupied by high richness mussel beds if the system was uncontaminated by lead .

Conclusions:

Results demonstrate the overall suitability of the Bourbeuse River as a conservative reference for establishing predicted presence of mussel beds in reaches of the Big River in the absence of lead. The Big and Bourbeuse rivers are similar in channel characteristics and physical habitats known to be important for the occupancy of high-richness mussel beds in this system (Key 2019). Average reach length of suitable habitat on the Big River is 60% longer than the Bourbeuse and Meramec rivers, and the prevalence of agriculture and the input of ammonia from human wastewater is greater in the Bourbeuse River relative to the Big River. These findings suggest calculations of lost high-richness mussel beds in Big River due to factors other than habitat - using the Bourbeuse River as a reference - are conservative. The Big River has 130 km of fundamentally suitable habitat, of which 104 km is located between river km 170.5-16.5 (Lindner and Key 2017) where lead contamination has adversely affected freshwater mussels (Roberts et al. 2016). Over the length of the modeled river channel on the Big River, we estimated loss of 30-33 river kilometers or an area of approximately 1.26 km^2 of river that, without the effects of lead, would otherwise be occupied by high-richness freshwater mussel beds.

Our estimate of freshwater mussel declines only include what we predict as the most species-rich mussel beds in the river and assume similar impact of human activities other than heavy metal mining (e.g., agriculture and wastewater) and similar habitat quality. In fact, we have multiple lines of evidence suggesting that, in the absence of heavy metal contamination, the Big River may be a higher-quality system that could support more mussel beds and species than the Bourbeuse River. An investigation into the available length of suitable habitat reveals less (53%) in the Bourbeuse River compared to the Big River (67%), when considered as a relative percentage of the modeled domain. Furthermore, the high percentage (83%) of mussel beds in suitable habitat in the uncontaminated portions of the Big River means that mussels are located at higher frequency in the remaining, uncontaminated fundamentally suitable habitat in the Big River. In the Bourbeuse River, high-richness mussel beds are established in only 61% of fundamentally suitable habitat as described by the model.

It is striking that, even with less suitable habitat relative to overall river length, smaller suitable habitat reach lengths, and higher N loads, the Bourbeuse River still contains higher mussel species richness and occupancy of suitable habitats than in the Big River (Key 2019; Roberts et al. 2016), suggesting factors other than poor habitat, agriculture or municipal water quality degradation as potential causative factors of mussel decline. Roberts et al. (2016) and Albers et al. (2016) found degraded mussel fauna in the Big River associated with elevated concentrations of lead. Besser (2010) and U.S. EPA (2020) have conducted laboratory toxicity tests with juvenile mussels that demonstrate a negative response to elevated heavy metals in Big River sediment. Remedial actions to address heavy metal contamination in the Big River that presents risk to the environment, including mussel fauna, are being evaluated by EPA. In addition, the natural resource trustees (U.S. Fish and Wildlife Service and the MoDNR) are considering what restoration opportunities, including potentially mussel reintroduction, are available in the Big River, once remedial action is complete (SEMORRP 2014) . Our results suggests that high quality, unoccupied habitat exists in the Big River, but that mussel restoration may be best contemplated after heavy metal contamination is addressed to avoid failure of restoration efforts. The application of the fundamental model and a spatially-explicit understanding of the fundamentally suitable habitat in these systems may allow for directed restoration activities with a higher potential for success.

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Appendix

Figure A1: Example reach from the Big River showing habitat suitability classes from the habitat suitability model used to quantify injury in this report (Key et al, *In Review*). The habitat suitability classes extend 530 river kilometers for the Big, Bourbeuse, and Meramec rivers.

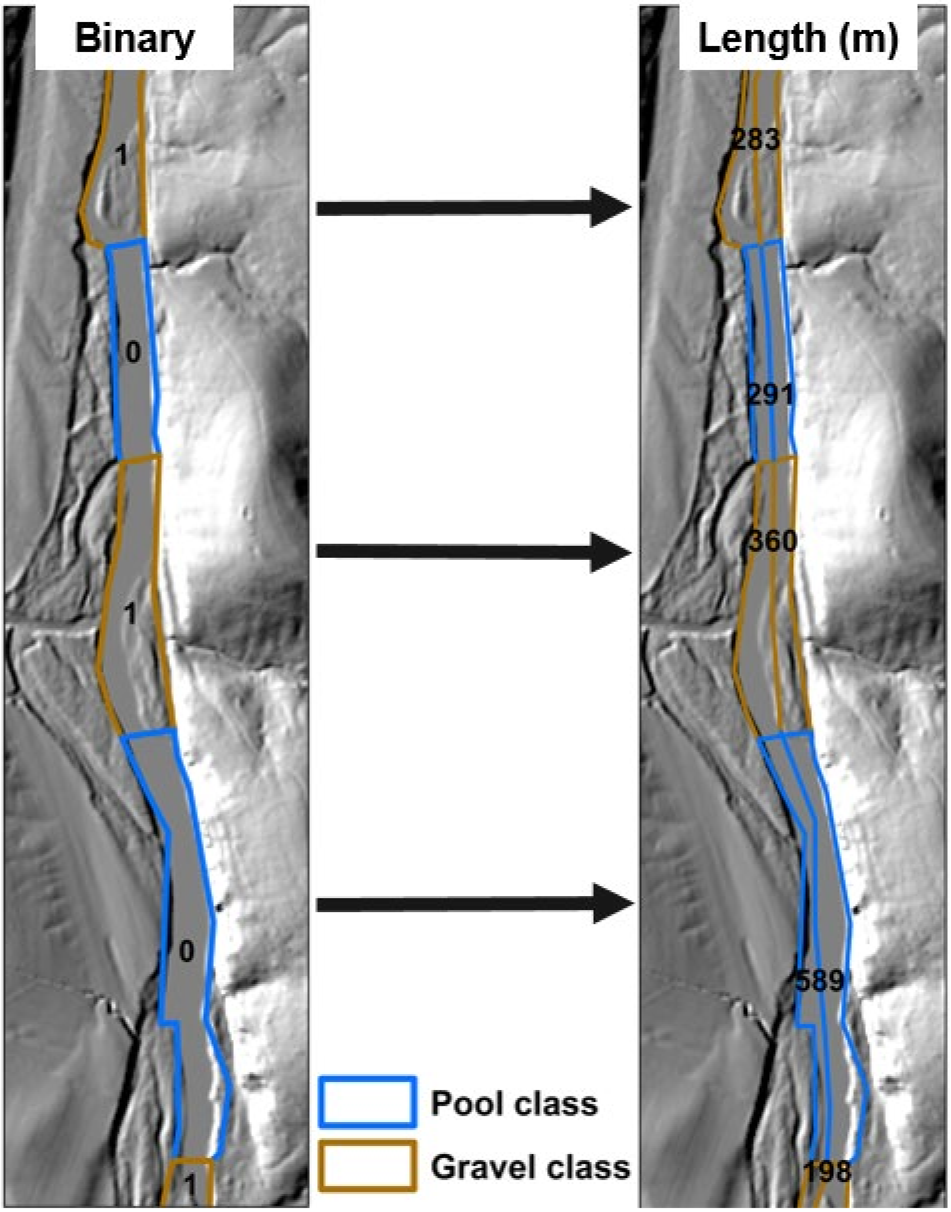

Figure A2: Example of binary channel characteristics (gravel and pool reach class spatial dataset) on the left, and the corresponding length of the reach polygons along the stream centerline on the right.

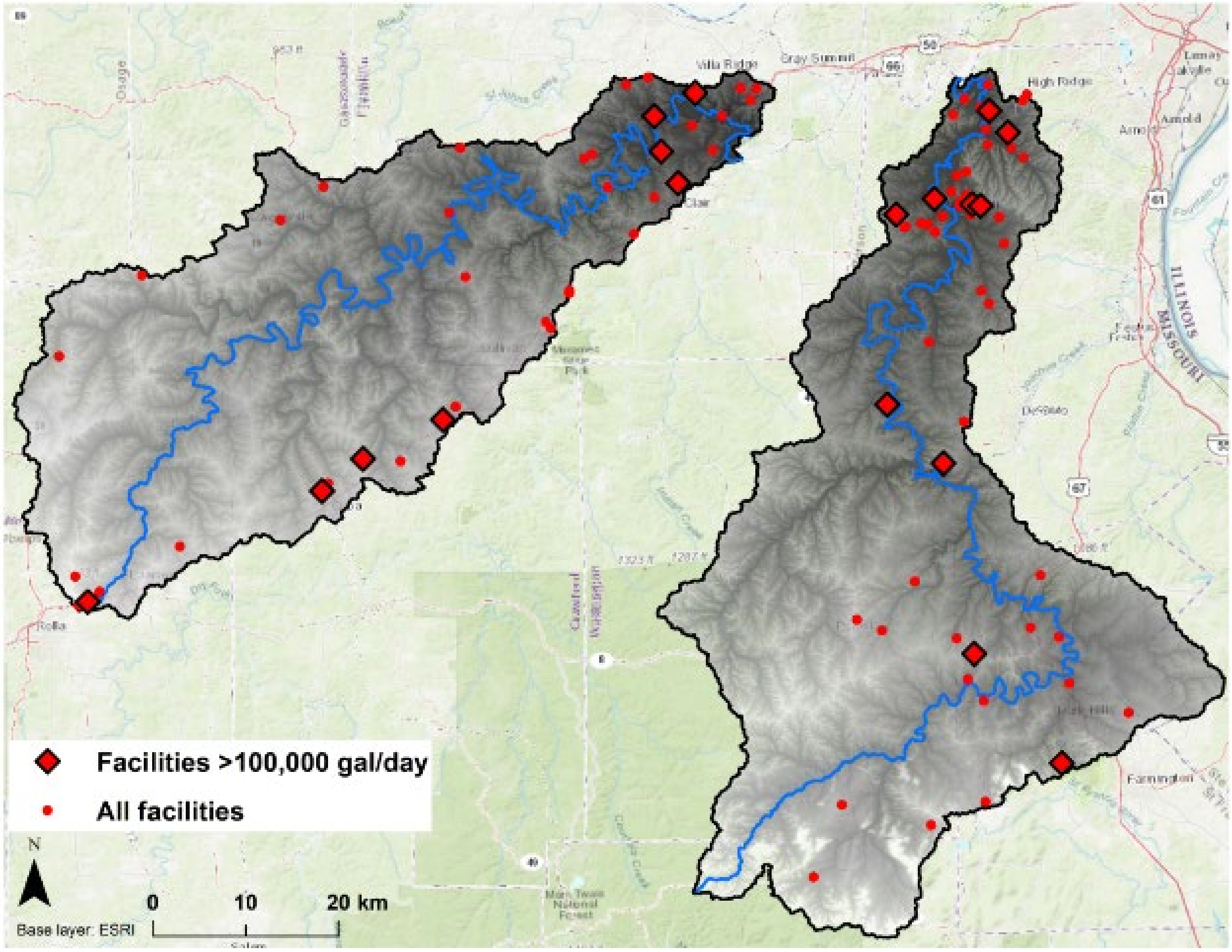

Figure A3: Spatial distribution of wastewater treatment plants in the Big and Bourbeuse river watershed

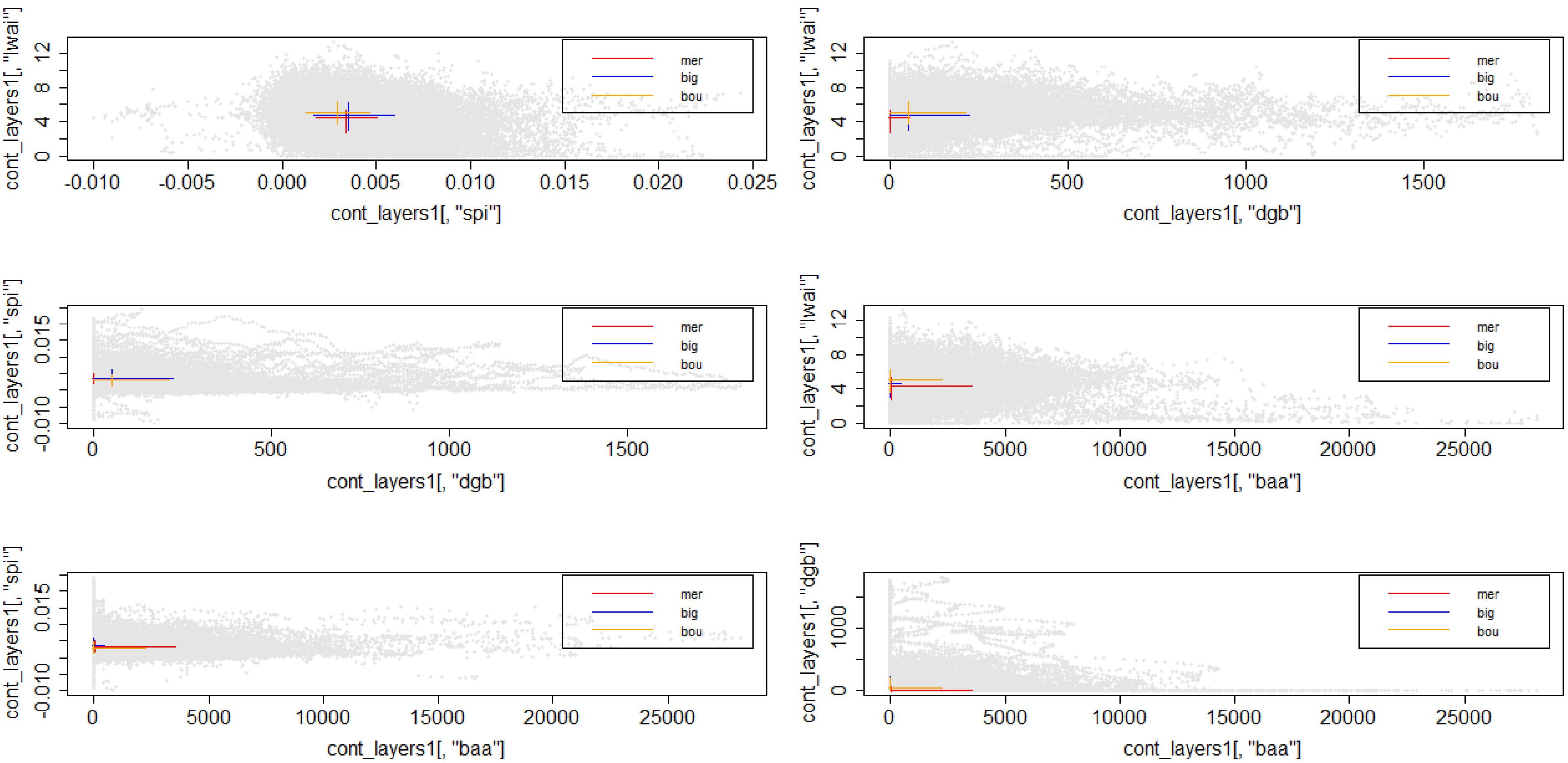

Figure A4: Scatter plots comparing the entire continuous datasets of physical channel characteristics (>40,000 data-points shown in light gray background coloring) with the median and interquartile range plotted as crosshair whiskers for the continuous datasets from the habitat suitability model. This shows that even though we have these vast datasets, the basic statistics between the datasets across all rivers are similar because of the close proximity of the crosshairs.

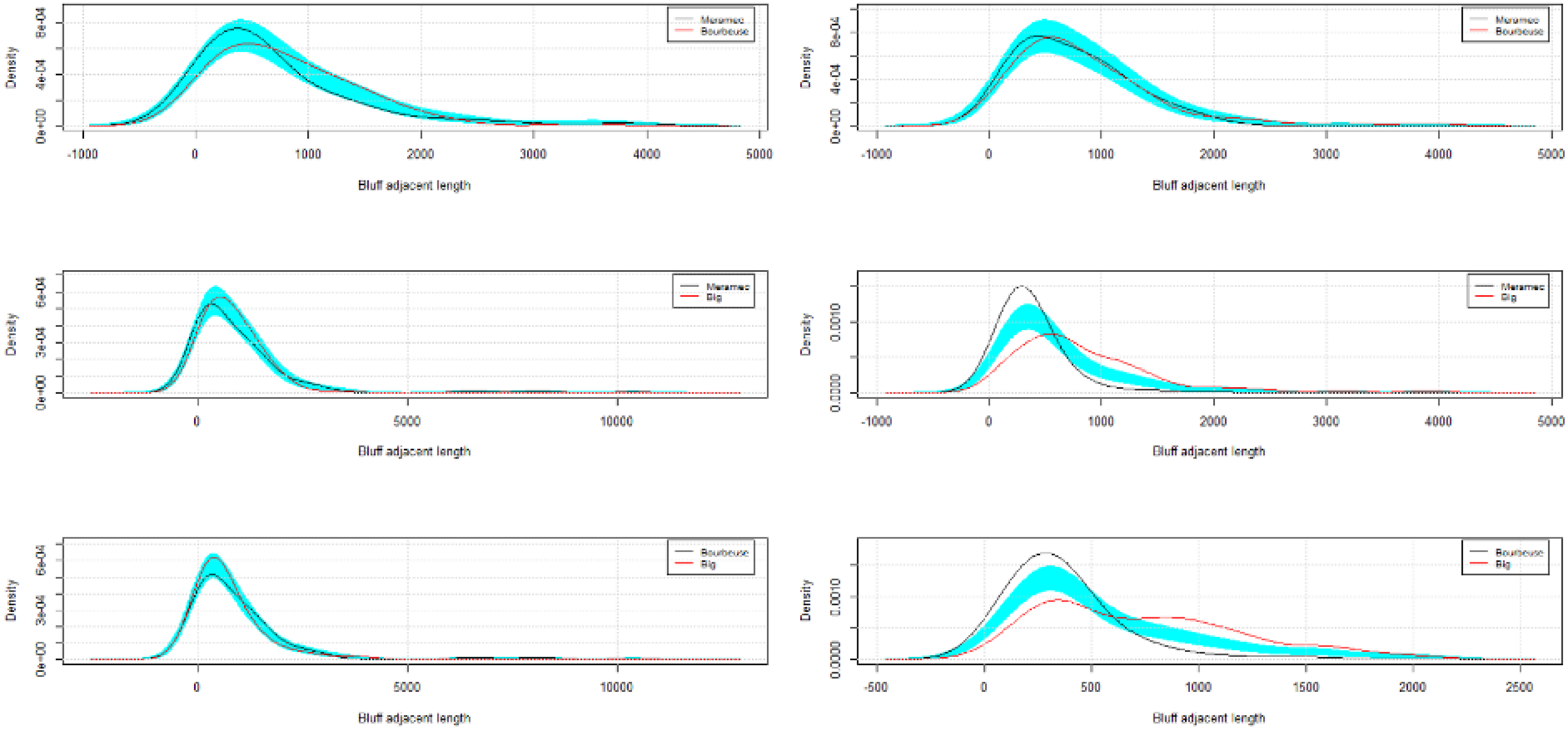

Figure A5: Paired comparisons of the probability density functions of the lengths of the bluff not-adjacent and bluff adjacent reach classes, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. There are between 176 and 252 data-points that comprise the curves.

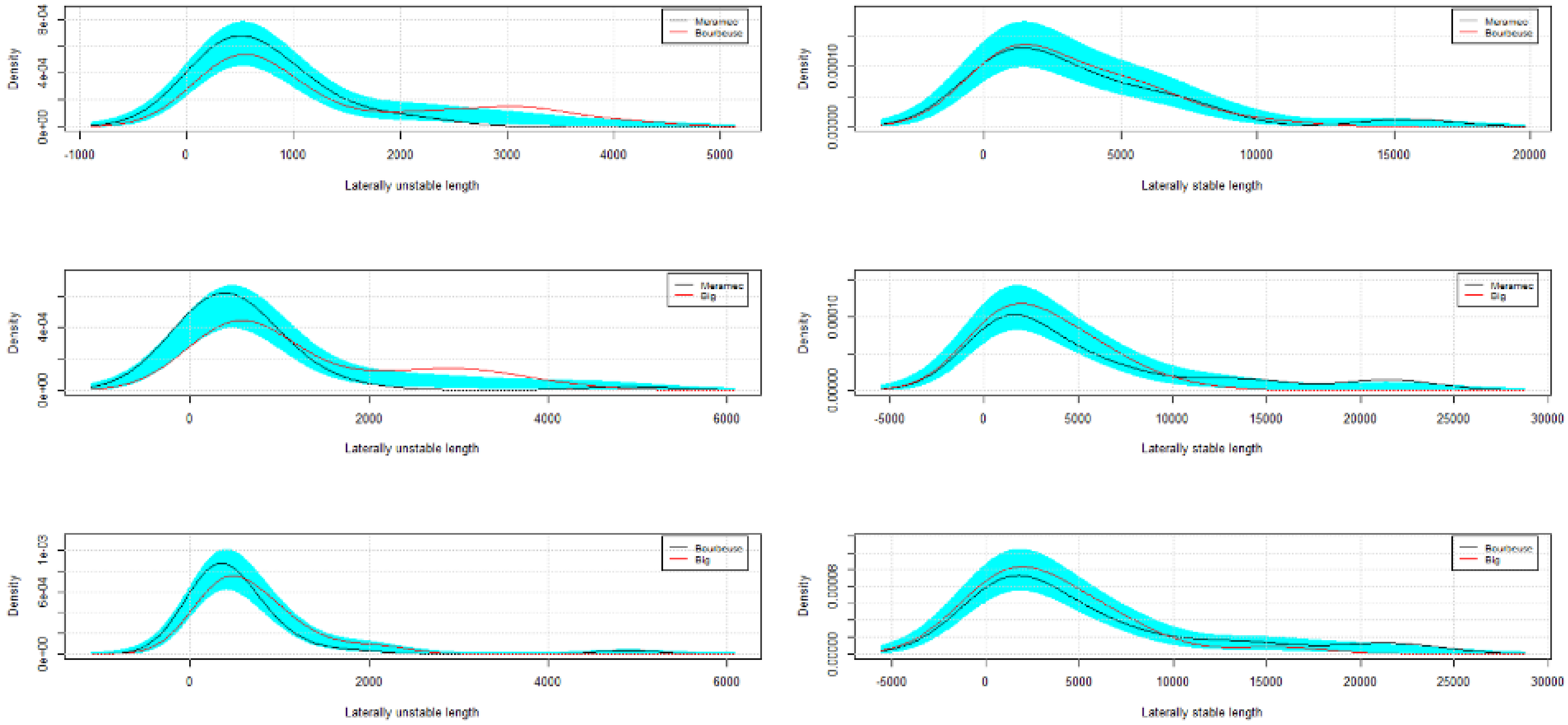

Figure A6: Paired comparisons of the probability density functions of the lengths of the laterally unstable and stable reach classes, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse rivers, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. There are between 58 and 69 data-points that comprise the curves.

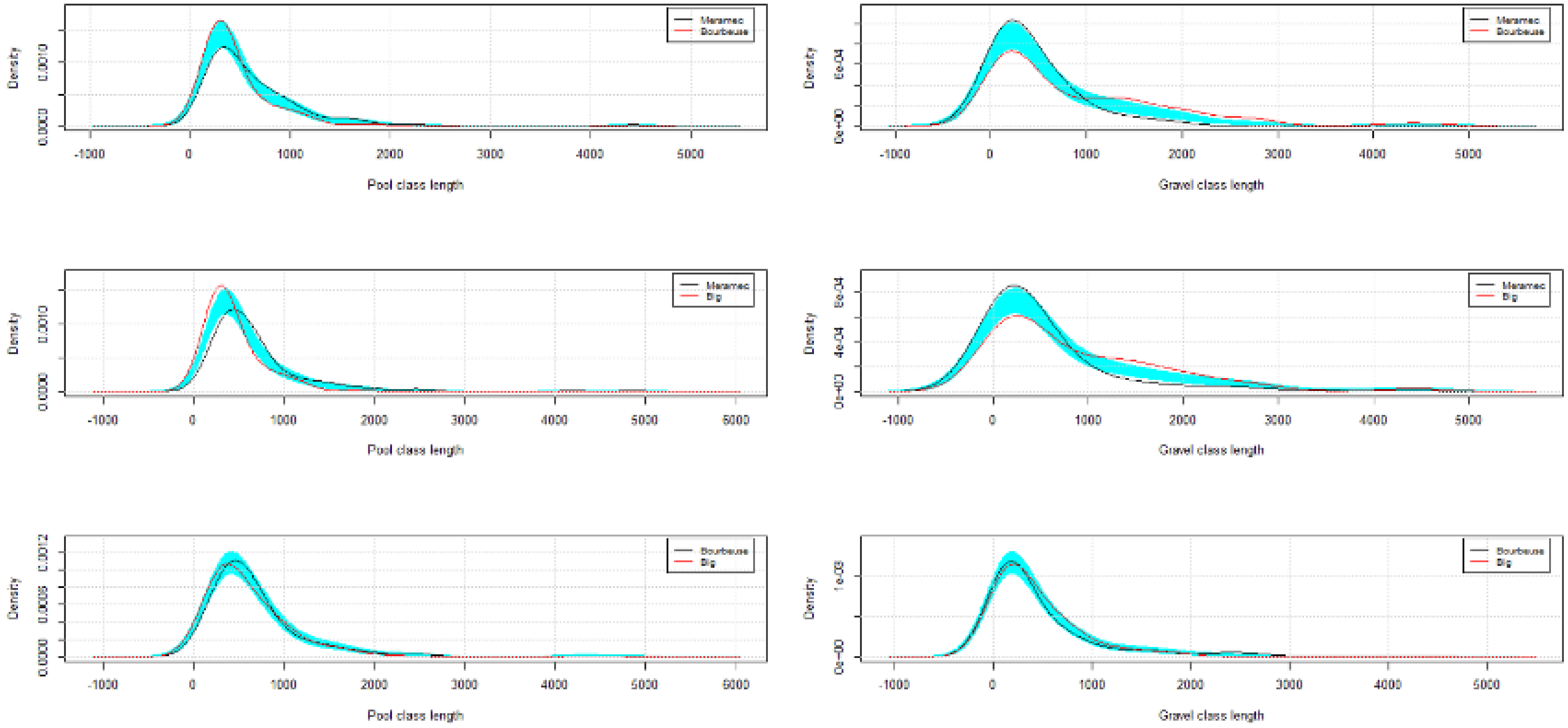

Figure A7: Paired comparisons of the probability density functions of the lengths of the pool and gravel reach classes, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse rivers, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. There are between 235 and 311 data-points that comprise the curves.

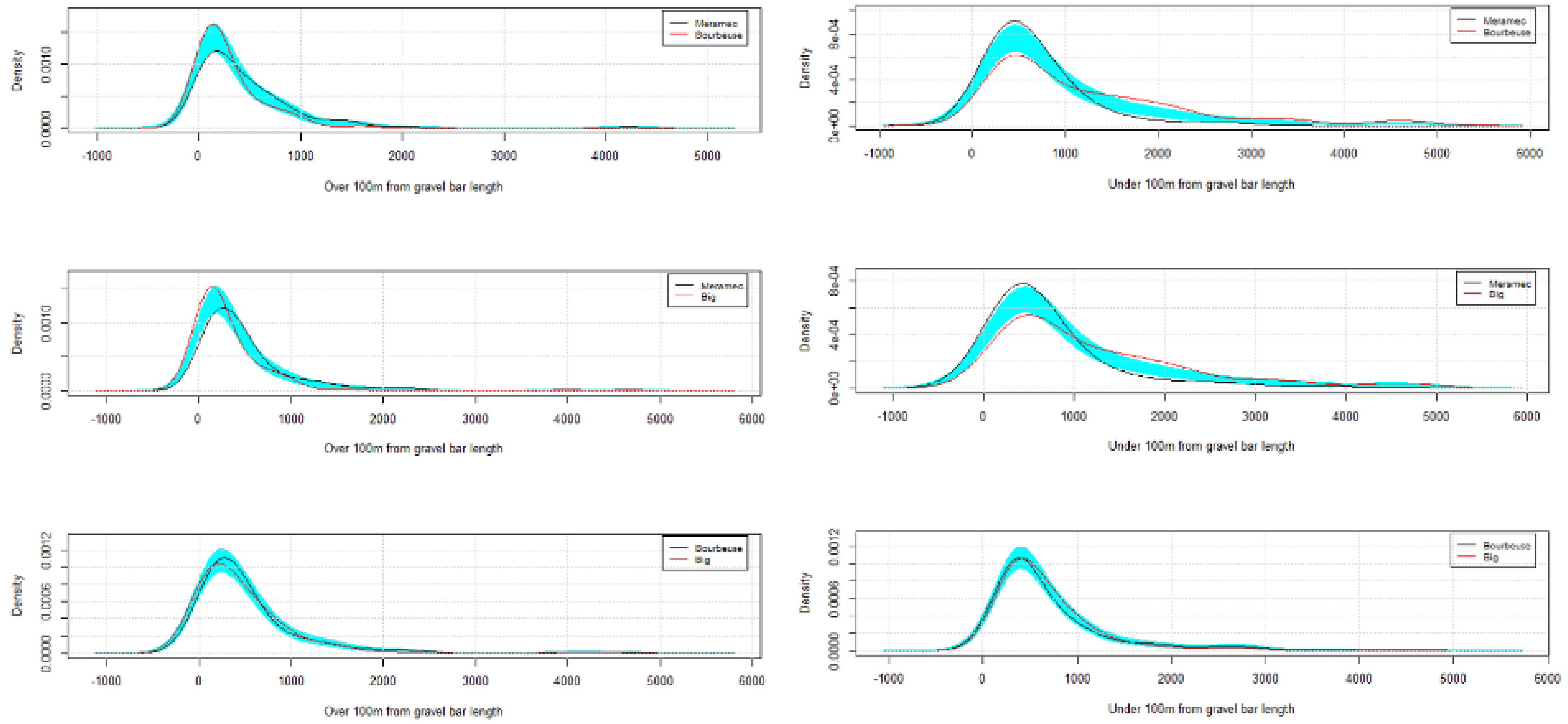

Figure A8: Paired comparisons of the probability density functions of the lengths of the reach classes over 100m and under 100m from a gravel bar, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse rivers, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. There are between 213 and 301 data-points that comprise the curves.

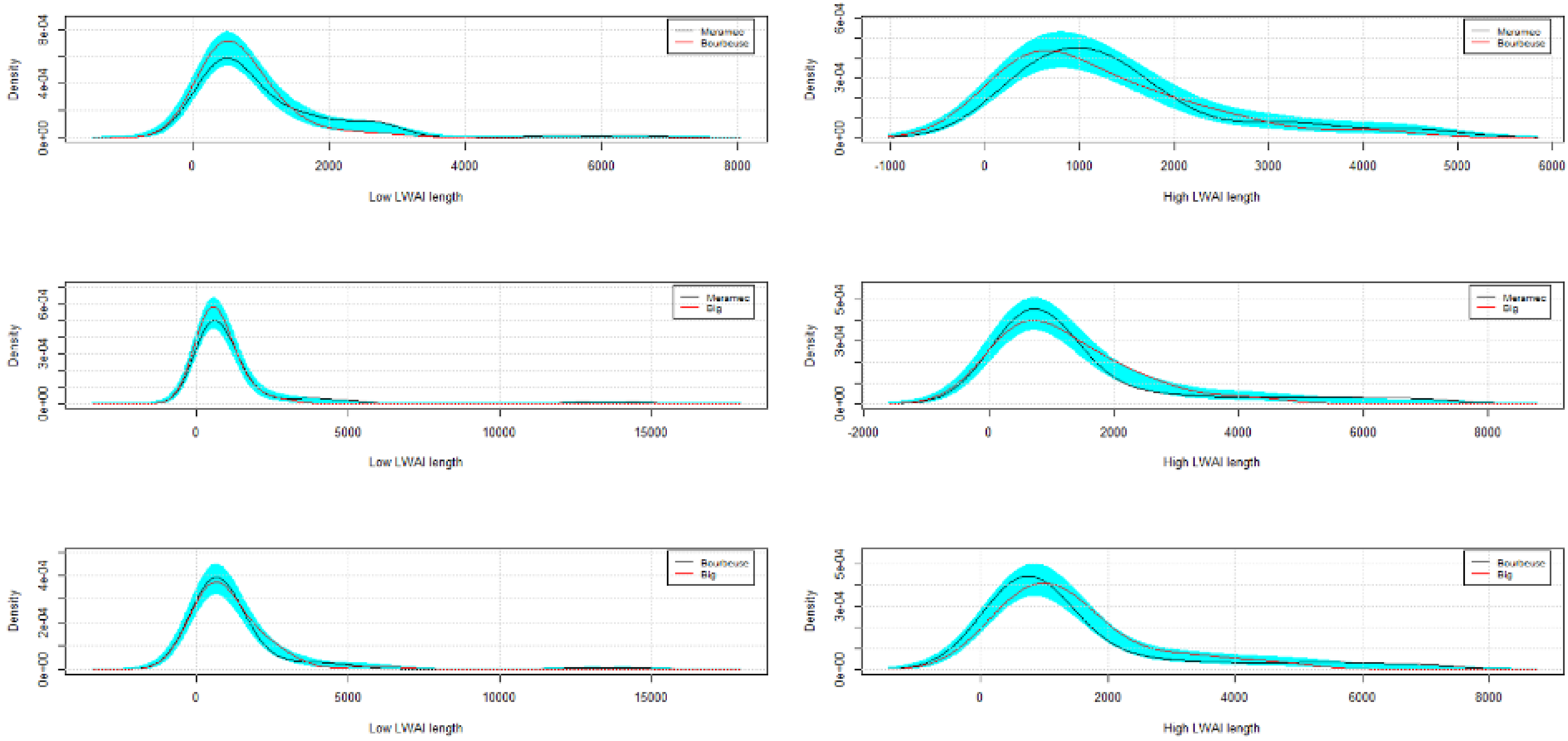

Figure A9: Paired comparisons of the probability density functions of the lengths of the low and high low-water availability reach classes, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse rivers, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. Between 119 and 131 data-points comprise the curves.

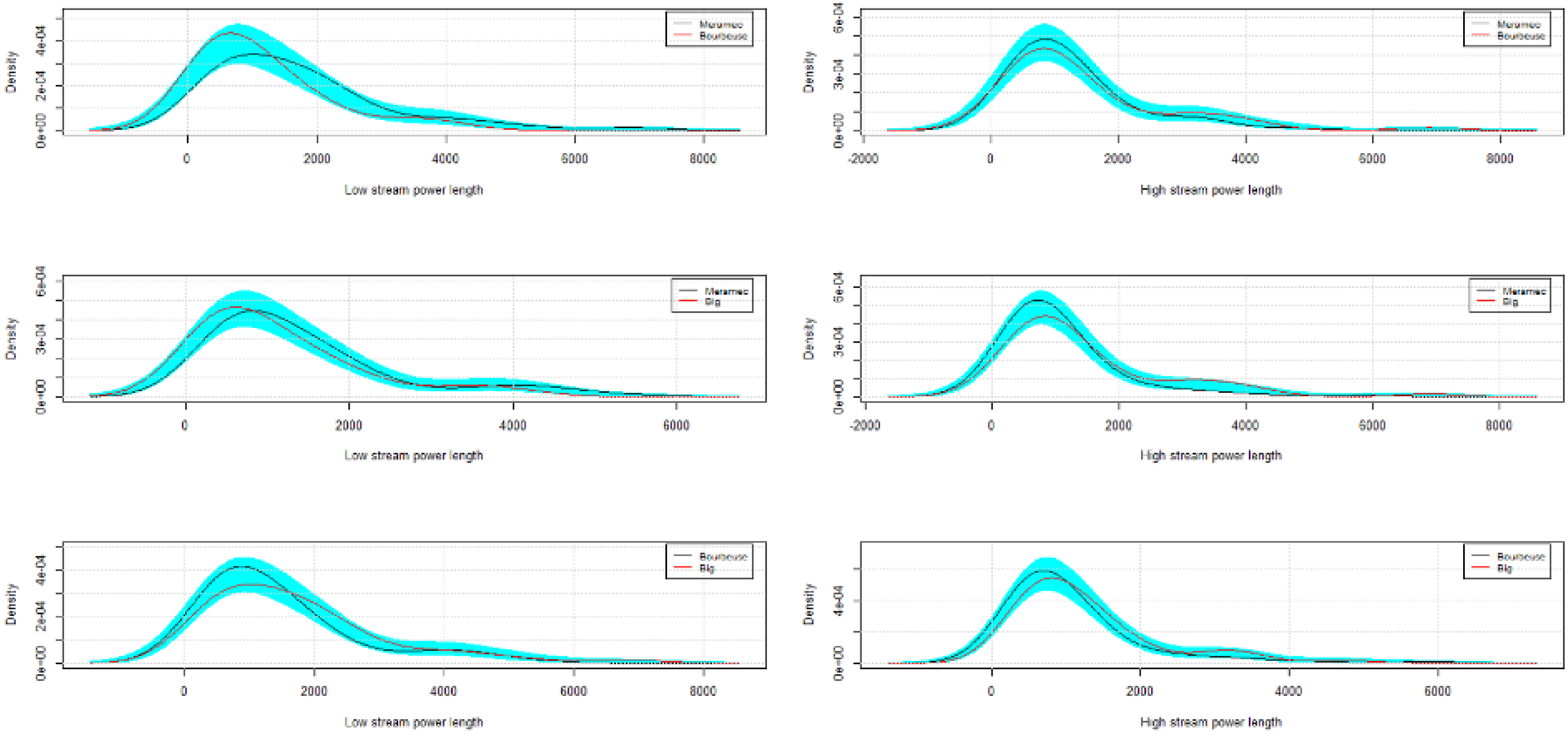

Figure A10: Paired comparisons of the probability density functions of the lengths of the low and high stream power reach classes, with the equality curves shown in blue. ANOVA scores from the bootstrap tests are in Table 2. The two bottom plots are comparing the data between the Big and Bourbeuse rivers, while the two middle and two upper plots compare the Meramec to the Big and Bourbeuse rivers, respectively. Between 95 and 133 data-points comprise the curves.

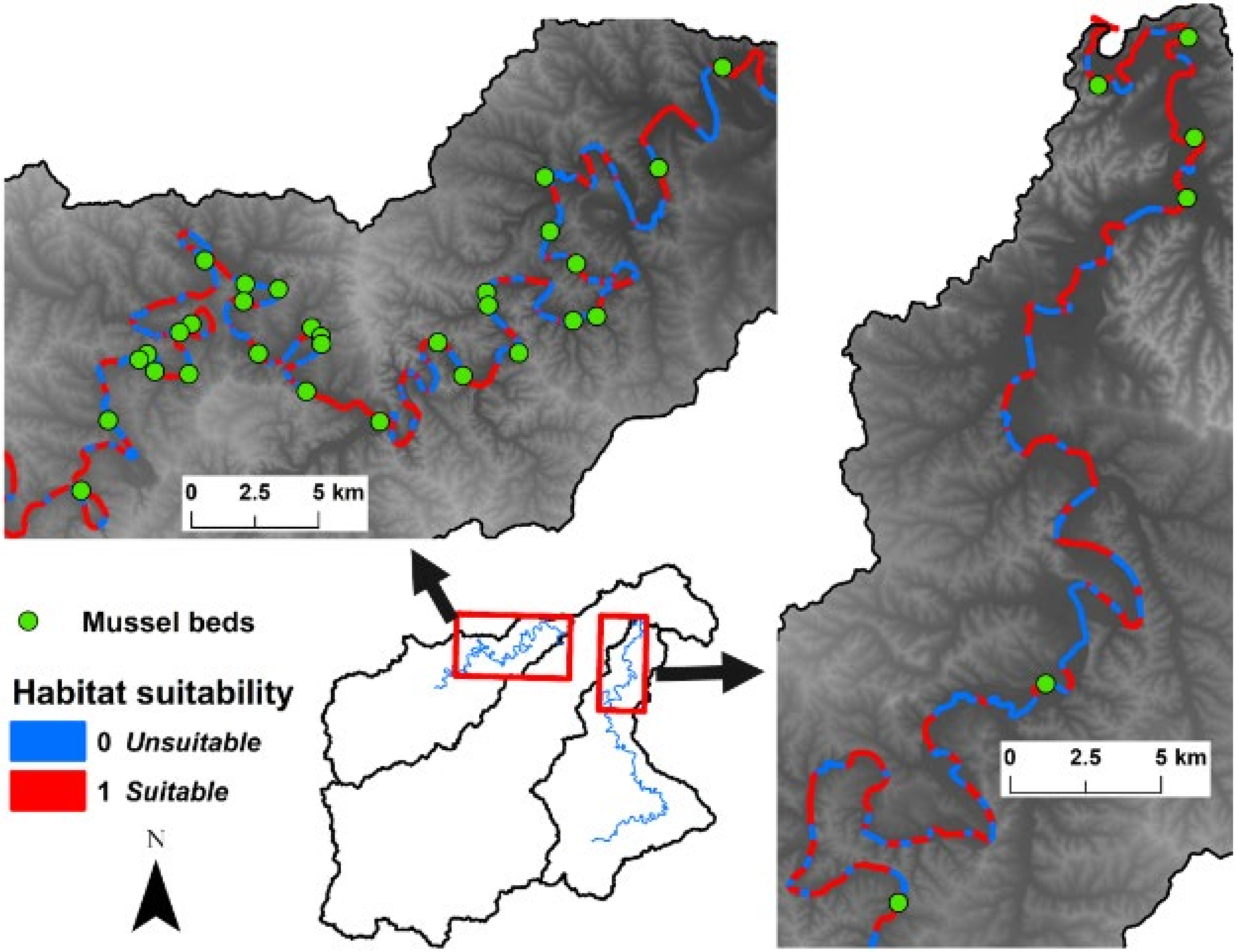

Figure A11: Distribution of mussel beds with species richness greater than the 50th quantile in the Big River (6 beds) and the Bourbeuse River (33 beds).

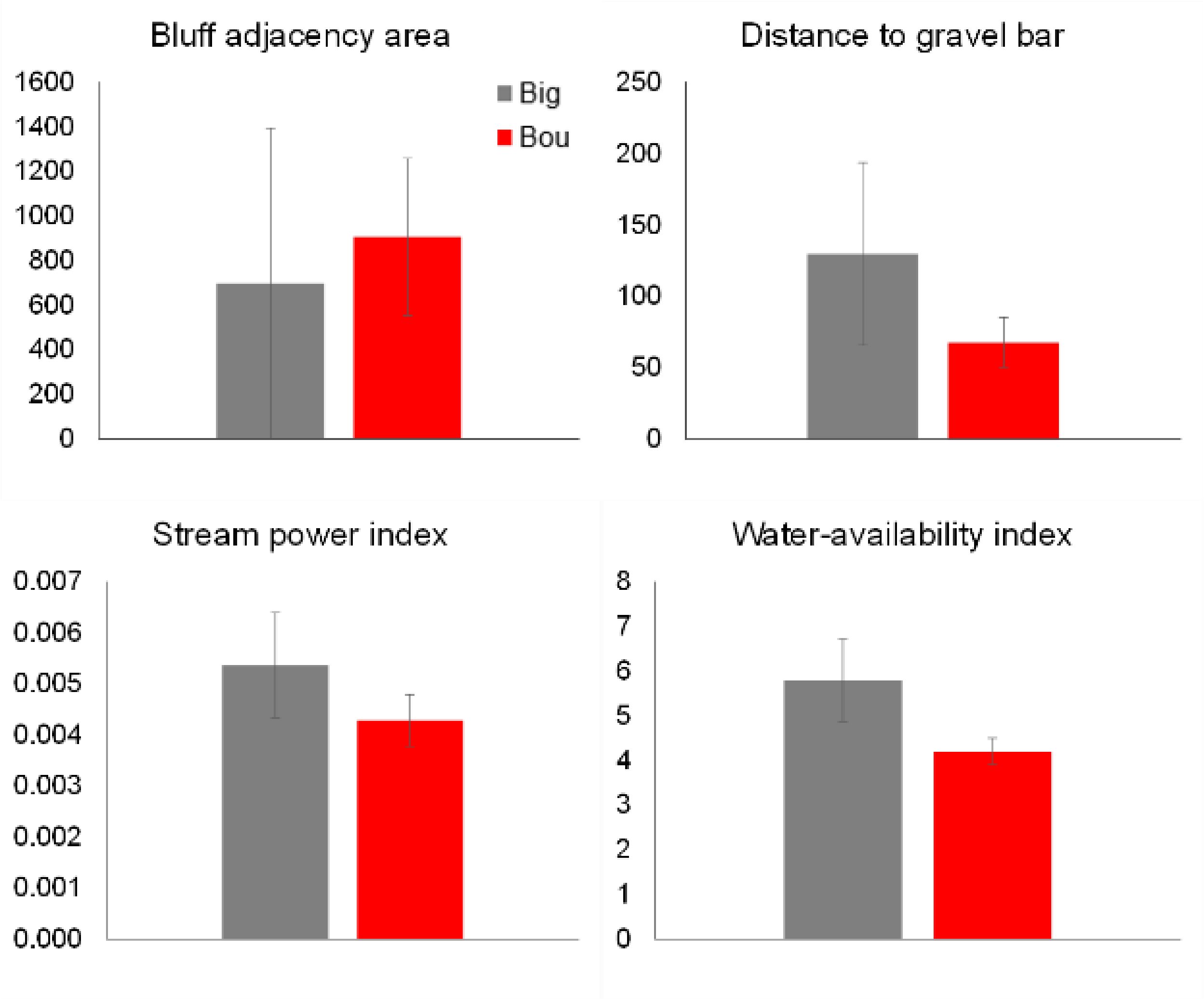

Figure A12: Mussel bed use of the 4 continuous physical habitat spatial datasets for the Big and Bourbeuse rivers, showing standard error bars; n=6 for the Big River and n=33 for the Bourbeuse River.

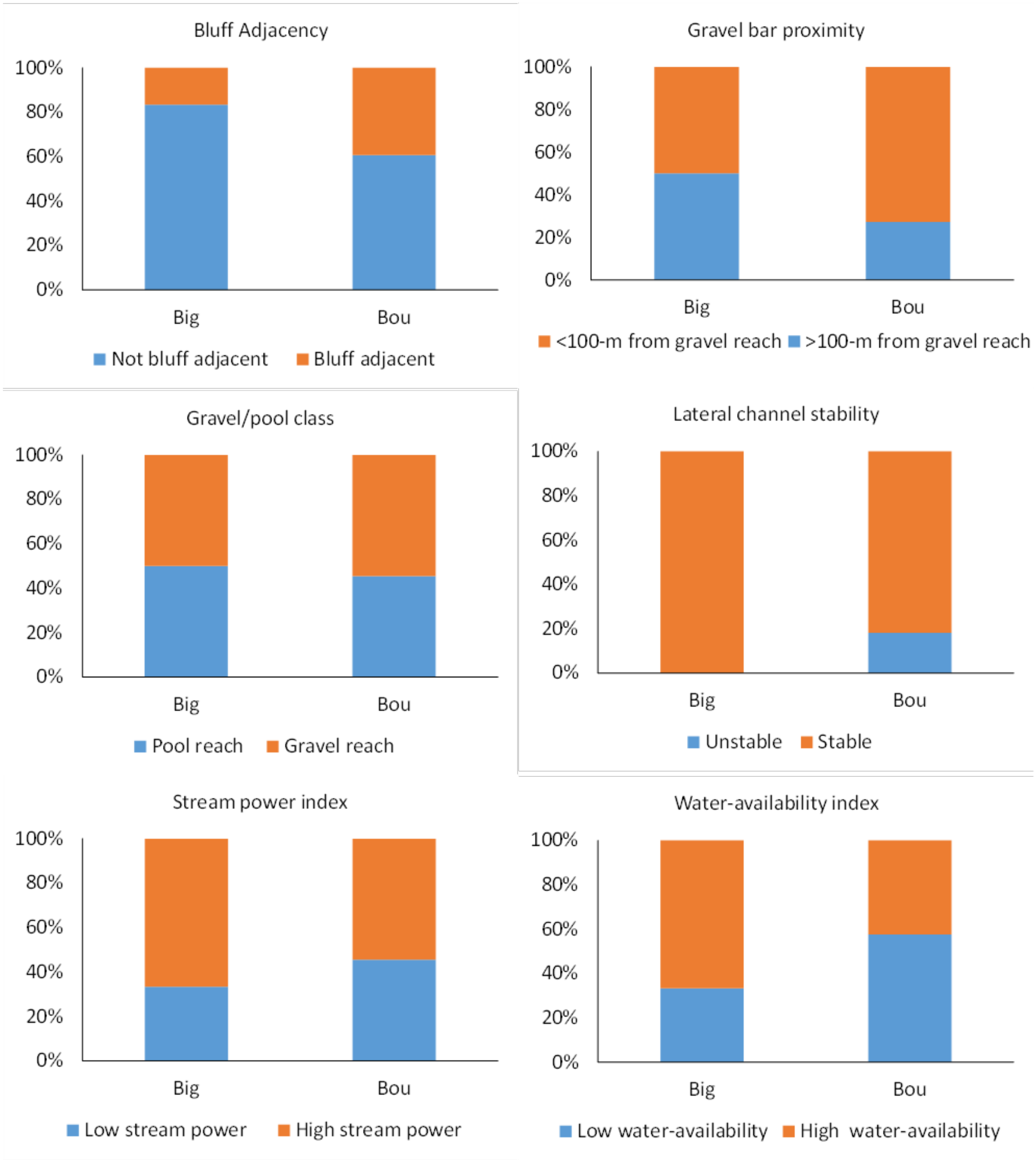

Figure A13: Percent use by mussel beds of the physical habitat characteristic classes of the 6 binary physical habitat spatial datasets in the Big and Bourbeuse rivers; n=6 for the Big River and n=33 for the Bourbeuse River.

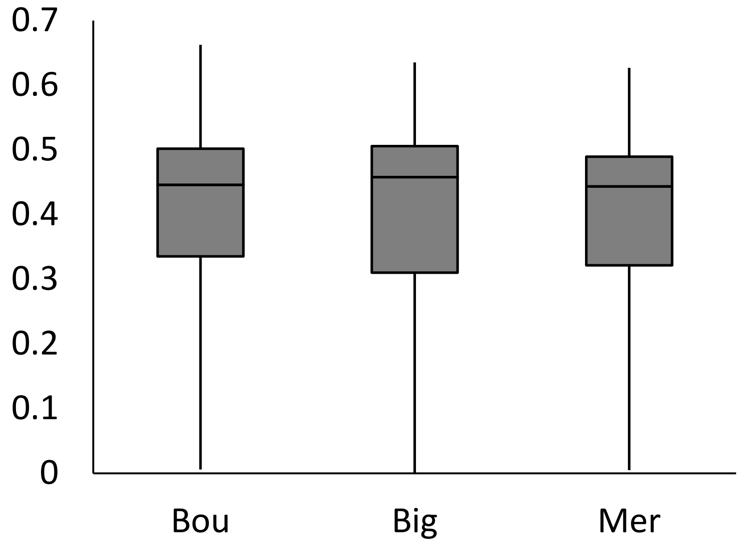

Figure A14: Box and whisker plots (range of data – lines – first and third quartile of data – box – and median – line in box) of habitat suitability scores from the suitability model spanning the full length (n>40,000 data-points) of the Meramec, Big, and Bourbeuse rivers, demonstrating overall similarity in average habitat characteristics among river systems.

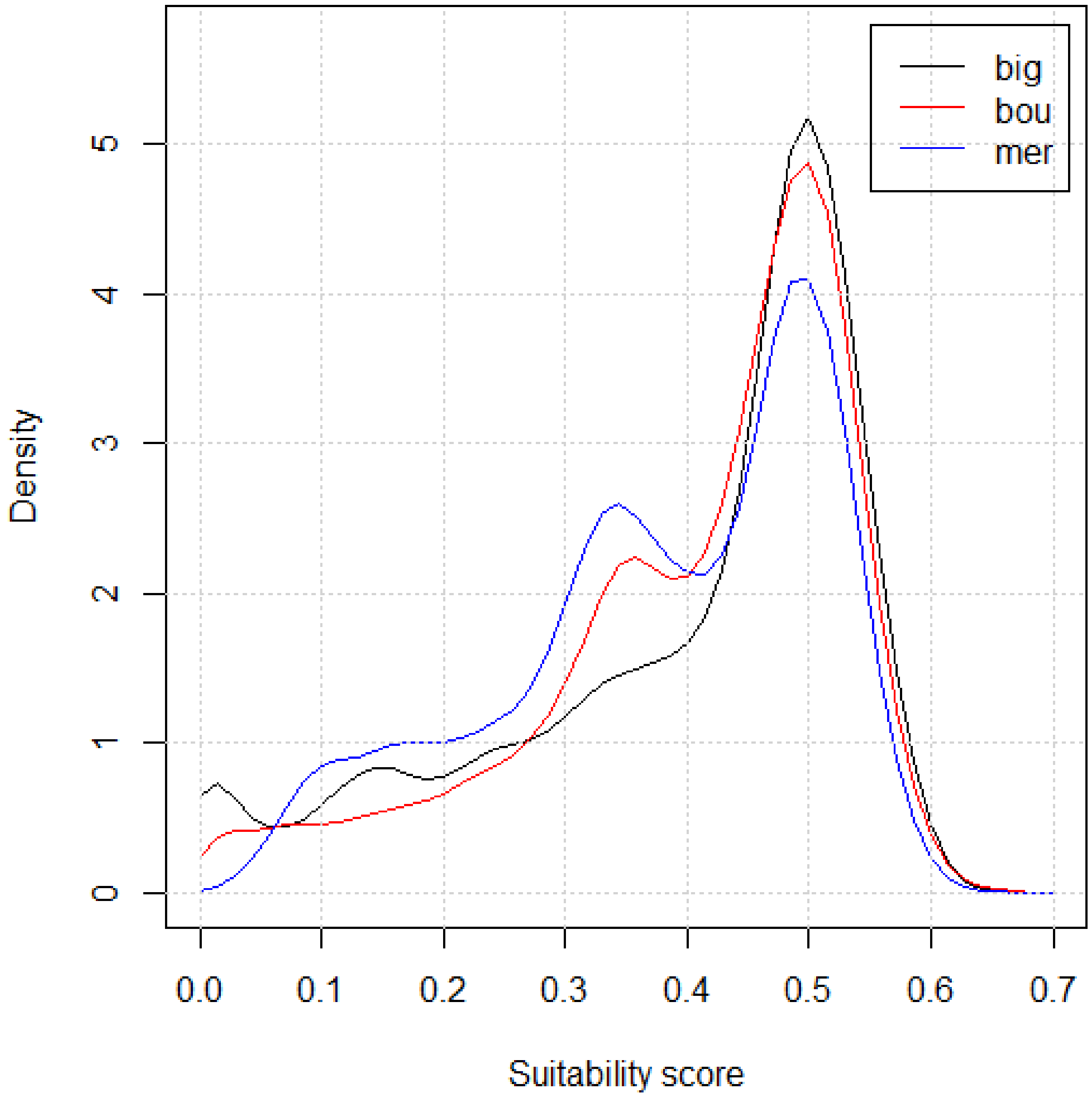

Figure A15: Probability density function of the suitability scores for the Meramec, Bourbeuse, and Big Rivers; n > 40,000 data-points total for the 3 curves.

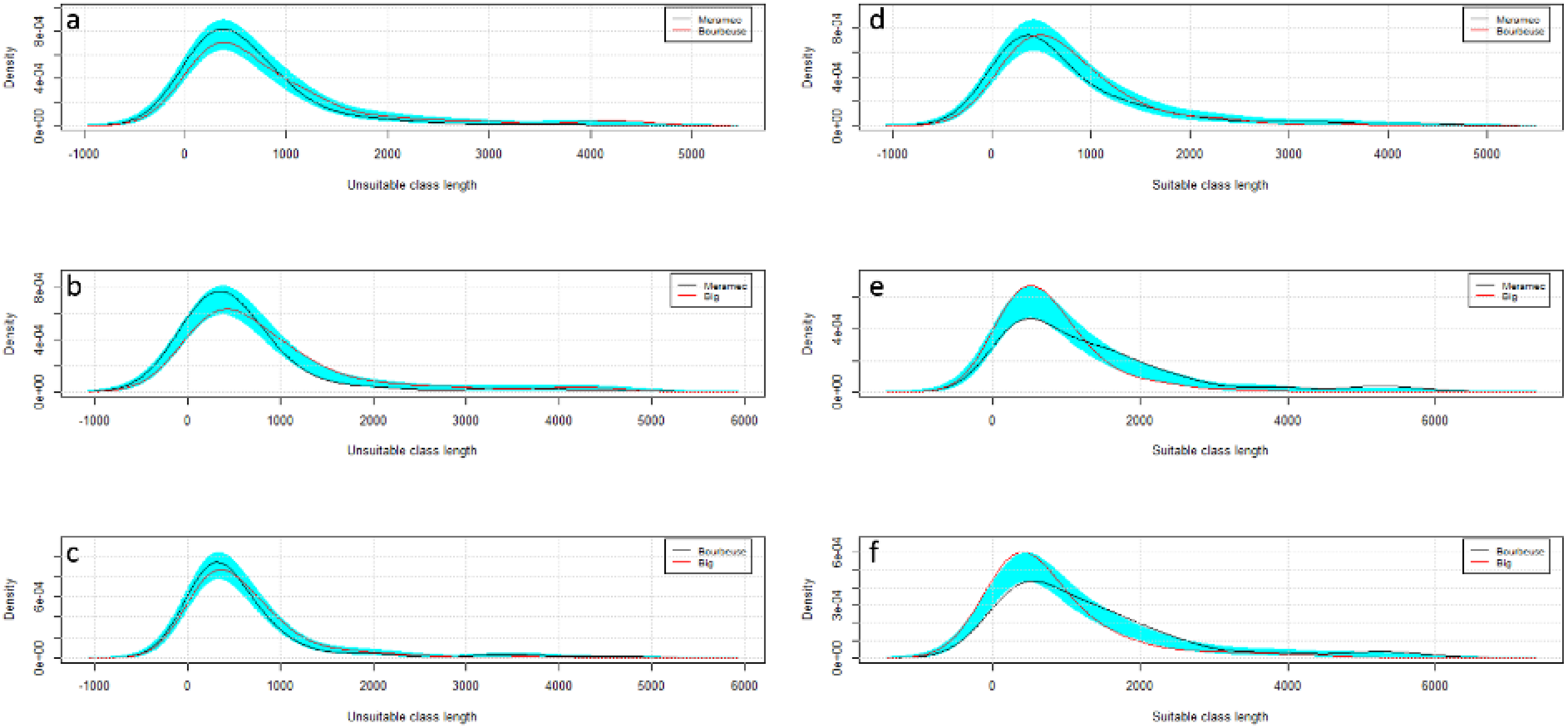

Figure A16: Paired comparisons of the probability density functions of the lengths of the unsuitable and suitable reach classes, with the equality curves shown in blue. Panels e and f show the Big River as having a distribution of suitable habitat distinct (outside the equality band) from the Bourbeuse and Meramec Rivers. In this case, the distinction is that the Big River has longer suitable reaches. Panels b and c show that the Big River has shorter unsuitable reaches than the Bourbeuse and Meramec Rivers. There are between 68 and 99 data-points that comprise the curves.

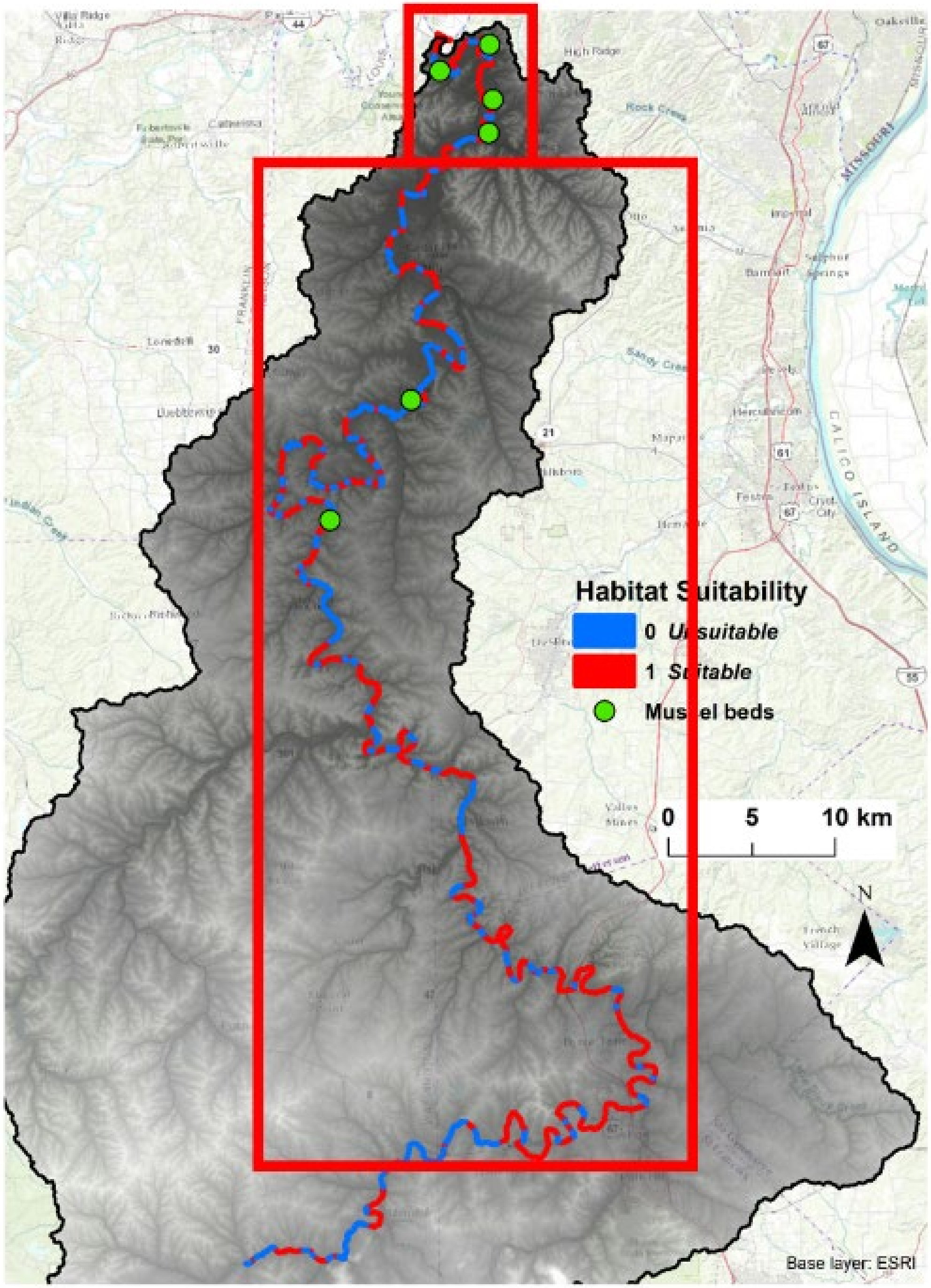

Figure A17: Spatial distribution of high-richness mussel beds in the Big River, showing the approximate boundaries in red boxes of the lower 16.5 km of the river channel that is well-populated with high-richness mussel beds (box at top of map), and the upper 154 km (river km 170.5 to 16.5, box at bottom of map) that is poorly populated with high-richness mussel beds.