

Article

Spawning Habitat Partitioning of Sympatric Salmonid Populations in the Upper Bois Brule River, Wisconsin [†]

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Abstract

Spawning habitat partitioning can be important for maintaining sympatric fish species. Likewise, critical spawning habitat loss may challenge the long-term persistence of sympatric fish species. The Bois Brule River, Wisconsin, USA, is a spring-fed, western Lake Superior tributary that supports five naturally reproducing populations of salmonids (native brook trout *Salvelinus fontinalis*; introduced brown trout *Salmo trutta*, rainbow trout *Oncorhynchus mykiss*, coho salmon *O. kisutch*, and chinook salmon *O. tshawytscha*). Given increases in recreational angler use and predicted climate-associated changes to trout stream habitat, a better understanding of species interactions during spawning is important to guide future management and conservation of these anthropogenically derived sympatric native and introduced salmonids. Our aim was to establish whether there was partitioning or overlapping in the redd site location preferences among native and introduced salmonids inhabiting the Bois Brule River. We mapped species-specific redd locations by canoe over a 15.3 river km section known to be important for salmonid spawning and evaluated physical, flow, and thermal conditions of these habitats of the Bois Brule River during 2021–2022. We found that spring spawning rainbow trout and fall spawning pacific salmonids and brown trout used the same spawning locations on mid-channel, larger gravel reefs downstream of riffle sections. Native brook trout spawned on smaller substrates with lower streamflow on the edges of the channel, with the highest spawning activity occurring in littoral areas of lentic portions of the river. Our findings provide valuable knowledge of critical spawning habitats for sympatric salmonids that may inform habitat conservation and enhancement efforts in the Bois Brule River and other Great Lakes tributaries with similar sympatric, naturally reproducing salmonids populations.

Keywords: habitat partitioning; brook trout; Salmonidae; Lake Superior tributary; spawning; redd

Key Contribution: Described spawning habitat partitioning between native brook trout *Salvelinus fontinalis* and introduced salmonid species in a Great Lakes tributary.

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1. Introduction

Sympatry in fishes is largely dictated by diverse habitats that allow for the completion of species-specific life histories, e.g., [1,2]. Often, the availability, diversity, and quality of species-specific spawning habitats is critical in the sustainability of sympatric fish populations and their ability to support commercial and recreational fisheries. Differences in spawn timing may also be an important factor contributing to sympatry, particularly if there is spawning habitat overlap among some species. Because of the reliance of sympatric fishes on critical habitat, abiotic (e.g., climate change), anthropogenic (e.g., residential development), and biotic (e.g., invasive species) disturbances resulting in habitat loss have and will continue to challenge sympatric fish populations [3–8]. These challenges have particularly manifested in sympatric salmonids. A better understanding of spawning habitat characteristics in sympatric salmonid populations may be critical for long-term persistence, to inform habitat conservation and enhancement efforts, and to support fisheries.

Successful salmonid spawning (i.e., nest (redd) creation, egg fertilization, egg incubation, embryonic development, and fry emergence) is reliant on intricate environmental and habitat conditions [9]. Conservation, enhancement, and restoration of these critical stream spawning habitats is foundational to an ecosystem-based management approach to support the persistence of sympatric salmonid populations [2,10]. Stream habitat restoration is a focus of natural resources management agencies to mitigate or improve physical, chemical, and biological habitat in degraded systems. Stream ecosystem restoration practices that enhance connectivity, reduce erosion, and provide additional habitat (e.g., coarse woody habitat, riparian plantings) may result in beneficial conditions for salmonids [11–14]. Modifications to in-stream habitat have been used as a fisheries management technique to support salmonid populations for decades [15]. Spawning habitat augmentation through the addition of preferred spawning gravels has been an effective strategy for the enhancement of salmonids globally [16], particularly when spawning habitat availability may be limited and when combined with woody habitat and riparian restoration [14]. Evaluation of life history processes that have direct reliance on specific habitat conditions (i.e., spawning) can provide insight on best management practices for stream restoration techniques with goals of positively influencing the entire aquatic biological community.

Spawning redd surveys and microhabitat data collection provide information on in-stream conditions needed for salmonids to naturally reproduce. Inventories of habitat selection can be a valuable tool for fisheries management agencies to estimate abundance, temporal distribution, and spatial distribution of actively spawning salmonids [17]. Traditionally, redd surveys and spawning habitat monitoring has been conducted for salmonids within their native ranges and spawning habitat preferences [10,18–20]. However, widespread introductions of salmonids outside of their native ranges have created sympatric native and non-native populations, where continued persistence may be heavily reliant upon spawning habitat partitioning. For rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, coho salmon *O. kisutch*, and chinook salmon *O. tshawytscha*, substrate size and condition, water velocity, and water depth have been noted as important factors defining spawning redd habitat [10,18,19,21,22]. Brook trout *Salvelinus fontinalis* and brown trout often prefer spawning locations associated with groundwater upwelling [20,23,24]. Redd superimposition, or the presence of multiple spawning redds at the same location, has been shown to decrease spawning success [25–28]. With similar preferences in spawning habitats, it is possible that sympatric salmonids partition habitats to ultimately influence the success of their spawning efforts.

Lake Superior is among the largest freshwater resources in the world, and the associated tributary network feeding the lake provides critical habitat for many fish

populations. Western Lake Superior tributaries are dynamic systems typified by high seasonal spring runoff driven by snow melt, with many streams having a range of baseflow conditions depending on associated aquifer connectivity. Stream habitat degradation resulting from logging and development and non-native species introductions have altered fish communities throughout the Laurentian Great Lakes region [29,30]. These negative influences have led to diverse, often non-native aquatic food webs [31], which include introduced populations of Pacific salmonids [32–34]. Lake Superior tributaries historically supported native brook trout populations with individuals exhibiting migratory “coaster” life-history behaviors. Coaster brook trout are individuals that partake in potamodromous movements to lacustrine habitats. There has been increased conservation interest to restore coaster brook trout populations that were historically common throughout the region [35,36]. The research efforts of enhanced fisheries in Lake Superior tributaries currently supporting anthropogenically derived sympatric (sympatry caused by human influence) native and non-native salmonids are important for native species management and conservation (e.g., brook trout) and maintaining diverse sportfishing opportunities.

Given the presence of native brook trout and recreational angling importance of the self-sustaining native and non-native sympatric salmonid populations of the Bois Brule River, Wisconsin (a pristine south shore tributary to Lake Superior), there is a critical need to better understand the spawning habitat use of these species for their long-term sustainability. Our aim was to establish whether there was partitioning or overlapping in the redd site location preferences among native and introduced salmonids inhabiting the Bois Brule River. We documented spawning redd microhabitat characteristics and locations for each salmonid species in historically important spawning areas within a 15.3 river km section of the Bois Brule River, Wisconsin during 2021 and 2022. Our objectives were as follows: (1) mapping active spawning redd locations of brook trout, brown trout, rainbow trout, coho salmon, and chinook salmon; (2) evaluating physical habitat, flow, and thermal characteristics of species-specific spawning redd locations; and (3) identifying whether spawning habitat partitioning occurred among the salmonids. We hypothesized that there would be overlap in redd site location preferences among species during spawning efforts. Alternatively, there may be spawning habitat partitioning that reduces overlap and allows for the coexistence of sympatric native and non-native salmonid populations in the Bois Brule River.

2. Materials and Methods

2.1. Study Site

The Bois Brule River (hereafter referred to as “Brule River”), Wisconsin, is a spring-fed, western Lake Superior tributary that supports five naturally reproducing salmonids: native brook trout and introduced brown trout, rainbow trout, coho salmon, and chinook salmon (Figure 1).

This sympatric assemblage of native and introduced salmonids that includes potamodromous and stream resident life histories creates the potential for overlap of natural reproduction strategies, spawning locations, and habitat requirements. The Brule River is a model system to address our objectives due to the presence of these naturally reproducing sympatric salmonids and its habitat heterogeneity (groundwater inflow, riparian zone) and connectivity (no barriers to migration ~71 river km), which is uncommon among Lake Superior tributaries.

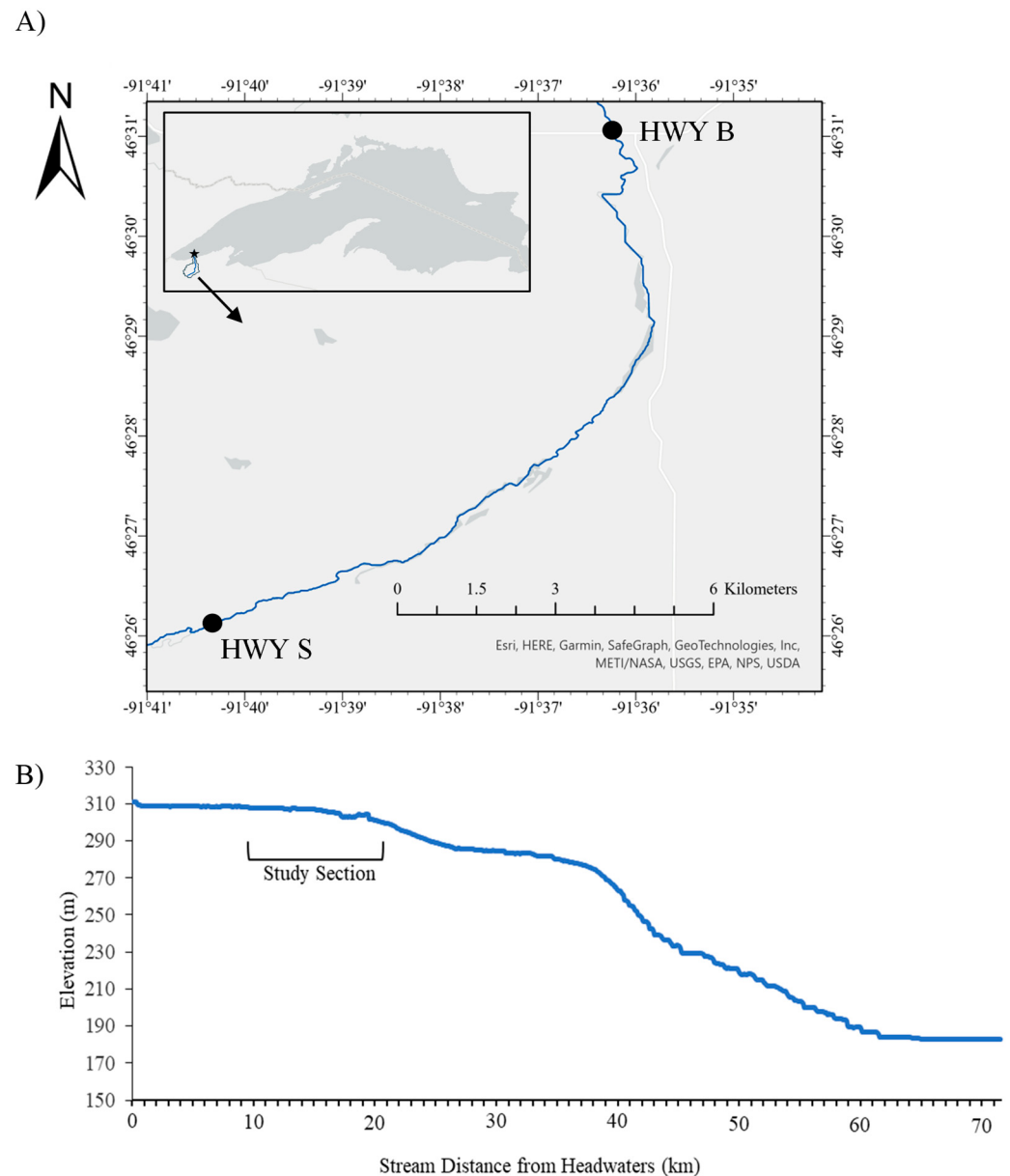


Figure 1. (A) Map of the Brule River, Wisconsin (star), with enhanced views of the study section within the upper river—15.3 river km section from the Highway S Road crossing, Stone’s Bridge canoe landing ($46^{\circ}26'3.28956''$ N, $-91^{\circ}40'28.04844''$ W) to the Highway B Road crossing, Winneboujou canoe landing ($46^{\circ}31'1.6176''$ N, $-91^{\circ}36'11.74284''$ W)—and (B) longitudinal elevation profile with the study section outlined.

The annual spawning runs of potamodromous salmonids into the Brule River from Lake Superior have been well documented by the Wisconsin Department of Natural Resources (WI DNR) through a fishway video camera installed at an invasive sea lamprey *Petromyzon marinus* barrier that has been in operation since the early 2000s. Interannual temporal phenology of spawning run timing has been relatively consistent and primarily related to water temperature and photoperiod. Spawning run timing has also fluctuated due to varying annual flow conditions [37]. Fall rainbow trout spawning runs usually begin during August and continue through late November; these fish typically overwinter in the river. Spring rainbow trout spawning runs begin in late March and continue to the end of May [37]. Chinook salmon spawning runs can range from July to mid-October; coho salmon spawning runs occur from late August through November; and migratory brown trout spawning runs begin in July and last through late October [37]. Visual confirmations of

coaster brook trout are rare in video surveillance efforts; however, runs have been historically known to occur in the Brule River [38]. Backpack electrofishing surveys in the Upper Brule River during 2009 and 2015 confirmed self-sustaining populations of resident brook and brown trout, and juvenile rainbow trout and coho salmon [39].

2.2. Redd Surveys: Redd Mapping and Covariates Measurement

Previous salmonid spawning surveys documented redd locations within the Brule River based on the presence of multiple species of spawning fish, available access, and sufficient visibility to identify redd locations [38,40,41]. Based on these previous surveys, we conducted redd surveys in the 15.3 river km section of the Brule River from the Highway S road crossing (Stone's Bridge canoe landing (46.4342471° N, -91.6744579° W)) to the Highway B road crossing (Winneboujou canoe landing (46.5171160° N, -91.6032619° W)). In 2021, we floated this river section by canoe every one to three weeks (condition dependent) during late March through late November to identify peak species-specific spawning activity. During identified peak periods of active spawning (early-April to the end of May, and mid-September to the end of November) during 2021 and 2022, we conducted surveys every 7–14 days, e.g., [17].

Two surveyors, one consistent among all redd surveys, traversed the river section in a canoe and recorded the latitude and longitude of identified redd sites with noticeable indentation and clean gravel with a Garmin 64st Global Positioning System (GPS) unit. The spawning fish present were visually identified to species to the best of our ability, and a suite of environmental covariates were recorded for a subset of redd locations. Species identification was relatively straightforward for the rainbow trout, brook trout, brown trout, and chinook salmon by physical characteristics and spawn timing. Identification was more challenging for the migratory brown trout and coho salmon. Two subpopulations of brown trout spawn in the Brule River. One subpopulation exhibits a migratory life history and the other is a stream resident subpopulation [38]. Visual identification of the two subpopulations was not possible; thus, stream resident and migratory brown trout subpopulation spawning was likely observed. Redds were not assigned to a species based on redd size, timing, or location; if fish were absent from a redd, we did not infer species identity. Previously marked redds that were not freshly cleaned and without the presence of new fish were not recorded in subsequent surveys.

In-field covariate redd measurements included metrics that had been previously identified as potentially important determinants of salmonid spawning habitat selection [17,42]. Redd diameter was the length (cm) of the cleaned gravel indentation. Mean substrate size was the mean grain size diameter measurement (mm) of 5–10 randomly chosen spawning gravels found in the tailspin section of the redd. Mean substrate diameter measurements were then assigned a Wentworth grain-size scale classification [43]; percent presence of coarse sand was estimated; water depth was recorded at the deepest (pot) and shallowest (tailspin) part of the redd; water velocity was taken with a Marsh McBirney Flo-Mate 2000 (Marsh-McBirney Inc., Frederick, MD, USA) at the depth where spawning fish would likely have their heads positioned; and water temperature and dissolved oxygen measurements were recorded using a YSI 85, YSI ProDO, or a YSI Pro20 (YSI Inc., Yellow Springs, OH, USA).

Environmental covariates that were assigned to GPS redd locations post hoc are as follows: riparian area classification from the Wiscland 2.0 database from the WI DNR [44]; percent surface slope of nearest stream bank calculated using the U.S. Geological Survey 1/3 arc-second digital elevation models (DEMs) using the "Surface Parameters" tool in ArcGIS Pro 3.1 [45,46]; and distance from groundwater upwelling sites along the stream reach. Upwelling sites were visually identified as cleared areas with sediment movement and marked with GPS. Due to the historical practice of fisheries managers of the Brule

River adding extensive amounts of gravel by raft and helicopter for improving salmonid spawning habitat [40], we created a categorical covariate of previously documented gravel addition location (binary, Yes/No). We also conducted thermal imaging in March 2023 with a drone-mounted Zenmuse XT2 thermal sensor (DJI, Shenzhen, CN; gain set to AUTO temperature/range/gain with an image size of 640×512 , “Fusion” color scheme) at a subset of prolific spawning sites to further explore potential groundwater influences. Differences in temperature between groundwater inflow and surface water at this time (warmer groundwater) were identifiable by visual differences in thermal imagery.

2.3. Data Analysis—Spawning Redd Locations

We examined the temporal extent of spawning redd development by tracking species-specific redd numbers found weekly during the fall active spawning seasons and biweekly during the spring active spawning seasons during 2021 and 2022. Peak spawning periods were defined as the dates with the highest number of active redd sites for each species. These data were then compared with average water temperature (from redd survey), average total daily discharge from USGS gauge station 04025500, and daily photoperiod (h) for Brule, Wisconsin, obtained from the meteor package in Program R version 4.3.0. [47,48].

We examined the spatial extent of spawning redds across the surveyed river section by importing latitude and longitude coordinates into ArcGIS Pro 3.1 [46] and developing spawning habitat utilization maps for each species. Numerical redd environmental covariates including redd diameter, pot depth, water velocity, water temperature, dissolved oxygen, and percent slope were summarized by calculating mean, ranges, and standard error by species with data collected during 2021 and 2022. Redd prevalence within each option of the categorical covariates (gravel addition presence, coarse sand presence (>5% of redd substrate), Wentworth substrate classification [43,49], riparian zone classification, and distance from nearest marked groundwater upwelling) were calculated for each species. Due to the non-parametric nature of the covariate data, we used Kruskal–Wallis Rank Sum Tests [50] to test for significant covariate differences among species. If statistically significant differences were observed, post hoc, Bonferroni corrected Dunn’s tests were conducted to test for significant differences among species [51]. We used an $\alpha = 0.05$ to determine statistically significant differences, with the null hypothesis of no species-specific differences between redd characteristics. All Kruskal–Wallis and Dunn’s tests were conducted in Program R [48].

Given non-normal data and numerical and categorical environmental covariates, we analyzed the redd data with conditional inference tree and forest models [52,53] to test for important environmental covariates influencing species-specific redd locations. Classification tree models (CART) [54] and random forest models [55] are especially useful in an ecological framework due to their ability to handle non-parametric data and provide useful importance metrics for covariates [56]. Conditional inference trees and forests are better suited to handle complex data where potentially highly correlated variables are present and are not as prone to overfitting [52]. Both methods perform recursive univariate splits of dependent variables from a set of covariates. Conditional inference trees use a quadratic test to determine tree splits, which provides a test statistic and p -value at each variable split determined to influence the response variable. Out-of-bag error for the trees was determined by calculating the percent accuracy between true and predicted redd classifications for each species. Conditional inference forests are an ensemble technique of multiple conditional inference trees, in which the algorithm samples without replacement, creating a random sample for each tree [53]. We used default parameters for our conditional forest model, aside from increasing the number of trees to 1000. Out-of-bag error rate for the model was determined by calculating the percent accuracy between true and predicted redd

classifications for each species. In addition, variable importance values were calculated for each covariate using the varimp function in the partykit package [48,57] to assess relative covariate influence on redd classification. Conditional inference tree and random forest models were conducted in Program R with the ctree and cforest functions in the party and partykit packages [48,57,58].

2.4. Data Analysis—Spawning Habitat Partitioning Among Species

We used the number of species-specific redds observed multiplied by 1.2 (to account for multiple redds per female) to approximate the number of spawning females located within the river section for each spawning season [17,59]. Species-specific redds were designated as geodesic, buffer polygons in ArcGIS Pro version 3.0.4. using the calculated average redd diameter for each species. An estimation of area of overlap was calculated in areas where multiple species were found spawning to simulate potential redd superimposition occurring within our study.

3. Results

3.1. Redd Mapping and Covariates Measurement

During 2021 and 2022, 15 redd surveys were completed annually. Surveys were conducted from the last week of March to the second week of November in both years (Tables 1 and 2). The number of species-specific redd observations and environmental covariates measured from the redds varied between years (Tables 1 and 2). Combining the two spawning seasons, covariate data from 68 rainbow trout, 20 chinook salmon, 77 coho salmon, 16 brown trout, and 80 brook trout redds were assessed (Appendix A Figure A1).

Table 1. Salmonid redd survey details during 2021 and 2022 in the Brule River, Wisconsin. Date of survey, number of redd locations observed by species, average daily stream discharge (m³/s) from USGS Gauge station 04025500, and daily photoperiod (sunlight hours) for Brule, Wisconsin are included.

Survey Date	Redds	Mean Daily Discharge (m ³ /s)	Mean Water Temp. (°C)	Daily Photoperiod (h)
1 April 2021	32 rainbow trout	6.99	5.66	12.76
15 April 2021	15 rainbow trout	7.87	7.56	13.52
4 October 2021	6 chinook salmon 2 brown trout	4.53	12.23	11.59
18 October 2021	2 coho salmon	3.91	8.25	10.84
24 October 2021	4 coho salmon	3.79	5.60	10.52
8 November 2021	66 coho salmon	3.71	6.91	9.77
15 November 2021	115 brook trout	5.27	3.13	9.46
2 April 2022	10 rainbow trout	5.27	5.08	12.82
9 April 2022	1 rainbow trout	7.45	3.70	13.20
22 April 2022	3 rainbow trout	9.88	4.83	13.89
29 April 2022	2 rainbow trout	8.04	7.2	14.24
6 May 2022	5 rainbow trout	7.90	9.94	14.58
16 October 2022	5 chinook salmon 4 brown trout 3 coho salmon	3.65	5.93	10.94

23 October 2022	6 chinook salmon 4 brown trout 14 coho salmon	3.45	7.66	10.57
30 October 2022	2 chinook salmon 4 brown trout 4 coho salmon	3.45	6.96	10.21
6 November 2022	2 brown trout 162 brook trout 12 coho salmon 1 chinook	3.57	6.86	9.87
13 November 2022	8 brook trout 3 coho salmon	5.47	2.0	9.55

Table 2. Redd microhabitat covariates by salmonid species during the 2021 and 2022 surveys in the Brule River, Wisconsin.

Species	Number of Redds	Season	Redd Diameter (cm) (Mean ± SD, Range)	Water Depth (cm) (Mean ± SD, Range)	Water Velocity (m/s) (Mean ± SD, Range)	Water Temperature (°C) (Mean ± SD, Range)
Brook Trout	80	Fall	29.35 ± 2.51, 24–37	54.58 ± 23.36, 14–124	0.055 ± 0.03, 0.012–0.149	5.95 ± 1.71, 2.8–7.5
Brown Trout	16	Fall	54.56 ± 7.74, 42–67	48.44 ± 15.62, 24–79	0.279 ± 0.060, 0.128–0.424	7.22 ± 2.24, 5.6–12.8
Chinook Salmon	20	Fall	104.30 ± 14.50, 75–126	69.35 ± 15.73, 41–94	0.308 ± 0.098, 0.128–0.469	8.9 ± 2.52, 5.7–12.8
Coho Salmon	77	Fall	77.12 ± 12.07, 53–103	52.92 ± 14.15, 28–86	0.347 ± 0.106, 0.130–0.760	6.99 ± 0.59, 5.6–8.3
Rainbow Trout	68	Spring	88.47 ± 23.02, 40–157	43.00 ± 12.05, 23–97	0.370 ± 0.114, 0.131–0.58	6.39 ± 1.68, 3.7–11.8
Species	Number of Redds	Season	Dissolved Oxygen (mg/L) (Mean ± SD, Range)	Surface Slope of Stream Bank (%) (Mean ± SD, Range)	Tailspin Substrate Wentworth Classification	Site of Previous Gravel Addition
Brook Trout	80	Fall	9.50 ± 1.09, 8.2–10.9	8.03 ± 6.28, 0.41–37.98	100% Medium Pebbles	31.25% Yes, 68.75% No
Brown Trout	16	Fall	9.33 ± 0.96, 8.3–11.6	1.57 ± 0.59, 0.35–2.91	18.75% Medium Pebbles, 68.75% Coarse Pebbles, 12.5% Very Coarse Pebbles	100% Yes
Chinook Salmon	20	Fall	9.62 ± 0.79, 8–10.9	3.16 ± 3.16, 0.35–11.97	90% Coarse Pebbles, 10% Very Coarse Pebbles	95% Yes, 5% No
Coho Salmon	77	Fall	8.86 ± 1.02, 7.8–10.8	3.47 ± 3.21, 0.14–11.97	92.2% Coarse Pebbles, 7.8% Very Coarse Pebbles	94.8% Yes, 5.2% No
Rainbow Trout	68	Spring	9.66 ± 0.95, 8.26–11.7	2.88 ± 3.22, 0.06–14.9	89.7% Coarse Pebbles, 10.3% Very Coarse Pebbles	83.3% Yes, 17.7% No
Species	Number of Redds	Season	Coarse Sand Presence in Redd	Riparian Zone Classification	Distance from Nearest Marked Groundwater Upwelling (m)	
Brook Trout	80	Fall	99.9% Yes, 0.01% No	70% Coniferous Forest,	43.75% within 25 m, 26.30% greater than 100 m,	

				21.25% Forested Wetland, 8.75% Mixed Deciduous Conifer Forest	25% within 50–75 m 5% within 75–100 m
Brown Trout	16	Fall	6.25% Yes, 93.75% No	25% Coniferous Forest, 75% Forested Wetland	93.75% greater than 100 m
Chinook Salmon	20	Fall	5% Yes, 95% No	40% Coniferous Forest, 60% Forested Wetland	75% greater than 100 m
Coho Salmon	77	Fall	13% Yes, 87% No	53.25% Coniferous Forest, 46.75% Forested Wetland	75.32% greater than 100 m, 22.08% within 75–100 m
Rainbow Trout	68	Spring	2.9% Yes, 97.1% No	42.65% Coniferous Forest, 57.35% Forested Wetland	80.88% greater than 100 m

Rainbow trout actively spawned from the last week of March through the second week of May. Fall spawning for several species generally began during the first week of October and continued through the second week of November. Species-specific spawning times were staggered, with timing for each species varying between years (Appendix B Figure A2). Discharge patterns were different between study seasons (Appendix B Figure A2); however, water temperature and photoperiod were consistent between years (Table 1).

Annual species-specific active spawning times differed between years. Distinct weekly periods of active spawning where a relatively large number of redds were identified for all salmonids were observed during 2021. During 2022, active spawning times spanned multiple weeks with less distinct weekly peaks. Peak spawning times for rainbow trout were the last week of March in 2021 and 2022. Chinook salmon were the first species to exhibit spawning activity in fall 2021 and 2022, which began during the first week of October. About 1–2 weeks after chinook salmon spawning was initiated, active spawning congregations of coho salmon were observed. A low number of brown trout redds were found in the survey area. During 2021, two brown trout redds were found, while fourteen were observed in 2022. Brook trout displayed the most consistent spawning behavior between years, with large numbers of redds observed during both years in early November (Tables 1 and 2, Appendix B Figure A2).

Non-native rainbow trout, chinook salmon, coho salmon, and brown trout exhibited similar spawning site preferences in terms of physical locations but differed in temporal phenology. Preferences of these species differed considerably in the physical characteristics and locations of spawning sites compared to native brook trout. Spring spawning rainbow trout and fall spawning chinook salmon, coho salmon, and brown trout were observed using the same spawning locations on larger gravel reefs in the higher flow thalweg, downstream of riffle sections. Some brown trout redds were found on smaller substrate and closer to the stream bank within those areas. Native brook trout were observed spawning on smaller substrates with slower streamflow on the edges of the stream channel, with large aggregations of spawning activity occurring in littoral areas of lentic habitat within the river near groundwater inflow (Figure 2).

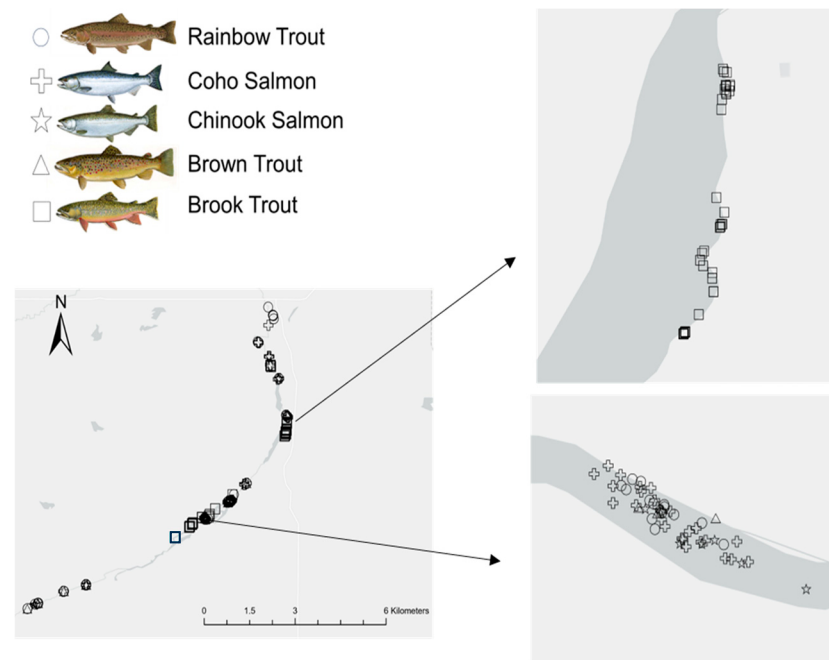


Figure 2. Distribution of salmonid redd sites in the Brule River, Wisconsin with enhanced views on prominent spawning areas during 2021 and 2022. The shape indicates species-specific redd sites (brook trout = square, rainbow trout = circle, coho salmon = cross, chinook salmon = star, brown trout = triangle). Fish images from the Wisconsin Department of Natural Resources by Virgil Beck.

3.2. Spawning Redd Locations

Of the 80 redds where microhabitat characteristics were measured, brook trout redds were the smallest in diameter and intermediate in pot depth compared to the other salmonid redds examined (Table 2, Figure 3). Water velocity and water temperatures at brook trout redds were the lowest compared to the other salmonids (Table 2, Figure 3). Dissolved oxygen concentrations at brook trout redds were relatively consistent among the salmonids, whereas the average percent slope of the nearest streambank was highest (Table 2, Figure 3). Brook trout spawning habitat sites were characterized by medium pebble-size substrates associated with upwelling sites in the forested riparian zones. About one-third of brook trout redds were on sites with previous gravel additions (Table 2, Figure 4).

Brown trout redd ($n = 16$) microhabitat characteristics were second lowest in diameter and pot depth compared to the other salmonids examined (Table 2, Figure 3). Water velocity at brown trout redds was the second lowest compared to the other salmonids, whereas water temperatures were similar to those of coho salmon redds (Table 2, Figure 3). Spawning dissolved oxygen concentrations were similar to the other salmonids and percent slope was lowest (Table 2, Figure 3). Brown trout redd site preferences included coarse-pebble substrates that lacked sand in areas away from upwelling sites in forested riparian zones (Table 2, Figure 4). All brown trout redds were located on sites of previous gravel additions (Table 2, Figure 4).

Twenty chinook salmon redds were examined and showed the highest redd diameters and pot depths compared to the other salmonids examined (Table 2, Figure 3). Water velocity at chinook salmon redds were similar to those of brown trout and coho salmon (Table 2, Figure 3). Water temperatures were greatest at chinook salmon redds (Table 2, Figure 3). Dissolved oxygen concentrations at chinook salmon redds were similar to the other salmonid redds examined, and percent slope was intermediate (Table 2, Figure 3). Chinook salmon redds were characterized by coarse pebble-sized substrates lacking sand, away from upwelling sites, in

forested riparian zones (Table 2, Figure 4). About 95% of chinook salmon redds were on sites of previous gravel additions (Table 2, Figure 4).

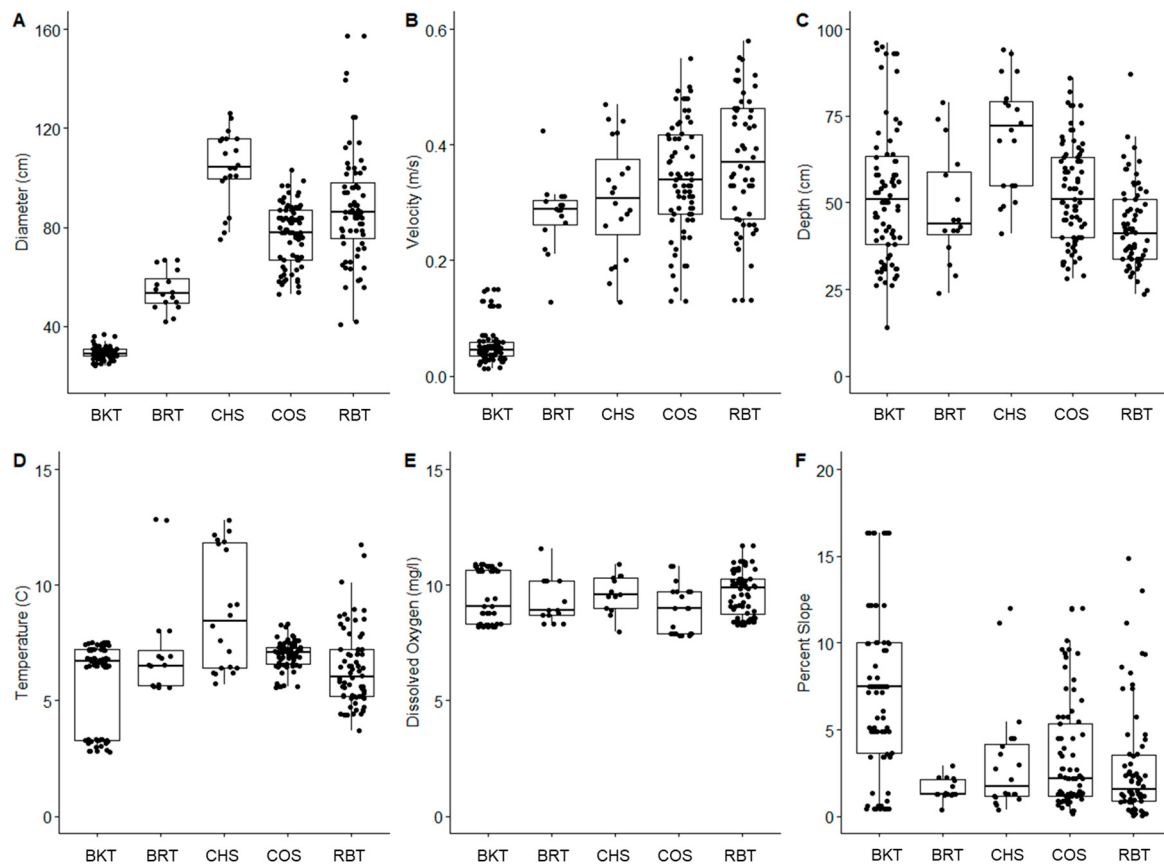


Figure 3. Redd diameter (cm) (A). Water velocity (m/s) (B). Water depth at redd pot (cm) (C). Water temperature (°C) (D). Percent surface slope of nearest streambank (%) (E). Dissolved oxygen (mg/L) (F) of each salmonid species (BKT = brook trout, BRT = brown trout, CHS = chinook salmon, COS = coho salmon, RBT = rainbow trout) redd measured during 2021 and 2022 surveys in the Brule River, Wisconsin.

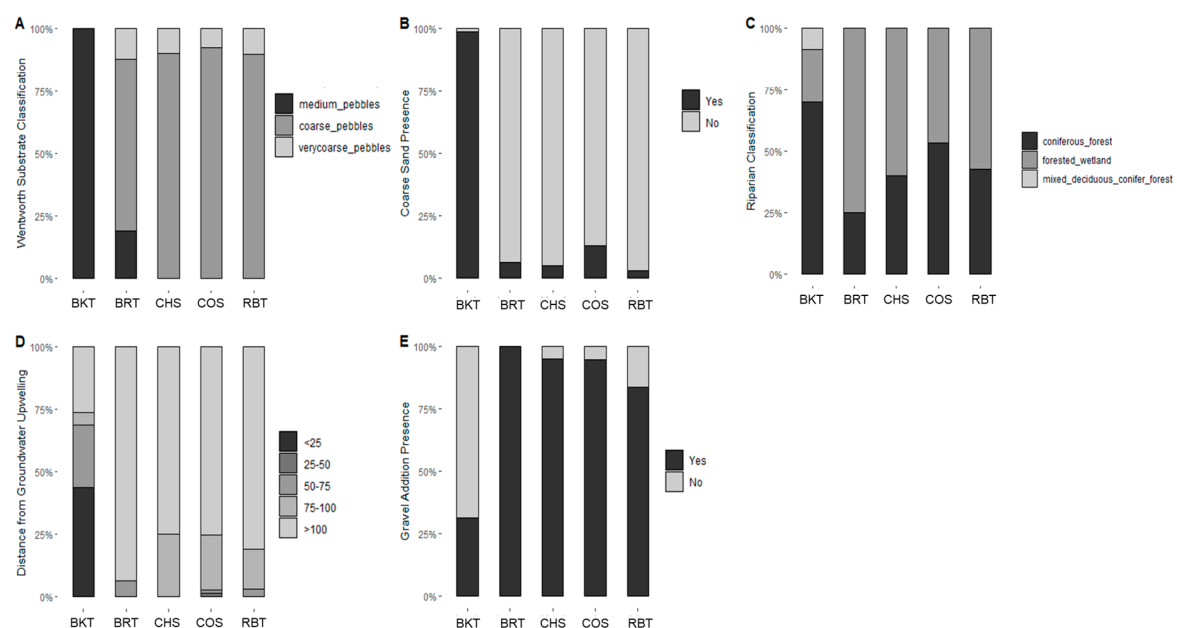


Figure 4. Percent of each salmonid species (BKT = brook trout, BRT = brown trout, CHS = chinook salmon, COS = coho salmon, RBT = rainbow trout) redd in Wentworth classifications of average

tailspin gravel substrate (A), each distance category from the nearest groundwater upwelling (B), with greater than 5% coarse sand present (C), each riparian zone classification (D), and area with previous gravel additions (E) during 2021 and 2022 surveys in the Brule River, Wisconsin.

Coho salmon redds ($n = 77$) measured had intermediate redd diameters and pot depths compared to the other salmonids examined (Table 2, Figure 3). Water velocity and temperature at coho salmon redds were similar to those of brown trout (Table 2, Figure 3). Dissolved oxygen at coho salmon redds were consistent with the other salmonids, and percent slope was the second highest (Table 2, Figure 3). Coho salmon redd site preferences included coarse pebble-sized substrates generally lacking sand, away from upwelling sites, with a forested riparian zone (Table 2, Figure 4). Similarly to chinook salmon, about 95% of coho salmon redds were associated with sites of previous gravel additions (Table 2, Figure 4).

Sixty-eight rainbow trout redds were measured and redd diameter and pot depth were second highest and lowest, respectively, compared to the other salmonids examined (Table 2, Figure 3). Water velocity at rainbow trout redds was highest, whereas water temperatures were second lowest (Table 2, Figure 3). Dissolved oxygen at rainbow trout redds was highest among the salmonids, whereas percent slope was second lowest (Table 2, Figure 3). Rainbow trout redd location preferences were similar to brown trout, chinook salmon, and coho salmon (i.e., coarse pebble-sized substrates lacking sand, away from upwelling sites, in forested riparian zones) (Table 2, Figure 4). Fewer rainbow trout redd sites were associated with previous gravel additions compared to brown trout, chinook salmon, and coho salmon (Table 2, Figure 4).

Kruskal–Wallis tests indicated that there were statistically significant differences among species for all 12 covariates measured ($df = 4$, all $p < 0.05$). The highest chi-square values were identified for the presence of sand ($\chi^2 = 202.18$), redd diameter ($\chi^2 = 196.86$), water velocity ($\chi^2 = 167.64$), non-sand substrate classification ($\chi^2 = 147.17$), and presence of previous gravel additions ($\chi^2 = 103.79$). Post hoc Dunn's tests indicated that redd diameter was the only covariate where all species were significantly different from each other (all $p < 0.05$). Post hoc Dunn's tests of water depth indicated significant differences between brook trout and chinook salmon, brook trout and rainbow trout, brown trout and chinook salmon, chinook salmon and coho salmon, chinook salmon and rainbow trout, and coho salmon and rainbow trout (all $p < 0.05$). Post hoc Dunn's tests of water velocity indicated a significant difference between brook trout and all other species (all $p < 0.05$). Post hoc Dunn's tests of water temperature indicated significant differences between brook trout and chinook salmon, brook trout and coho salmon, chinook salmon and rainbow trout, and coho salmon and rainbow trout (all $p < 0.05$). The only statistically significant difference in dissolved oxygen concentration at redd sites occurred between coho salmon and rainbow trout ($p < 0.05$). A significant difference in percent slope was also observed between brook trout and all other species (all $p < 0.05$).

Two conditional inference tree models were constructed using redd and covariate data, with the first using all 12 measured environmental covariates. Important covariates that determined tree splits were spawning season, Wentworth substrate classification, redd diameter, and water temperature (Figure 5, Table 3). Initially, spring spawning season separated and classified all rainbow trout spawning redds. Physical characteristics of redds including substrate size and redd diameter classified the majority of remaining redds by species. In general, longer fish created larger redds and spawned on bigger substrate (80% of chinook salmon > 97 cm, 100% of brook trout < 33 cm and on medium pebbles). Due to relatively similar redd diameters and substrate size between brown trout and coho salmon, water temperature was used to classify the remaining redds. Overall, redd classification accuracy was 100% for brook trout, rainbow trout, and chinook salmon, 87% for coho salmon, and 69% for brown trout.

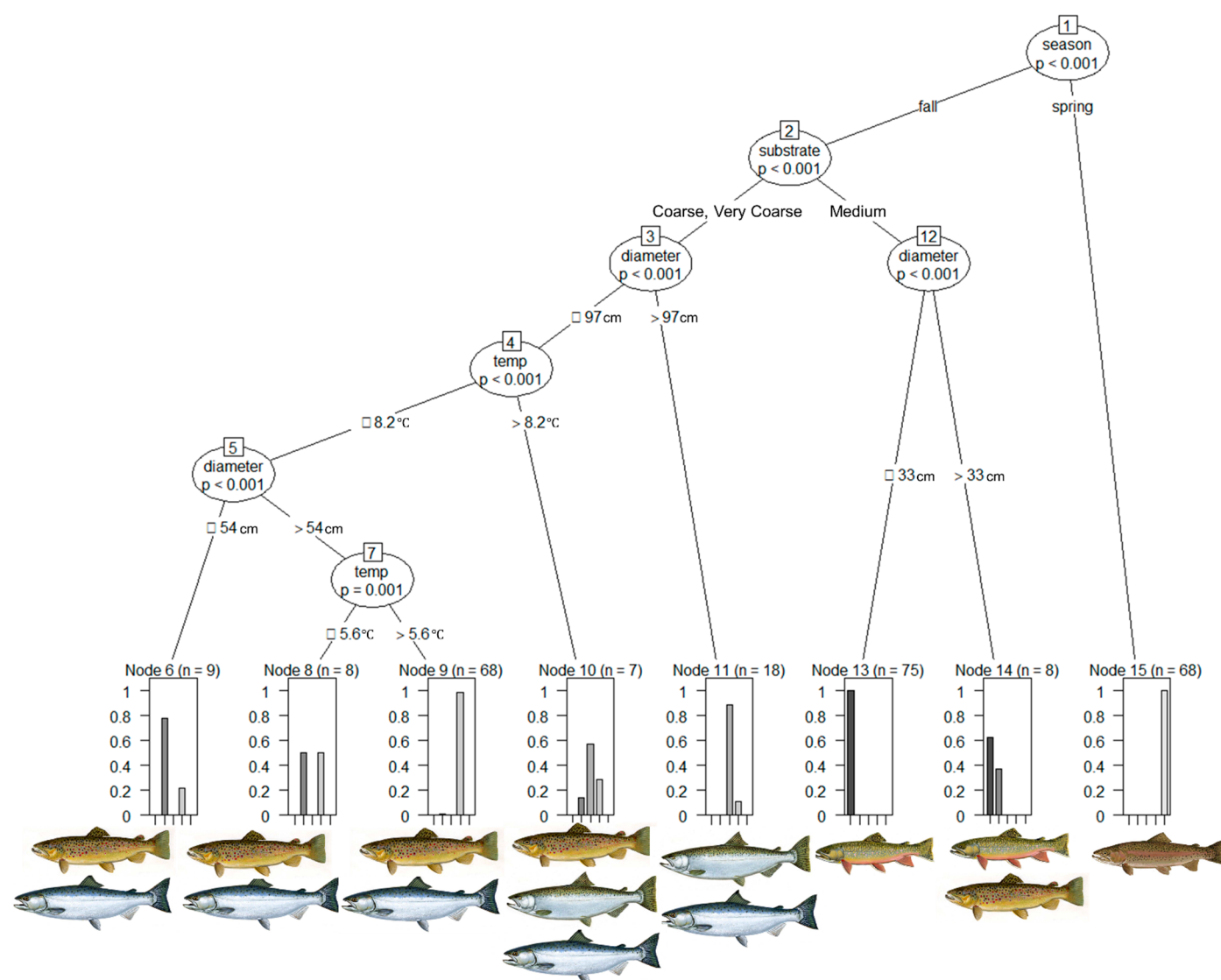


Figure 5. Summary of decisions for conditional inference tree model classification for all 12 microhabitat covariates of salmonid spawning redds in the Brule River, Wisconsin during 2021 and 2022. Boxed numbers indicate the specific tree split.

Table 3. Summary of decisions for conditional inference tree model classification for all 12 microhabitat covariates of salmonid spawning redds in the Brule River, Wisconsin during 2021 and 2022.

Tree Split	Covariate Split Decision	Chi-Square	p-Value	Covariate, Node Number	Redds Classified	Percent of Total Redds
1	Season—Spring vs. Fall	260	$p < 0.001$	Season = Spring, 15	68 Rainbow Trout	100% of Rainbow Trout
2	Substrate—Medium vs. Coarse and Very Coarse Pebbles	183.3	$p < 0.001$			
12	Medium Pebbles, Redd Diameter—<33 cm vs. >33 cm	61.7	$p < 0.001$	Diameter ≤ 33 cm, 13	75 Brook Trout	93.75% of Brook Trout
				Diameter ≥ 33 cm, 14	5 Brook Trout	6.25% of Brook Trout
					3 Brown Trout	18.75% of Brown Trout
3	Redd Diameter, >97 cm vs. <97 cm	60.7	$p < 0.001$	Diameter ≥ 97 cm, 11	16 Chinook Salmon	80% of Chinook Salmon

					2 Coho Salmon	2.5% of Coho Salmon
4	Redd Diameter ≤ 97 cm, Water Temperature— <8.2 °C vs. >8.2 °C	52.52	$p < 0.001$	Water Temperature ≥ 8.2 °C, 10	4 Chinook Salmon	20% of Chinook Salmon
					2 Coho Salmon	2.5% of Coho Salmon
					1 Brown Trout	6.3% of Brown Trout
5	Water Temperature $\leq$ 8.2 °C, Redd Diameter— <54 cm vs. >54 cm	26.52	$p < 0.001$	Diameter ≤ 54 cm, 6	7 Brown Trout	50% of Brown Trout
					2 Coho Salmon	2.5% of Coho Salmon
7	Redd Diameter ≥ 54 cm, Water Temperature— <5.6 °C vs. >5.6 °C			Water Temperature ≤ 5.6 °C, 8	4 Brown Trout	25% of Brown Trout
					4 Coho Salmon	5.2% of Coho Salmon
				Water Temperature ≥ 5.6 °C, 10	1 Brown Trout	6.3% of Brown Trout
					67 Coho Salmon	87% of Coho Salmon

The second tree was a subset model that excluded redd diameter and season and used 10 covariates to further test for differences between rainbow trout, brown trout, coho salmon, and chinook salmon. The model detected several covariate differences among the species, which included, water velocity (m/s), pot water depth (cm), and dissolved oxygen concentration (mg/L) (Figure 6, Table 4). Without redd diameter, brook trout redds remained highly classified by substrate size ($\chi^2 = 249.89$, $p < 0.001$) and slower water velocities ($\chi^2 = 60.73$, $p < 0.001$). Lower quadratic test scores were found in the second model, indicating that differences among the species were weaker and classification was less accurate with redd classification accuracies of 100% for brook trout, 90% for chinook salmon, 66% for coho salmon, 60% for rainbow trout, and 0% for brown trout.

Table 4. Summary of decisions for conditional inference tree classification for subset (excluding redd diameter and season) of 10 microhabitat covariates of salmonid spawning redds in the Brule River, Wisconsin during 2021 and 2022.

Tree Split	Covariate Split Decision	Chi-Square	p-Value	Covariate, Node Number	Redds Classified	Percent of Total Redds
1	Substrate—Medium vs. Coarse and Very Coarse Pebbles	249.80	$p < 0.001$			
11	Substrate = Medium Pebbles, Velocity—<0.13 m/s vs. >0.13 m/s	60.73	$p < 0.001$	Water Velocity $\leq$ 0.13 m/s, 12 Water Velocity $\geq$ 0.13 m/s, 13	76 Brook Trout 4 Brook Trout 3 Brown Trout	95% of Brook Trout 5% of Brook Trout 18.75% of Brown Trout
2	Water Depth—<67 cm vs. >67 cm	44.73	$p < 0.001$	Water Depth ≥ 67 cm, 10	13 Chinook Salmon 13 Coho Salmon 3 Brown Trout 2 Rainbow Trout	65% of Chinook Salmon 16.88% of Coho Salmon 18.75% of Brown Trout 2.94% of Rainbow Trout
3	Water Depth ≤ 67 cm, Water Temperature—<11.3 °C vs. >11.3 °C	38.99	$p < 0.001$	Water Temperature ≥ 11.3 °C, 9	7 Chinook Salmon 1 Brown Trout 1 Rainbow Trout	35% of Chinook Salmon 6.25% of Brown Trout 1.47% of Rainbow Trout
4	Water Temperature ≤ 11.3 °C, Water Depth—<43.82 cm vs. >43.82 cm	15.68	$p = 0.013$	Water Depth $\geq$ 43.82 cm, 8	43 Coho Salmon 24 Rainbow Trout 2 Brown Trout	55.84% of Coho Salmon 35.29% of Rainbow Trout 12.50% of Brown Trout

					2 Chinook Salmon	10% of Chinook Salmon
	Water Depth ≤ 43.82 cm,			Dissolved Oxygen	8 Coho Salmon	10.39% of Coho Salmon
5	Dissolved Oxygen— <8.2 mg/L vs. >8.2 mg/L	16.85	$p = 0.002$	≤ 8.2 mg/L, 6	44 Rainbow Trout	64.70% of Rainbow Trout
				Dissolved Oxygen	13 Coho Salmon	16.88% of Coho Salmon
				≥ 8.2 mg/L, 7	4 Brown Trout	25% of Brown Trout

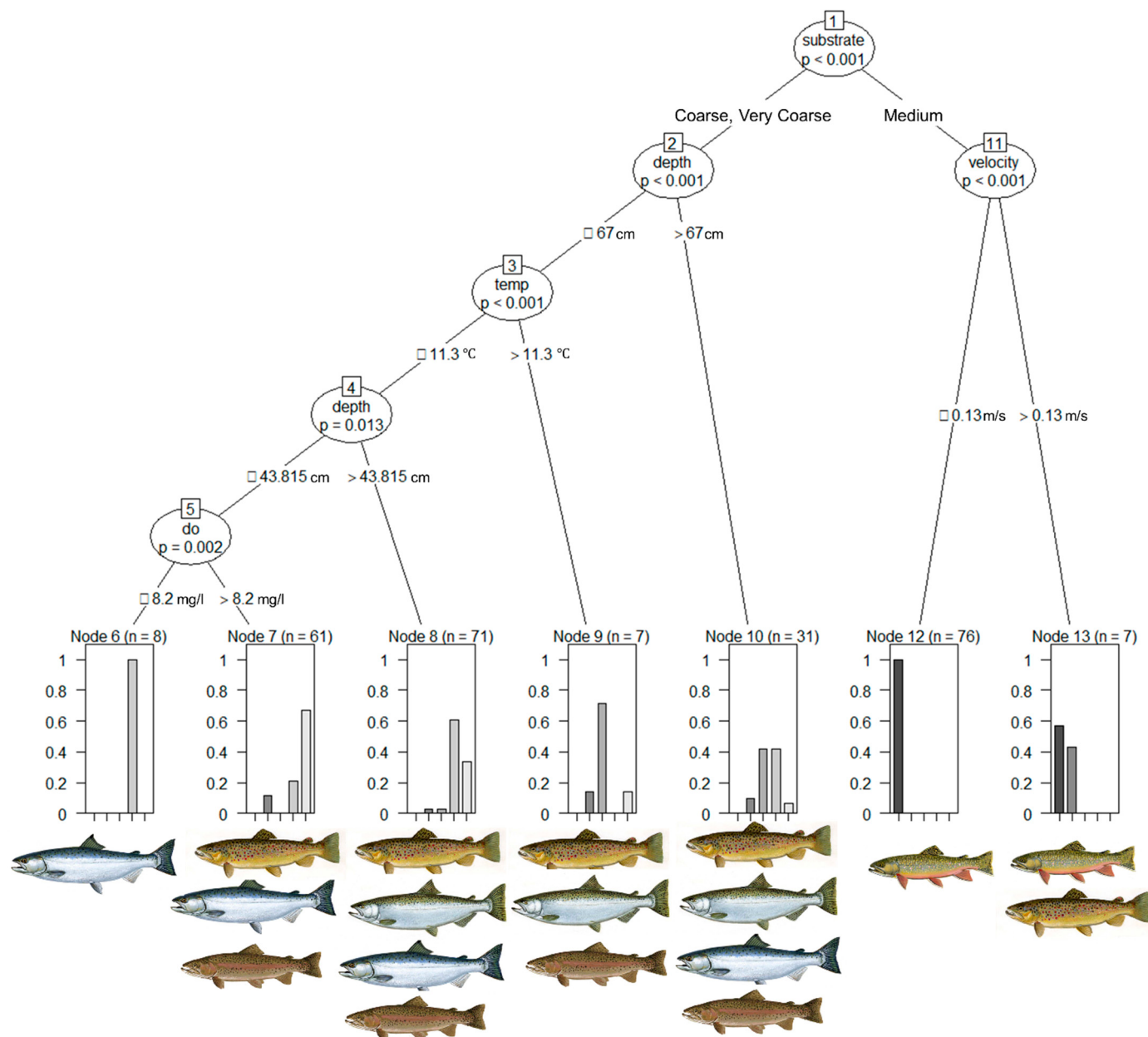


Figure 6. Summary of decisions for conditional inference tree subset model classification for subset (excluding redd diameter and season) of 10 microhabitat covariates of salmonid spawning redds in the Brule River, Wisconsin during 2021 and 2022. Boxed numbers indicate the specific tree split.

Importance values calculated from the conditional inference forest model showed similar patterns to the tree models and indicated that the most influential covariates on species-specific redd classification were season (3.084), redd diameter (1.652), and substrate classification (0.596) (Appendix C Figure A3). Water temperature (0.338) and water velocity (0.229) rated similarly and ranked relatively high. Redd pot water depth (0.089), dissolved oxygen concentration (0.078), presence of coarse sand (0.038), nearest riparian area classification (0.034), presence of previous gravel addition (0.027), distance

from groundwater upwelling (0.011), and percent slope (channel gradient) (0.009) ranked lower. The two survey years had varying conditions, and this was deemed a relatively important indicator (0.334).

3.3. Spawning Habitat Partitioning Among Species

The number of spring spawning rainbow trout females estimated within the study reach during 2021 and 2022 was 56 and 25, respectively. The number of spawning females estimated during the 2021 fall season was 138 brook trout, 86 coho salmon, 7 chinook salmon, and 2 brown trout. During the 2022 fall season, the estimated number of spawning females within the study reach was 204 brook trout, 43 coho salmon, 17 brown trout, and 17 chinook salmon.

Brook trout spawned in different areas from the other salmonids and showed preferences for springs and areas of groundwater inflow (Table 2, Figure 7, Appendix D Figure A4). There was evidence of spawning habitat overlaps among brown trout, coho salmon, and chinook salmon during fall spawning, and faster riffle sections supported most of the spawning redd locations for these species. Specific cases of inter-specific redd superimposition were rare, with buffer overlap occurring between one coho salmon and one chinook salmon redd (overlapping area = 0.11 m²). Fall redd microhabitat characteristics were relatively similar among brown trout, coho salmon, and chinook salmon. Spring spawning rainbow trout showed similar microhabitat characteristics to fall spawning brown trout, coho salmon, and chinook salmon and there was evidence of overlap between areas of rainbow trout and coho salmon redds (overlapping area = 0.24 m²). Both locations were found in the same riffle section densely populated with spawning redds (Figure 1).



Figure 7. Enhanced view of single riffle section heavily used by multiple salmonids for spawning redd construction in the Brule River, Wisconsin during 2021 and 2022. Please note the absence of native brook trout spawning redds at this location (Appendix D Figure A4).

4. Discussion

Salmonid redd location and distribution in the Brule River, Wisconsin showed several differences in associated habitat features that may reduce redd overlap among the sympatric salmonids. Redd characteristics differed in variables including diameter, which was the most important physical indicator of salmonid-specific redd traits in the Brule River. Much of this was likely due to species-specific differences in fish length (primarily chinook salmon) and not due to spawning habitat selection; however, the observational nature of our study precluded direct measurements of species-specific salmonid lengths. Our findings were consistent with observations of redd characteristics of introduced, migratory chinook salmon, coho salmon, brown trout, and rainbow trout in a Lake Ontario tributary where redd characteristic differences were the result of differing sizes of redds among the species [60]. Redd size has previously been used to determine redd sites of other sympatric rainbow trout, coho salmon, and chinook salmon populations in California [21] and used to decrease misidentification rates of sockeye salmon *Oncorhynchus nerka* and pink salmon *O. gorbuscha* in Alaska [10] so this information is useful for future redd classification in the Brule River.

The most influential environmental determinants of salmonid species classification of redd sites in the Brule River were substrate, water velocity, and water temperature. Ranges of environmental covariates for the salmonid populations predictably fell within ranges of measurements from the previous research conducted within their native and introduced ranges [60–64]. The presence of a native brook trout population within the Brule River allowed for valuable comparisons between introduced and native salmonids and provided insight to potential brook trout restoration efforts in tributaries with a similar salmonid species assemblage.

Temporal separation among fall spawning Brule River salmonids occurred during 2021, but not 2022. Chinook salmon occupied known spawning areas the earliest in each fall. Chinook and coho salmon peak spawning times were separated by about two weeks during 2021. During 2022, however, chinook salmon, coho salmon, and brown trout were found spawning concurrently in similar locations for multiple weeks, likely due to earlier temperature declines and colder water temperatures. Our findings were consistent with other salmonids such as sockeye salmon, which have been shown to migrate at different times due to water temperature and streamflow in Alaska [65]. Water temperature was significantly lower for brook trout spawning compared to the other fall spawning salmonids. No water temperature difference was observed between chinook salmon, coho salmon, and brown trout. Brook trout spawned about two weeks after peaks of coho salmon and brown trout spawning aggregations. Johnson et al. (2010) [60] found spawn timing overlap to be the greatest between chinook and coho salmon. Similarly, we found overlap in spawn timing between coho and chinook salmon in the Brule River; however, an interaction between coho salmon and brown trout was also common. Although our observations at individual redd sites were short in duration, we did not witness any aggression or redd usurpation during periods of spatial or temporal overlap in salmonid spawning.

Prominent fall spawning areas selected by introduced Pacific salmon and brown trout were the same main channel riffle areas used by spring spawning rainbow trout. Spring spawning rainbow trout populations had similar redd characteristics when compared to brown trout, coho salmon, and chinook salmon, likely due to spawning in the same areas. Overlap was relatively low and redd superimposition was rare during actual spawning efforts due to differing temporal onset of spawning; however, this has the potential to influence successful hatching and growth to ontogeny for brown trout, coho salmon, and chinook salmon, should rainbow trout excavate existing redds and displace incubating eggs. In this case, chinook salmon redds would be most vulnerable to

superimposition by the later fall spawning coho and brown trout and particularly by spring spawning rainbow trout before chinook fry emergence. Given that rainbow trout fry typically swim up weeks after spawning, the estimated hatch time for rainbow trout fry would be during July or just before fall spawning, indicating that fall spawning species redd superimposition would be least influential on rainbow trout. Potential inter-specific competition at the juvenile stage among the introduced salmonids may be directly limiting populations of brown trout, coho salmon, and chinook salmon and may influence patterns in annual run sizes. The potential for inter-specific competition between juvenile brook trout and the other salmonids would likely be reduced due to juvenile brook trout remaining in the Brule River for several years post-hatching compared to brown trout and the Pacific salmon, which smolt more rapidly. Future research on interactions among these species during the first year of their life may yield insights into the reproductive success of each species in the Brule River ecosystem.

Interactions between brook trout and other salmonids during spawning were not common due to different spawning habitat selection and locations for redd construction. Most spawning redds observed for salmonids, other than brook trout, were in sites that had previous gravel and cobble additions for spawning habitat enhancement. Brook trout were the only species with most redd sites not found in artificial gravel additions. Brook trout used a greater amount of available habitat for spawning efforts and tended to select locations with different characteristics than the other species. Brook trout redds were smaller in diameter, in relatively deeper water, and were found in slower flow velocities. Spawning aggregations were more focused on discrete littoral zones of lentic habitats near groundwater discharge within the river. The Brule River is a unique tributary in the fact that it has multiple slow flow and lacustrine-like regions within our survey section [29]. These slow flow areas can provide refugia for brook trout during temperature extremes [66] and foster more rapid growth rates in some cases [67], potentially explaining subpopulations of brook trout being present within/near lacustrine areas in the Brule River during spawning efforts. Littoral spawning of brook trout has been documented previously near areas of groundwater flow [68]. Brook trout redd sites in the Brule River were closer to groundwater inflow locations, and in areas of significantly higher stream bank slopes. Varying preference for groundwater upwelling by brook trout during spawning efforts has been previously documented in several studies [20,63,69]. Higher slope gradient along the Brule River likely increases hydrogeological interactions with the underlying sandy aquifer and results in more groundwater inflow to the stream. This relationship was also found in preferred spawning areas for brook trout in a Massachusetts stream [20]. Brook trout redd site selection included more coarse sand, small gravel, and fine material rather than cobble and large gravel. Significant presence of fine material has also been shown in other wild populations of brook trout in Georgia [70] and suggests that native brook trout eggs are seemingly tolerant of marginal egg incubation conditions in redds better than introduced salmonids. Brook trout redds also displayed the most variability in use of different riparian areas, again suggesting a more generalized use of available habitats by the native species compared to the introduced salmonids.

Although brook trout successfully spawn without much interference from other salmonids in the Brule River, our findings have implications for the restoration of coaster brook trout populations. Recent genetic research has confirmed that brook trout populations in specific river systems are likely multiple subpopulations that interact during spawning efforts to induce gene flow [71]. Population-level research on brook trout has found that 3–5% of individual brook trout undertook relatively long-distance migrations near the timing of spawning efforts [69,72]. Movement over longer distances is likely epigenetic [72] and may contribute to the coaster life history. Coaster brook trout making migrations into Lake Superior could consist of those 3–5% of individuals with the

epigenetic signal to travel long distances. Although this may be a small percentage of individuals within an entire population, historical brook trout populations in the Laurentian Great Lakes were likely much larger before the introductions of non-native salmonids and other invasive species and before disturbances such as habitat loss, pollution, and climate change, resulting in more brook trout exhibiting coaster life history strategies. The high use of larger riffle areas with gravel present for redd construction by other salmonids now in most Laurentian Great Lakes tributaries may restrict larger brook trout attempting to use similar spawning areas. Salmonid spawning in the Brule River suggests that potential inter-specific competition may limit the restoration of larger migratory brook trout populations exhibiting coaster life history variations in systems that have sympatric salmonid populations present.

Visual species identification and counting errors are possible during redd surveys [17]. Follow-up surveys of identified redds in an attempt to identify eggs or juveniles may have reduced any visual species identification errors but were outside of the scope of our study. Weather conditions and streamflow characteristics can greatly influence water clarity. Assessing redd sites visually in flowing water limited the time of visibility before spawning fish may have been disturbed off spawning redds and can be influenced by faster streamflow (i.e., in riffle sections). Identifying prime spawning areas during pre-spawn surveys allowed for a better approach to maximize visibility on subsequent outings. The distinction between coho salmon and the migratory brown trout population during fall spawning surveys proved to be the most visually challenging species identifications due to similar fish lengths and coloration. However, consistent surveyors across both years likely reduced misidentifications of spawning salmonids. We also acknowledge differences in spawning participation among years by brown trout in our study area. This may be related to intermittent spawning participation by adult brown trout, nocturnal spawning (and thus missed by daytime surveys) or selection of areas upstream and downstream of our study location. Additional research on brown trout in the river is needed to clarify this variability. Precautions to reduce counting errors included the labeling of each individual redd site and only identifying new redds during surveys. Surveyors conducting redd surveys via canoe/kayak should also have experience with swift river conditions and proper training for safely traversing rivers during cold weather periods. Conducting redd surveys by walking streambanks can successfully index spawning salmonids [69] and may provide a more appropriate and safer approach for management agencies depending on stream riparian zone characteristics, river flow conditions, and instream habitat.

5. Conclusions

Redd surveys proved to be an efficient way to identify occupied spawning areas in the Brule River for each Salmonid species. Pairwise comparisons of recorded environmental covariates at each redd microhabitat were effective in determining significant differences that existed among species. Conditional tree and forest models provided further exploration and interpretation of important redd site characteristics. Spatial analysis in ArcGIS was able to assess overlapping spawning habitats among species and identify important spawning locations in the Brule River for native and introduced salmonids. Future use of the suite of statistical models to classify wide-scale habitat measurements may be able to be used to estimate habitat abundance and quality for potential stocking efforts and to identify limiting variables that may be addressed with habitat enhancements. Furthermore, our models may potentially prove useful for classifying vacant redds to census overall spawning activity within larger spatial reaches of the river. Cobble and gravel additions as well as channel alterations may prove beneficial in streams that have limited spawning habitat for non-native brown trout and

Pacific salmonids, and potentially native coaster brook trout, but would not be recommended for native non-coaster brook trout based on the findings of our study.

The combination of techniques used within our study allowed for thorough analysis of the chosen spawning habitats of sympatric salmonids. Our results suggested that redd site selection may have implications regarding overall population maintenance for introduced species, and partitioning of spawning habitat may be occurring within the Brule River. Native non-coaster brook trout spawning did not seem to be influenced by the presence of introduced salmonids in our study reach based on the segregation of spawning sites between the introduced salmonids and brook trout. Spawning habitat partitioning is likely occurring among the introduced species and may explain why the species which occupies the prolific spawning riffles the latest (rainbow trout) is able to maintain more robust populations. That said, current spawning habitat availability for salmonids in the Brule River did not appear limiting given the lack of redd superimposition and the robust, naturally reproducing populations of salmonids observed in our study. Historical gravel additions proved to be an important factor for spawning locations of introduced salmonids, and continued upkeep and enhancement can potentially provide more spawning habitat for those species, and potentially for the restoration of coaster brook trout, while also considering the conservation of more generalized native non-coaster brook trout spawning habitat.

Conservation and restoration efforts of native species and management of introduced sportfish populations should be considered together and may influence fish community population maintenance ability. Continued monitoring of river ecosystem conditions (e.g., water temperature, streamflow, sediment transport, riparian zone change) [73] in addition to localized monitoring of critical life history behaviors of fish will be necessary to effectively manage native and introduced salmonids of the Brule River and other Laurentian Great Lakes tributaries into the future.

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Data Availability Statement: Data from this study used to formulate results are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

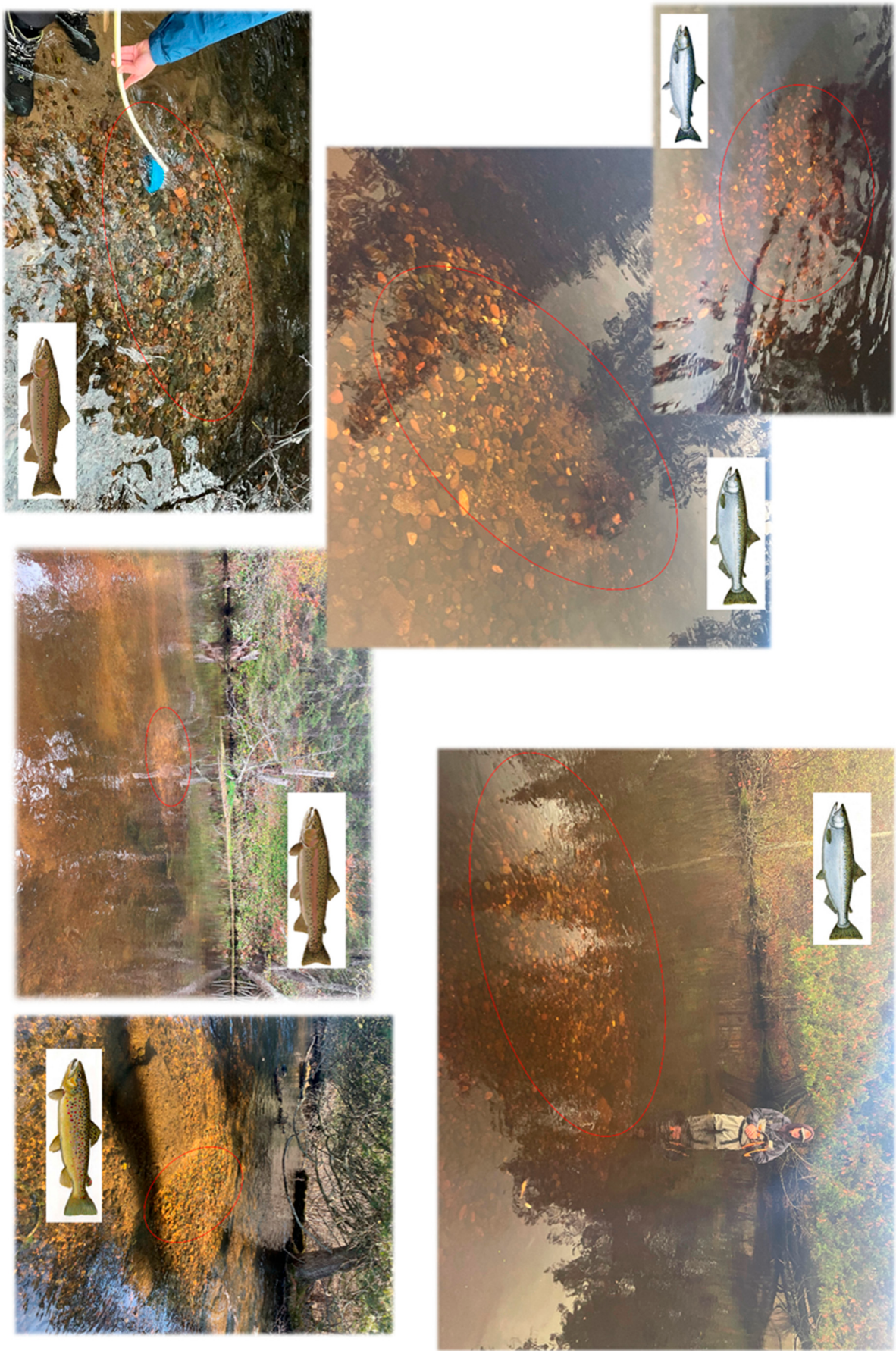




Figure A1. Images of salmonid redd sites (circle) in the Brule River, Wisconsin, during 2021 and 2022, identified by species.

Appendix B

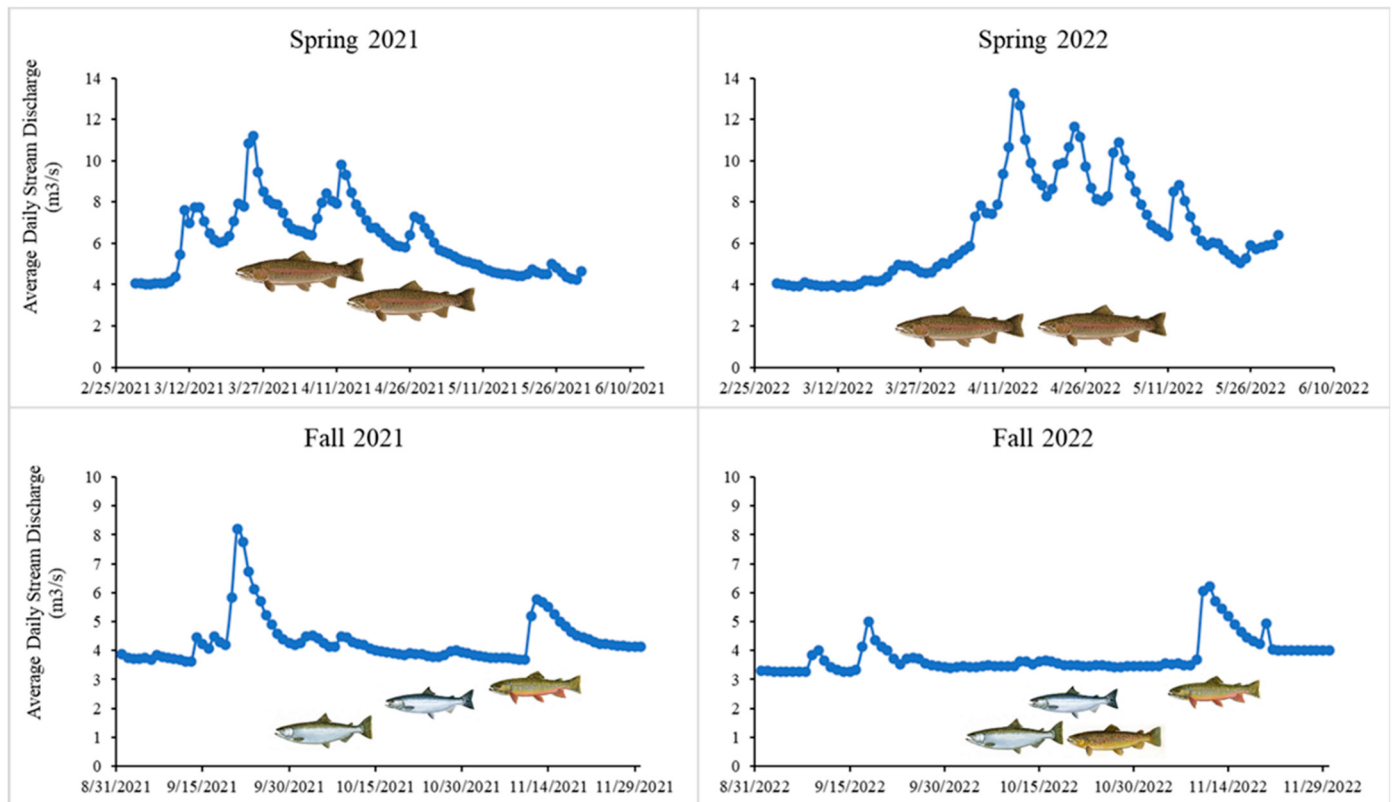


Figure A2. Streamflow patterns in the Brule River, Wisconsin during survey periods during 2021 and 2022, with images of salmonid species redd found.

Appendix C

Covariate Influences on Species Classification of Redd Site

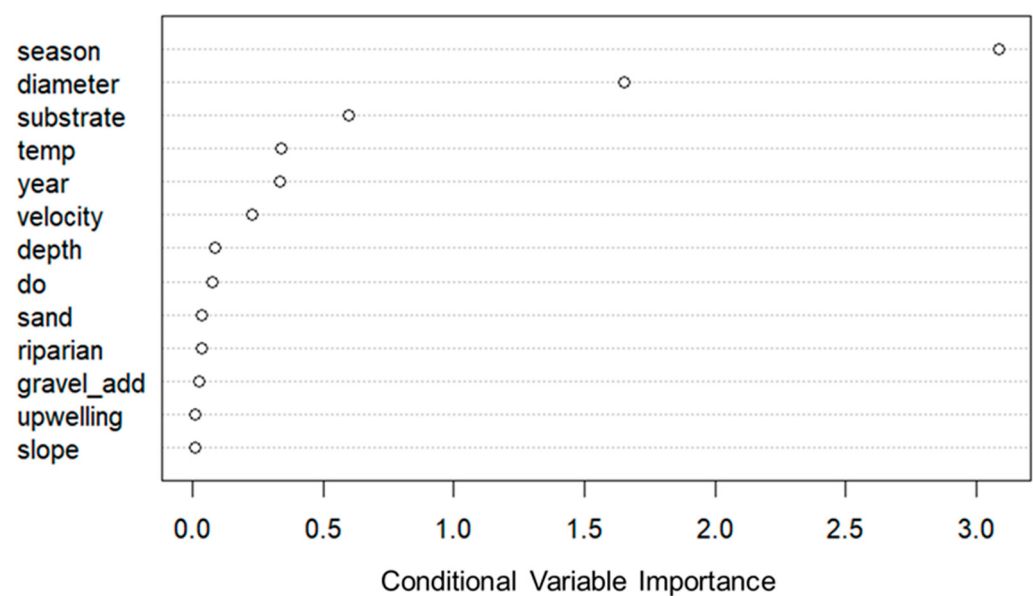


Figure A3. Conditional inference forest model calculated importance values for all 12 microhabitat covariates of salmonid spawning redds in the Brule River, Wisconsin, during 2021 and 2022.

Appendix D

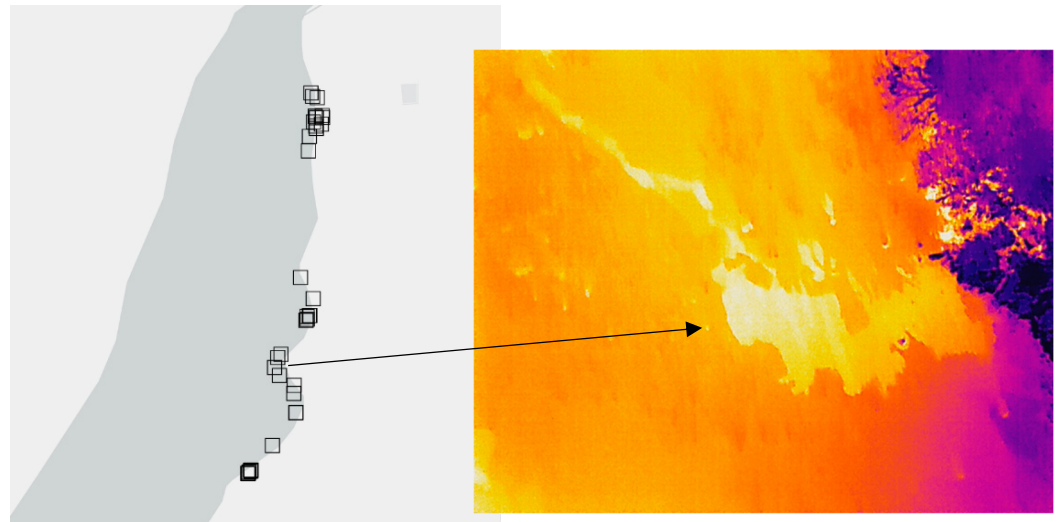


Figure A4. Thermal image taken by drone of largest aggregations of brook trout *Salvelinus fontinalis* spawning redds (square) in the Brule River, Wisconsin, showing large groundwater inflow area. White/yellow indicate warmer temperatures.

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