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The presence of quality suitable habitat is important for the success of naturally reproducing Brook Trout populations. Therefore, measuring redd habitat Brook Trout select for was the focus of this study. Brook Trout redds were observed during the spawning season of 2016. We identified a total of 18 redds in the Necktie River in late October and early November. Specific environmental variables associated with redd selection were measured: velocity, in-stream cover, canopy cover, bank overhang, river width, substrate type and size, temperature, and max-depth. The same environmental variables were measured for 30 randomly selected sites. Redds were distributed in riffles 85% of the time, and runs 15% of the time; random sites were distributed evenly throughout the system (43% in riffles and 57% in pools). Significant environmental variables associated with redds were substrates (P < 0.01), cover (P = 0.02), conductivity (P < 0.01), and dissolved oxygen (P < 0.01). All other variables showed no differences which indicated Brook Trout were not selecting for these variables. Although Brook Trout are more tolerant to ecosystem changes than other species of salmonids, they appear to be selecting for larger substrates. Therefore, future management should be focused on preserving or creating areas of suitable substrates to ensure maximum reproductive potential of the population.

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Introduction

Brook Trout Salvelinus fontinalis sustain naturally occurring populations in streams across the continent (MacCrimmon and Campbell 1969). Understanding wild Brook Trout habitat requirements is essential for conserving existing populations and restoring habitats no longer self-sustaining populations supporting (Mollenhauer et al. 2013). Although there are numerous accounts on reproductive ecology of Brook Trout, the nature, extent, and importance of these interactions are not fully understood (Witzel and MacCrimmon 1983).

Abiotic factors have an immense influence on spawning behavior. Reproductive behavior of Brook Trout has been closely associated with groundwater inputs (Scott and Crossman 1998). A previous study by Kamler (2002) indicated temperature was the most important factor affecting the rate of embryonic development in salmonids during spawning cycles. Other abiotic factors of likely significance include substrate size, adequate streamflow, and overhead cover. In aquatic ecosystems, specific habitat dynamics are important for reproductive success of individual fish populations.

Habitat suitability indices for spawning locations (redds) of Brook Trout are specific to their habitats and ecosystem dynamics (Raleigh 1982). Brook Trout spawning habitat is characterized as clear, silt-free, cold spring-fed water, with rocky substrates in a 1:1 riffle-run ratio, with slow deep water. Quality redd habitat also includes vegetated stream banks, abundant instream cover, stable water flow and temperature regimes, and undercut banks (Raleigh 1982). A lack of suitable habitat may influence populations through effects on individual fitness and may limit abundance if certain habitats needed to complete critical life stages are limited or lacking (Rosenfeld and Hatfield 2006).

Knowledge of Brook Trout habitat can be used to help (1) direct conservation efforts to areas with habitat conditions deemed necessary for sustaining Brook Trout populations, (2) assist with restoration programs by providing targets for stream restoration efforts, and (3) predict the consequences of potential habitat changes and management actions (Scheuerell et al. 2006; Knudby et al. 2010). Therefore, this study aims to determine suitable Brook Trout habitat selection for redds in the Necktie River, Bemidji, MN.

Methods

Study site

This study was located on the Necktie River between 279th Ave and County Road 45 in Hubbard County, MN. The total length of the study site is 2.82 km (Table 1). After surveying the entire Necktie River (32.2 km), this section of the river was the only area where redds were present. Brook Trout redds were located during the spawning season of 2016, September-December. Study area surveying and temperature logging occurred from 21 September - 27 October 2016. Environmental variable measurements occurred on November 20-23 and December 3-6. Brook Trout are the only salmonid that inhabit the Necktie River. *Tactics*

Redds were visually located while walking the banks of easements or kayaking stream sections. At all redds and at 30 random sites temperature, velocity (thalweg), max depth, bank-full width, substrate type and size, dissolved oxygen, conductivity, pH, bank undercut, percent canopy, and percent cover (in stream) were measured. *Measurement of Habitat Variables*

Velocity (nearest 0.01 cm/sec) was measured using an FH950.10020 Portable Velocity Meter (Hach Company., Loveland, Ohio), at the thalweg. Max depth (m) was measured at the thalweg using a meter stick. Bank-full width was measured from the start of vegetation on one bank to the other, at a 90° angle to which the direction of the river was flowing. One-hundred randomly selected substrate particles were collected along a transect across the stream at each site. In deeper pools, an aquatic net (500 µm) was used to obtain substrate samples. From each collected sample, the 30 largest particles had the diameter measured to determine mean particle size. All samples were weighed (nearest 0.01 g) then run through a series of sieves (sieve sizes = 4.36, 3.36, 2.38, 2.00, 1.68, 0.42, and pan < 0.420 mm) using a CSC Scientific Sieve Shaker 18480 (CSC Scientific Company, Inc., Fairfax, VA.). Cumulative retained weight (CRW) in each

TABLE 1. Necktie River location and characteristics.

sieve was used to determine percent fine (sum of CRW of sieve sizes ≤ 2.38 mm/total weight $\cdot 100$). Dissolved oxygen (nearest 0.01 mg/L), conductivity (μ S/cm), pH, and water temperature (nearest 0.1 °C) were recorded using a YSI 6050000 Professional Plus Multiparameter Meter (YSI Inc., Yellow Springs, Ohio). Bank undercut was measured using a meter stick inserted into the undercut (nearest mm) under the overhang until bank contact was made. This was recorded perpendicular to redd and random sites. Percent canopy was the average of estimates recorded by two people independently. This was done by standing directly above redd, looking up through a 5.08 cm diameter PVC to estimate percentage of canopy. The same procedure used to measure canopy was applied to measure cover, however, instead of looking up, we looked down through the PVC at redd to estimate percentage of cover.

Data Analysis

Cumulative frequency plots were generated for visual comparisons between redd and random sites across each environmental variable. A Shapiro-Wilk test (Shapiro and Wilk 1965) was used across all variables to test for normality. If results were normal (P > 0.05), a standard t-test was used to test for differences between redd and random sites. If results were abnormal (P < 0.05), a Wilcoxon test was used to determine differences in means (Wilcox 1945). All percentage data were arcsine transformed prior to analysis.

Results

Brook Trout redd selection

A total of 18 Brook Trout redds were identified in the study area. Redds were only found in a 2.82 km stretch. Redds appeared in late October at 5.5 $^{\circ}$ C and no new redds were identified after 6 November 2016 at 3.3 $^{\circ}$ C (Figure 1).

Velocity

Thalweg velocity associated with redds averaged 0.19 cm/s (SD = 0.09). At random sites, thalweg velocity averaged 0.22 cm/s (SD = 0.06). There was no significant difference between thalweg velocity at redd and random sites (W = 338, P = 0.15; Figure 2).

		Study Area			
Descriptive		Length of River	Study Reach	Gradient	
feature	Location	(km)	(km)	(m/km)	Sinuosity
	47°23'21.20"N,				
Necktie River	94°46'43.28"W	32.2	2.8	0.53	1.6



FIGURE 1. Temperature readings six weeks prior to Brook Trout spawn occurrence from 21 September - 27 October 2016 on the Necktie River, MN.



FIGURE 2. Cumulative percents plotted for velocity at redds and random sites measured at the thalweg in the Necktie River, MN, for Brook Trout between 3-6 December 2016.

Max depth and bank-full width

Average max depth was 0.54 (SD = 0.21) and 0.57 m (SD = 0.15) at redd and random sites, respectively (Table 2). Max depth at redd and random sites were not significantly different (T = 0.51, df = 26.81, P = 0.61; Figure 3; Table 3). Average bank-full width at redds was 4.58 m (SD = 1.18) and 4.65 m (SD = 1.10) at random locations. Bank-full width was not significantly different between the two site types (T = 0.21, df = 33.96, P = 0.84).

Substrates

At redds, the dominant substrates found were cobble, gravel, and fines. Average percent fines at redds was 42% (SD = 19). At random sites clay and sand were more prevalent. Average percent fines at random sites was 88% (SD = 23). There were significant differences in mean particle size between redd and random sites (W = 28, P < 0.01; Figure 4). There were significant differences in percent fines between redd and random sites (W = 442.5, P < 0.01).

Dissolved oxygen, conductivity, and pH

Dissolved oxygen in areas associated with redds were significantly different from random sites (W = 120, P < 0.01). Dissolved oxygen averaged 11.43 mg/L (SD = 0.62) at redds and 10.86 mg/L (SD = 0.35) at random sites. Conductivity was significantly different between groups (W = 67, P < 0.01). Mean conductivity was 309.07 µS/cm (SD = 28.33) at redds, and 270.74 µS/cm (SD = 6.85) at random sites (Table 2). There was not a significant difference in pH (W = 236, P = 0.48) with averages of 9.10 (SD = 0.69) at redds and 9.05 (SD = 0.94) at random sites.

Canopy and cover

In areas associated with redds, mean percent canopy was 16.11% (SD = 27.11) while cover was 29.17% (SD = 30.99) (Table 2). In areas associated with random sites, mean percent canopy was 15.3% (SD = 27.80) while cover was 19.43% (SD = 33.33) (Table 2). There was no significant difference in canopy between groups (W = 193.5, P = 0.09; Figure 5), however, there were significant differences in cover (W = 166.5, P = 0.02; Figure 6).



FIGURE 3. Cumulative percent plot of max depth at redd and random sites measured at the thalweg in the Necktie River, MN, for Brook Trout between 3-6 December 2016.



FIGURE 4. Cumulative particle weights plotted by sieve size for redd and random sites, on the Necktie River, MN, for Brook Trout between 20-23 November 2016.

	Necktie Study Area		
Descriptive feature	Redd Sites	Random Sites	
Latitude/Longitude	47°23'21.20"N, 94°46'43.28"W	47°23'21.20"N, 94°46'43.28"W	
Substrate size (mm)	16 (5-43) ± 4.09	$2(5-28) \pm 94.98$	
Percent fines	42 (7-81) ± 19	88 (31 – 100) ± 23	
Dissolved oxygen (mg/L)	$11.43~(10.66\text{-}12.60)\pm0.62$	$10.86~(10.22\text{-}11.56)\pm0.35$	
pН	$9.10~(7.47\text{-}10.82)\pm0.69$	$9.05~(7.35\text{-}11.30)\pm0.94$	
Conductivity (uS/cm)	$309.07(272.9-342.7) \pm 28.33$	$270.74~(238.7\text{-}275.8)\pm 6.85$	
Temperature (°C)	$1.33(0.9-3.7) \pm 0.87$	$0.65 (-0.01 - 1.1) \pm 0.44$	
Percent canopy (%)	$16.11~(0.080.0)\pm27.11$	$15.30~(0.0\text{-}90.0)\pm27.80$	
Percent cover (%)	$29.17~(0.0\text{-}90.0)\pm 30.99$	$19.43~(0.0\text{-}98.0)\pm33.33$	
Velocity (cm/s)	$0.19~(0.12\text{-}0.43)\pm0.09$	$0.22~(0.09\text{-}0.48)\pm0.06$	
Depth (m)	$0.545~(0.23\text{-}0.86)\pm0.21$	$0.574~(0.11\text{-}0.84)\pm0.15$	
River width (m)	4.58 (2.9-6.7) ± 1.18	4.65 (3.2-7.3) ± 1.10	
Undercut (m)	$0.40~(0.0\text{-}0.81)\pm0.22$	$0.44~(0.0-0.89)\pm0.22$	

TABLE 2. Measured environmental variables reported as mean, range in parenthesis, and ± standard deviation.



FIGURE 5. Percent canopy plotted for redd and random sites measured along the banks on the Necktie River, MN, for Brook Trout between 20-23 November 2016.



FIGURE 6. Percent cover plotted at redd and random sites measured in the river on the Necktie River, MN, for Brook Trout on 20-23 November 2017.

Bank undercut

There were no significant differences between groups for bank undercut (T = 0.61, df = 35.82, P = 0.55; Figure 7). Average measured undercut was 0.40 m (SD = 0.22) at redds and 0.44 m (SD = 0.22) at random sites.



FIGURE 7. Cumulative percent plotted for bank under-cut at redd and random sites, on the Necktie River, MN, for Brook Trout between 20-23 November 2016.

Discussion

Since the cessation of stocking in 2012, Brook Trout populations have sustained solely by natural reproduction within the Necktie River. The study area riparian zone was dominated by agriculture, deciduous hardwoods, and evergreen tree species. However, there was insufficient canopy which provides protection from predators and provides cool water temperatures. Cutting of trees from Beaver (*Castor canadensis*) activity could greatly reduce canopy. Beaver dams were dominant throughout, which decreases velocity increasing substrate deposition, and coarse woody debris which aids in controlling gradient, providing essential in-stream cover, and diversifying necessary spawn habitat for rearing salmonids (Pierce et al. 2015).

TABLE 3. T-test and Wilcoxon analysis results.

Environmental Variables	Analysis Result	
Dissolved oxygen (mg/L)	W = 4, P = 0.0014	
pH	W = 236, P = 0.48	
Conductivity (uS/cm)	W = 67, P < 0.01	
Substrate size (mm)	W = 28, P < 0.01	
Fines (%)	W = 442.5, <i>P</i> 0.0002	
Canopy (%)	W = 193.5, P = 0.09	
Cover (%)	W = 166.5, P = 0.02	
Velocity (cm/s)	W = 338, P = 0.15	
Max depth (m)	T = 0.51, P = 0.61	
Bankfull-width (m)	T = 0.21, P = 0.84	
Undercut bank (m)	T = 0.61, P = 0.54	

There were significant differences in substrates at redd and random locations. Redds were dominated by gravel and cobble and random sites were dominated by clay, sand, fines < 2.36 mm, and small woody debris. Reproductive success of Brook Trout decreases with increasing amounts of fine grained sediments (Peters 1965; Waters 1995). McRae and Diana (2005) reported unsuitable substrates for spawning site selection at < 2 mm indiameter. Roghair et al. (2002) also reported unsuitable spawn substrates to be < 2 mm and suitable substrates > 3.00 mm. Wentworth (1922), reports sand and other fines to be < 2.0 mm. Similarly, Bain et al. (1985) reported unsuitable substrates < 2.0 mm. Results from this study indicate Brook Trout are selecting for substrates with a mean particle size of 16 mm. Many studies have suggested that substrate size is directly correlated with spawning site selection for Brook Trout (Witzel and MacCrimmon 1983; Guillemette et al. 2011). Raleigh (1982) reported particle size optimal for Brook Trout spawning to be 3.4 - 50.5 mm and Duff (1980) reported 30 - 80 mm whereas Witzel and MacCrimmon (1983) reported a mean particle size of 6.9 mm. Similarly, our averages for percent fines and particle size match the criteria reported in other studies. Efforts are underway for the removal of beaver dams throughout the system. Dams are one major issue that affect habitat and can be the single most contributor to declining Brook

Trout abundance, therefore limiting suitable habitat for redd selection.

There were no significant differences in velocity. Wesche (1980) described optimal velocity for Brook Trout spawning to be < 0.15 cm/s. This was not evident from our results. This could be explained by insufficient in-stream habitat (woody debris, cover, bank shear) or excessive flooding caused by Beavers.

Max depth between redd and random sites were not significant. Thus, indicating Brook Trout were not selecting for specific depths. Redds were found in riffles where depth is known to be shallow. The lack of differences in bank-full width between groups indicates Brook Trout are selecting for other environmental variables for spawning. One suggestion to account for insignificance in bank-full width is insufficient velocity due to reach conditions.

Between both groups, dissolved oxygen was significantly different. Dissolved oxygen levels are not well documented in studies related to Brook Trout habitat spawning. Raleigh (1982) did report with age, required oxygen levels vary. Brook Trout require high oxygen concentrations near saturation. At temperatures < 15 °C, oxygen concentrations should be > 7 mg/L and > 10 mg/L at temperatures > 10 °C. Our findings indicate dissolved oxygen levels were within this range. Certain habitat features were documented but not measured as criteria for selection; near redds, natural springs were present in-stream, and along banks; this could be a contributor of increased oxygen levels where the system average was lower than the average at redds.

Specific conductance and pH are important variables for Brook Trout reproductive success. In the Necktie River, there are differences in conductance, attributed to increased ionic composition within the system. Ionic composition can be explained due to dissolved salts, chlorides, sulfides, and/or carbonate compounds. pH seems to be uniform throughout the river and the range measured during this study were suitable for Brook Trout. Other studies have shown Brook Trout adapting to pH levels as low as 6.0 ppm. Anything < 6.0 ppm can lead to unsuccessful reproduction (Raleigh 1982).

Canopy and cover aid in the success of Salmonids by providing cover for fry. Other uses for cover include relatively cool waters, provide allochthonous material, and provide shade during the mid-day hours. Limited overhead canopy can have adverse effects on a system by raising the temperature out of optimal range and reducing productivity. Instream cover such as logs, substrates, and vegetation provide shelter, nutrients, and much needed vertebrate and invertebrate nutrient source for Salmonid species like Brook Trout. Results indicated differences in percent cover showing selectivity by Brook Trout, but did not show differences in percent canopy. Bank undercut functions like cover; it provides a basis of cover from light, predators, and can be a suitable area for substrates to aggregate. Results from this study indicate no differences between variables among groups. Canopy, cover, and bank undercut can be explained by the highly variable morphology of the Necktie River. There were few instances where variables were consistent in selection. Often, stream width fluctuated greatly reducing the probability of suitable redd habitat.

Overall, there seems to be insufficient suitable habitat for spawning within the Necktie River. Substrates, velocity, canopy, and cover are important environmental variables that aid in successful Brook Trout spawning. Beaver dams degrade habitat; removal of dams can increase habitat suitability by increasing substrate deposition and stabilizing velocity. In 2005, a Habitat Improvement Project was headed by Tony J. Standera. This project anchored Christmas trees within the water column North of County Road 45 along with Tamarack and Spruce trees planted along stream banks to provide canopy in the future. The project resulted in increased in-stream cover, woody debris, stable flow regimes, and increased substrate loads outside the controlled reach. We recommend continuing the habitat improvement project, removing beaver dams, and monitoring suitable spawning habitat for redds. This original study for the Necktie River in Bemidji MN serves to provide a baseline data set for reproductive selection areas within the river.

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