**Title:** Broadscale population structure and hatchery introgression of Midwestern brook trout (*Salvelinus fontinalis*)

**Running title:** Midwestern brook trout population genetics

*In preparation for Transactions of the American Fisheries Society*

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**Abstract**

Brook Trout (*Salvelinus fontinalis*) have faced significant declines throughout their native range and have been stocked in Midwestern waters since the late 1800's to offset such losses. Several studies have investigated the genetic effects of these stockings, but these efforts have been confined to relatively small spatial scales. In this study, we compiled 8,454 Brook Trout microsatellite genotypes from 188 wild Midwestern populations and 26 hatchery strains to provide novel insights of broadscale population structure, regional patterns of genetic diversity, and estimates of hatchery introgression for inland Wisconsin populations. Our results indicate high levels of differentiation among our study populations, a lack of hydrological population structuring, lower estimates of genetic diversity in the Driftless Region, and that hatchery introgression has been largely confined to regions of inland Wisconsin that have been heavily affected by anthropogenic disturbances (i.e. the Driftless Region). We also provide support that populations may be able to purge hatchery-derived alleles, discuss plausible mechanisms behind this phenomenon, and consider their relevance to accurate estimation of hatchery introgression. Collectively, these results summarize the genetic effects of over a century of anthropogenic disturbance on native trout populations and emphasize the importance of integrating historical data into contemporary genetic research of intensively managed species.

## Introduction

Supplemental stocking is one of the most ubiquitous fisheries management strategies in North America (Halverson 2008). This approach is commonly used to mitigate anthropogenic disturbances (e.g. dams, overharvest, habitat degradation) by artificially increasing the abundance of recreationally or commercially valuable species (Jackson et al. 2004). However, the interbreeding between native and hatchery-derived conspecifics, hereafter referred to as introgression, can disrupt local adaptations of indigenous populations and reduce the resiliency of metapopulations through the homogenization of the regional gene pool (Carvalho 1993). Therefore, to maintain robust fisheries, natural resource managers require a more comprehensive understanding of the extent to which wild populations have historically introgressed with hatchery sources and the anthropogenic and natural factors that modulate hatchery introgression on the landscape.

Previous research suggests that domestication selection can lead to rapid heritable divergence of wild and domesticated populations (Huntingford 2004; Christie et al. 2012, 2014; Milot et al. 2013), with declines in relative fitness of 30% for each generation spent in captivity (Araki et al. 2007, 2008). The heritability of these domesticated traits makes it plausible that deleterious alleles may persist in a supplemented population for long periods of time following the cessation of stocking, particularly if the strength of natural selection is low or gene flow from adjoining native populations is limited (Lynch and O'Hely 2001). Compounding the problem of domestication selection, hatchery strains are often derived from source populations that are genetically divergent from the populations they are intended to augment (Humston et al. 2012; Bruce et al. 2018; Kazyak et al. 2018; White et al. 2018). Therefore, exogenous hatchery strains likely do not share similar local adaptations with the indigenous populations they are

86 supplementing (Fraser et al. 2011). Further, the process of local adaptation often involves
87 coadapted gene complexes that can be disrupted by introgression and result in lower fitness or
88 even hybrid incompatibilities (Orr 1995; Turelli and Orr 2000).

89 The extent of hatchery introgression is also highly dependent on anthropogenic and
90 natural factors affecting the relative demographic contributions of wild and stocked fish. For
91 example, measures of stocking intensity (e.g. total number of fish stocked) have been shown to
92 be positively correlated with increased rates of hatchery introgression (Almodódovar et al. 2006;
93 Marie et al. 2010; Lamaze et al. 2012; Létourneau et al. 2018). Also, the relative reproductive
94 output of hatchery fish, in comparison to native conspecifics, can further govern the extent and
95 persistence of hatchery alleles in natural systems (Emlen 1991). Finally, populations may be
96 able to purge deleterious hatchery-derived alleles following stocking cessation, likely through a
97 combination of natural selection, gene flow, and genetic drift (Perrier et al. 2013; Harbicht et al.
98 2014; Valiquette et al. 2014; Erdman et al. 2018; Létourneau et al. 2018).

99 Intensive land use (e.g. agriculture, timber harvest) and introduced species (e.g. Brown
100 Trout, *Salmo trutta*; Rainbow Trout, *Oncorhynchus mykiss*) have greatly reduced abundance or
101 caused local extirpations of Brook Trout (*Salvelinus fontinalis*) from many Midwestern streams
102 (Vetrano 2017). Natural resource management agencies have utilized supplemental stocking to
103 offset population declines since the late 19$^{th}$ century (United States Bureau of Fisheries 1888).
104 Unfortunately, historical extirpations and stocking practices have been incompletely
105 documented, thereby leading to the possibility that many contemporary populations may have
106 complete or partial hatchery ancestry. Uncertainties about historical hatchery introgression can
107 be problematic for management agencies as it hampers their ability to prioritize the conservation
108 of native populations. Fortunately, modern genetic and genomic tools now afford the ability to

109 examine the genetic structure of native populations as well as the effects of historical and
110 ongoing supplemental stocking (e.g. Halbisen and Wilson 2009; Turnquist et al. 2017).

111 Brook Trout genetic studies have been conducted in a number of areas across the
112 Midwest including Lake Superior (Sloss et al. 2008; Wilson et al. 2008; Stott et al. 2010;
113 Scribner et al. 2012; Leonard et al. 2013) and the Driftless Regions of Wisconsin (Hughes 2008)
114 and Minnesota (Hoxmeier et al. 2015). These studies utilized the microsatellite marker set
115 developed by King et al. (2012), and thus afford an opportunity to aggregate data from multiple
116 research efforts to gain novel insights of broadscale patterns of genetic structure in the region.
117 Here, we combine previously collected data from across the Midwest with new genotypes from
118 Wisconsin and Iowa to create a dataset of 8,454 individual Brook Trout genotypes, representing
119 188 wild populations across the Midwest and 26 hatchery strains of both regional and eastern
120 origin. These data were used to address four specific objectives: 1) investigate broadscale
121 population structure of Brook Trout in the Midwestern United States, 2) examine fine-scale
122 population structure of inland Wisconsin Brook Trout populations, 3) estimate rates of hatchery
123 introgression for inland Wisconsin Brook Trout populations, and 4) determine whether stocking
124 practices modulate rates of hatchery introgression in these populations.

125 **Methods**

126 *Ecological context*

127 The last major glaciation affecting the Midwestern United States occurred approximately
128 14,000 years ago and enveloped all contemporary Brook Trout habitat in the region with the
129 exception of the unglaciated Driftless Region of Minnesota, Wisconsin, and Iowa (Figure 1;
130 Bailey and Smith 1981). Early postglacial watersheds in the region were starkly different than

131 the large, single watershed of the contemporary Great Lakes. For example, glacial Lake Duluth
132 (now Lake Superior) and glacial Lake Chicago (now Lake Michigan) drained south into the
133 Mississippi River, glacial Lake Iroquois (now Lake Ontario) drained into the Hudson River and
134 provided a route for Atlantic fishes to colonize the Great Lakes, and glacial Lake Maumee (now
135 Lake Erie) had both westward and eastward connections to the Mississippi and Hudson Rivers,
136 respectively (Bailey and Smith 1981).

137 Prior to the 1800s, the upper Midwest was characterized by dense forests in the north,
138 prairie in the flatland areas to the south, and tall grass prairie and oak savanna in the Driftless
139 Region (Finley 1951). Extensive land clearing began in the 1850s and led to widespread
140 watershed impacts that reduced Brook Trout abundance, particularly in the Driftless Region
141 (Trimble and Lund 1982; Thorn et al. 1997). Stream conditions began to remediate during the
142 1930's following improved land-use practices. Indeed, pastures and cultivated crops remain a
143 prominent part of the landscape, and continued efforts have been made over the past few decades
144 to restore stream habitat and mitigate the effects of agriculture (Trimble and Lund 1982). These
145 restoration efforts have been successful in reestablishing many self-sustaining populations.
146 However, restoration efforts are still ongoing, and stocking is still necessary to maintain
147 adequate fisheries in many streams.

148 *Stocking history in study system*

149 Brook Trout stocking began in the Midwest during the late 19$^{th}$ century through the
150 cooperation of the federal government, private hatcheries, conservation groups, and eventually
151 state agencies (United States Bureau of Fisheries 1888; Thorn et al. 1997). The origin of early
152 hatchery strains is largely unknown. Although, there is speculation these strains were derived
153 from local populations (Fields and Philipp 1998; Hoxmeier et al. 2015). However, the use of

these strains occurred during an era when Brook Trout were readily transferred across state, provincial, international, and hydrological boundaries, thereby leading to the possibility that these putatively native strains underwent periodic infusions of alleles originating from divergent sources (United States Bureau of Fisheries 1888; Wisconsin State Conservation Commission 1930). These original hatchery strains were ultimately abandoned in the 1970-1980's in favor of strains developed by hatcheries located along the Atlantic seaboard (Fields and Philipp 1998; Hoxmeier et al. 2015). The most commonly stocked strain in Wisconsin since this time has been the St. Croix Falls hatchery strain. Other eastern-origin strains that have been used in the Midwest include the Owhi, Rome, and Assinica strains (Hunt 1979). In addition, several locally derived hatchery strains have been used since the 1990's (Hoxmeier et al. 2015; Wisconsin Department of Natural Resources 2019). Finally, wild Brook Trout translocations occurred within and among drainages during the late 1970's to 2007 to supplement lower abundance populations and refound putatively extirpated populations (G. Van Dyck, Wisconsin Department of Natural Resources, pers. comm.). Here, we focus on hatchery introgression in Wisconsin populations where genetic data is most complete, fewer eastern origin stocking sources were used (e.g. compared to Minnesota), and reliable stocking records were available post-1972 (Table S1).

*Genotyping and data standardization*

Brook Trout were genotyped at all or a subset of the following microsatellites: *Sfo28*, *Sfo38*, *Sfo52*, *Sfo86*, *Sfo88*, *Sfo113*, and *Sfo115* (Table S2). Most genotypes from Brook Trout populations outside Wisconsin were obtained from the following studies: Wilson et al. (2008), Stott et al. (2010), Leonard et al. (2013), Hoxmeier et al. (2015), and Kazyak et al. (2018). We also included previously unpublished data from 19 additional populations which were genotyped

177 by the above laboratories (see Table S3 for source of genotypes for each population).
178 Laboratory-specific DNA extraction, amplification, and genotyping methods can be found in
179 their respective publications (Table S3). Brook Trout from Wisconsin and Iowa were genotyped
180 at the Molecular Conservation Genetics Laboratory (University of Wisconsin-Stevens Point)
181 using methods similar to Ruzich et al. (2019). DNA was extracted with the Promega Wizard®
182 Genomic DNA purification kit (Promega Corp., Madison, Wisconsin), quantified using a
183 Nanodrop® ND-100 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware), and
184 normalized to a final concentration of 20 ng/µL. PCR amplification was conducted to amplify
185 microsatellite DNA loci, an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, California)
186 was used to separate PCR amplicons and determine allele sizes, and allele sizes were visually
187 verified using Genemapper and Geneious software packages (Life Technologies, Carlsbad,
188 California and Biomatters Inc., San Diego, California, respectively).

189 Genotypes were standardized across laboratories by comparing allele frequencies of
190 populations genotyped by two or more laboratories. If this was not possible, genetic samples
191 were exchanged among laboratories and independently genotyped to ensure consistent scoring
192 criteria (e.g. Wilson et al. 2008). Loci were removed from further analysis if they displayed high
193 rates of genotyping error or were not genotyped by all laboratories. This resulted in final
194 Midwest and inland Wisconsin datasets consisting of five and seven loci, respectively. Only
195 individuals with complete multilocus genotypes were included in this study. Additionally, only
196 populations with ≥ 20 individuals were retained for subsequent analysis. The resulting dataset
197 consisted of 8,454 individuals representing 188 wild populations (n = 7,367) and 26 hatchery
198 strains (n = 1,087; Table S3; Figure 1). All genotypes have been deposited on ScienceBase
199 (accession # to be assigned).

Loci and populations were tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using Genepop 4.5.1 (Raymond and Rousset 1995). Linkage disequilibrium and significant departures from Hardy-Weinberg equilibrium were evaluated using the false discovery rate method (Benjamini and Hochberg 1995).

*Diversity statistics*

*F*-statistics were calculated for loci included in Midwest and inland Wisconsin datasets using Genepop 4.5.1 (Weir and Cockerham 1984). Estimates of observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), and allelic richness rarefied to a sample size of 20 ($A_r$), were calculated for each population using the R package 'diveRsity' (Keenan et al. 2013). Effective population sizes ($N_e$) were calculated using NeEstimator v2.01 with an allele frequency cutoff of 0.02 and confidence intervals adjusted using the jackknife method (Waples and Do 2008; Do et al. 2014). The effective number of alleles per locus ($A_e$) and inbreeding coefficients ($F_{IS}$) were calculated for each population using GenAlEx 6.502 (Peakall and Smouse 2006, 2012). Estimates of genetic diversity were compared among state political boundaries and watersheds using pairwise Wilcoxon rank sum tests. Political boundaries included in these analyses serve as surrogates for differences in management strategies between state agencies. Watershed spatial extents considered in our genetic diversity comparisons range from the HUC2 level (e.g. Mississippi River and Great Lakes basins) to HUC6 (e.g. Mississippi River headwaters and Wisconsin River basins). Eastern-origin hatchery strains were also included as a group in all comparisons to investigate differences between wild populations and hatchery strains. Significance of these tests was assessed via the false discovery rate method (Benjamini and Hochberg 1995).

*Population structure*

223 Genetic population structure was assessed for both Midwest and inland Wisconsin
224 datasets through a combination of neighbor-joining trees, pairwise comparisons of $F_{ST}$, and
225 principal coordinate analysis (PCoA). Neighbor-joining trees were constructed using Nei's
226 genetic distance ($D_A$) and 1,000 bootstrap replicates in POPTREE2 and MEGA 6.0 (Nei et al.
227 1983, Felsenstein 1985, Takezaki et al. 2010, Tamura et al. 2013). Pairwise estimates of $F_{ST}$ were
228 calculated according to Weir and Cockerham (1984) and significance of differentiation was
229 assessed using exact *G* tests ($\alpha = 0.001$) in Genepop 4.5.1 (Tables S4 and S5; Raymond and
230 Rousset 1995; Goudet et al. 1996). The PCoA was performed on $F_{ST}$ matrices using GenAlEx
231 6.502. Analysis of molecular variance (AMOVA) was performed on the Midwest dataset using
232 state boundaries, HUC2, HUC4, and HUC6 watersheds as grouping criteria in the R package
233 'poppr' 2.8.5 (Kamvar et al. 2014, 2015). We included eastern origin hatchery strains as a
234 separate group in all comparisons. Similar tests were conducted for Wisconsin populations in the
235 Mississippi River basin. However, HUC4 and HUC6 watersheds are synonymous for our inland
236 Wisconsin study populations so estimates may be used interchangeably in this instance.

237 *Hatchery introgression*

238 Quantitative estimates of hatchery introgression for inland Wisconsin populations (i.e.
239 Mississippi River Basin) were obtained through discriminate analysis of principal components
240 (DAPC). However, the extensive, and often incomplete, stocking histories of the study
241 populations necessitated that we define native and hatchery assignment groups *a priori*.
242 Therefore, we applied methodology similar to the approach used by Kazyak et al. (2018) and
243 White et al. (2018) to identify putatively native populations. We calculated pairwise measures of
244 Nei's genetic distance ($D_A$) between all inland Wisconsin populations and the St. Croix Falls
245 strain, the most ubiquitous exogenous hatchery strain used in Wisconsin (Table S6). The average

246 genetic distance between each population and all other populations sampled across inland
247 Wisconsin was then compared to the genetic distance between each population and the St. Croix
248 Falls hatchery strain. Populations were considered putatively native if the genetic distance
249 between the St. Croix Falls hatchery strain was larger (i.e. more differentiated) than the average
250 genetic distance to the other inland Wisconsin populations.

251 Twenty individuals, corresponding to the minimum sample size, were randomly sampled
252 from each putatively native population and used to simulate a metapopulation of 500 native
253 Brook Trout using the *hybridize()* function in the R package 'adegenet' (Jombart 2008; Jombart
254 and Ahmed 2011). Similarly, multilocus genotypes from the St. Croix Falls strain were used to
255 simulate a population of 500 trout that was used as the hatchery assignment group. The resultant
256 DAPC model generated from these two assignment groups was subject to cross-validation where
257 the number of principal components retained minimized the mean square error. We then
258 introduced the empirical data from wild populations to this DAPC model and determined each
259 individual's probability of belonging to the simulated native and hatchery populations.
260 Population-level estimates of hatchery introgression were derived by averaging individual
261 assignment probabilities within populations. Adequacy of this classification framework was
262 assessed using classifications as groups with PCoA and an AMOVA and comparing results to
263 similar analyses assessing hydrological population structure.

264 Note that we limited our inferences to inland Wisconsin populations (i.e. Upper
265 Mississippi River basin) as pairwise comparisons among watersheds lacking hydrological
266 connections (i.e. no potential for natural gene flow) may upwardly bias average wild-wild
267 comparisons if sampling effort is not equally distributed among watersheds, as is the case here.
268 We also acknowledge that decades of genetic drift may lower the accuracy of introgression

269 estimates when analyzing contemporary samples of historically stocked populations and
270 hereafter refer to these introgression estimates as 'hatchery identity' (i.e. resemblance to St.
271 Croix Falls strain) to account for this uncertainty.

272 *Modeling the relationship between introgression and stocking practices*

273 Nested beta regressions were implemented in the R package 'betareg' to examine how
274 varying stocking practices affect the extent of hatchery identity in supplemented populations
275 (Cribari-Neto and Zeileis 2010). Covariates posited to influence rates of hatchery identity
276 included the total number of stocking events; the total number of fry (length ~ 20 mm),
277 fingerlings (length ~ 100 mm), and adults (length > 200 mm) stocked; and the amount of time
278 elapsed between stocking events and sample collections. However, defining the amount of time
279 elapsed between stocking events and sample collections is particularly challenging as most
280 populations have been repeatedly stocked and population-specific stocking timelines are highly
281 variable. We therefore adopted the approach of Létourneau et al. (2018) and calculated the
282 amount of time elapsed since the mean year of stocking and sample collection dates. Model
283 parsimony was assessed by comparing second-order Akaike information criterion (AICc) across
284 models with the expectation that the most plausible models possessed AICc values within two
285 units of the best approximating model and had biologically relevant parameter estimates (Arnold
286 2010).

287 **Results**

288 *Hardy-Weinberg and linkage disequilibrium*

289 Locus-specific tests for conformation to Hardy-Weinberg equilibrium resulted in 23 and
290 3 significant departures for the full Midwest and inland Wisconsin datasets, out of 1,070 and 462

tests, respectively. There were no discernable patterns for these departures with a maximum of three population-specific departures (Rome hatchery strain and Camp Hazard Creek) and 11 locus-specific departures (*Sfo115*) in the Midwest dataset. Similar patterns were observed for the inland Wisconsin dataset, with a maximum of one population-specific and locus-specific departure. Similarly, no discernable patterns were observed with the respect to the 29 and 20 population-specific locus pairs exhibiting linkage disequilibrium in the full Midwest and inland Wisconsin datasets, respectively. As such, all loci and populations were retained for subsequent analyses.

*Diversity statistics*

Wild populations in both the Great Lakes and Upper Mississippi watersheds were generally more diverse than eastern hatchery strains (Table 1; Table S3). We also found evidence that wild populations in Iowa are generally less diverse than wild populations in Minnesota and Wisconsin (Table 1; Table S3). Estimates of genetic diversity were variable between HUC4 watersheds, with less diverse populations being found in the Driftless Region (Table 1; Table S3). Overall, estimates of genetic diversity from populations in Iowa, the Black-Root drainage, and the Maquoketa-Plum drainage are more comparable to eastern hatchery strains than other states/drainages (Table 1; Table S3). Median effective population size estimates were 70.6, 112.5, and 85.3 for Midwestern, inland Wisconsin, and hatchery populations/strains, respectively (Tables S3 and S7).

*Population structure*

The neighbor-joining tree depicting genetic relationships among Midwestern Brook Trout collections suggests that populations are highly differentiated and that the underlying genetic

313 variation is not strongly aligned to watershed topology. This is evident as branches for individual
314 populations are long, distances between nodes are relatively small (in comparison to branch
315 lengths), and branching patterns do not reflect the HUC4 drainage basins of the sampling
316 locations (Figure 2). Similarly, pairwise estimates of $F_{ST}$ corroborate that wild populations are
317 significantly differentiated ($p < 0.001$; mean $F_{ST} = 0.15$) and the PCoA of this matrix did not
318 reveal any discernable patterns of hydrological population structure (Table S4; Figure 3c).
319 AMOVA results suggest that there is considerably more variation among populations within a
320 watershed (21.75-23.52%) than between watersheds (6.03-6.63%; Table 2). State boundaries
321 explained slightly more variation than watersheds. However, there was still substantially more
322 variation within states than between states.

323 The neighbor-joining tree depicting genetic relationships among inland Wisconsin Brook
324 Trout collections suggests a lack of hydrological population structure like that observed in the
325 full Midwestern dataset (Figure 4). However, we defined six distinct groups (A-F) in Figure 4
326 which we suggest may reflect distinct stocking histories. These groups were defined with respect
327 to the resolved tree topology, stocking records, and hatchery identity estimates discussed in the
328 subsection below. Hydrological population structure was largely absent in these groups (with the
329 exception of Group B). Results from PCoA also suggest minimal hydrological population
330 structure and high degrees of divergence within drainages, as opposed to among drainages
331 (Figure 3a). Our AMOVA results suggest weaker hydrological structuring in Wisconsin
332 compared to the broader Midwest with 2.26% and 20.75% of variance explained between and
333 within HUC4/HUC6 drainages, respectively (Table 2).

334 *Hatchery introgression*

A total of 41 putatively native and 24 putatively introgressed inland Wisconsin populations were identified via the genetic distance classification framework (Table S6; Figure 3b). Estimates of hatchery identity were highly variable and ranged from 0.0 – 59.4% (mean = 12.5%, SD = 14.5%) for the full inland Wisconsin dataset, 0.0 – 16.2% (mean = 4.0%, SD = 3.5%) for putatively native populations, and 10.5 – 59.4% (mean = 27.0%, SD = 14.7%) for putatively introgressed populations (Table S7; Figure 5). Estimates of hatchery identity for the six groups identified in the inland Wisconsin neighbor-joining tree were also highly variable (Table 3). The majority of populations with relatively high estimates of hatchery identity are located in the Driftless Region. In fact, only two populations located outside of the Driftless Region (South Fork of the Kinnickinnic River and Engle Creek) had hatchery identity estimates greater than 10% (Figure 5). The assignment accuracy of our DAPC approach was high for our assignment groups with 98.4% and 99.6% of the native metapopulation and hatchery strain being correctly reassigned, respectively. AMOVA results suggest that the classification framework explains approximately twice the amount of variation as hydrological structure (Table 2). Similarly, results from the genetic distance classification framework PCoA suggest that putatively native and putatively introgressed classifications explain more variation than hydrological structure (Figures 3a and 3b).

*Modeling the relationship between introgression and stocking practices*

Seven total models were constructed to investigate the relationship between stocking histories and hatchery identity (Table 4). The best performing model included only the number of years since the mean stocking event and was negatively correlated with the extent of hatchery identity. This model had a pseudo-$R^2$ value of 0.24 and possessed an AICc value 7.19 units lower than the next best fit model (Table 4; Figure 6).

## Discussion

*Diversity statistics*

Wild populations included in this study were generally more genetically diverse than hatchery collections. This was evident at multiple spatial scales including the state-level and HUC2, HUC4, and HUC6 watershed scales. The main exceptions to this trend were populations in the Black-Root and Maquoketa-Plum watersheds of the Driftless Region which displayed lower levels of genetic diversity more typical of eastern hatchery strains. Interestingly, we also observed higher estimates of hatchery identity in the Driftless Region. However, moderate levels of hatchery introgression (e.g. < 50%) are expected to increase the genetic diversity of populations through the introduction of exogenous alleles (Ozerov et al. 2016). Thus, it is plausible that higher estimates of hatchery identity and concurrently low estimates of genetic diversity in this region are the result of a complex history of habitat degradation and supplemental stocking. For example, there was a severe loss of quality trout habitat in many streams in the Driftless Region during the late 19$^{th}$ and early 20$^{th}$ centuries which likely reduced abundance and resulted in genetic bottlenecks. In this scenario, stocking would serve as artificial gene flow and partially mask a historical genetic bottleneck through the introduction of exogenous alleles, whereby historical bottlenecks or introgression would only be evident based on the presence of hatchery derived alleles.

We also found evidence that populations from the Great Lakes basin are slightly more diverse than populations from the Mississippi River basin as we observed significantly higher estimates of allelic richness in the Great Lakes basin. However, comparisons of the number of effective alleles per locus, observed heterozygosity, and expected heterozygosity were not statistically significant. These findings indicate that populations in the Great Lakes basin possess

a greater number of low frequency alleles. The occurrence of these low frequency variants may be indicative of higher connectivity or larger effective population sizes, which lessen the effects of genetic drift in the Great Lakes basin. Conversely, this may also be an artifact of poor land use practices in the Driftless Region that resulted in genetic bottlenecks or founder effects that effectively reduced genetic diversity.

Our findings also support other studies that suggest hatchery strains can be less diverse than wild populations (Leitwein et al. 2017; White et al. 2018; Beer et al. 2019). While the direct mechanisms behind reduced diversity in our hatchery collections are not known, they may be attributable to founder effects, mating strategies, or artificial selection resultant of either intentional or unintentional hatchery operations (Allendorf et al. 1987). These findings reiterate the importance of proper animal husbandry practices and genetic monitoring programs in hatchery settings.

*Population structure*

Populations included in this study were highly differentiated with very little of the underlying variation partitioned among drainages or political boundaries. Several factors may have contributed to the lack of hydrological population structure. First, we may have lacked the statistical power to detect such a relationship, as we used a limited number of genetic markers and Brook Trout populations are prone to genetic drift (due to low effective population sizes). However, this is unlikely a primary explanation as we observed high rates of genetic divergence among populations, and other endemic species display genetic population structure consistent with contemporary hydrological boundaries (Westbrook 2012; Euclide et al. 2020). Second, the complex glacial and hydrological history of the Midwest may have facilitated secondary contact between Brook Trout residing in Atlantic and Mississippian refugia and subsequently muddled

historical population structure (Danzmann et al. 1998). However, this is also unlikely as the contemporary boundary of the Great Lakes and Upper Mississippi watersheds has persisted for several millennia and thus would have yielded sufficient time for low abundance Brook Trout populations to differentiate. Third, low and heterogenous effective population sizes may have obscured population structure as genetic drift would be allowed to proceed at high and variable rates both within and among drainages. We did not directly investigate this possibility as the vast majority of our study populations had a large amount of uncertainty in effective population size estimates (i.e. 95% confidence intervals that spanned several orders of magnitude or included infinity; Tables S3 and S7) and thus would lead to vastly different evolutionary scenarios (Allendorf and Phelps 1981). Finally, the extensive history of supplemental stocking and translocations from heterogenous sources could have served to muddle the regional gene pool to levels which obscure natural population structure. We suggest that stocking, both from native and non-native strains, is the most likely explanation for the lack of hydrological population structure as several other studies in the Midwest have documented similar findings with intensively managed species (e.g. Hammen 2009; Westbrook 2012; Turnquist et al. 2017). However, these scenarios are not mutually exclusive, and a combination of these scenarios could explain the observed lack of hydrological population structure.

The genetic structure of inland Wisconsin populations paralleled what we observed across the Midwestern United States with highly differentiated populations on small spatial scales, high amounts of variation within watersheds, and relatively little variation partitioned among watersheds. While these patterns are contrary to theoretical expectations that the dendritic structure of hydrological systems restricts gene flow and gives rise to hierarchical genetic population structure, we suggest that the observed lack of hydrological structuring may be a

result of the extensive and complex stocking histories of the study populations. For example, we observed six distinct population groupings which we classified as primarily eastern hatchery ancestry (A & C), native ancestry (B & F), and mixed ancestry (D & E; Figure 4).

Group C strongly suggests introgression of the St. Croix Falls hatchery strain that has been extensively stocked from 1972 to the present. This group contains the St. Croix Falls strain as well as Ash Creek, which was used as a locally derived broodsource from 2001 to 2017. Collectively, these two strains account for approximately 85% of Brook Trout stocking events in Wisconsin waters from 2001 to 2017. Several populations in this group (Harker, Seas Branch, Upper Duncan, Gran Grae, Fancy, and Ash creeks) were stocked with undocumented strains from 1972 to 1990. However, the St. Croix Falls strain has been used to produce the majority of Brook Trout since its transfer to Wisconsin hatcheries in 1972. Therefore, it is likely that the genetic affinities of these populations and the St. Croix Falls strain are a result of introgression. We also observed several populations in this group (Brush, Fancy, Hynek Hollow, and Hay creeks) which have been stocked with Ash Creek progeny and may represent the inadvertent spread of St. Croix Falls alleles through the use of an introgressed broodsource. Finally, we observed several populations in this group (Harker, Seas Branch, Maple Dale, and Gran Grae creeks) that received wild brook trout translocations from 1991-2001. The wild source of these translocations is largely undocumented; although, they likely originated from Upper Duncan Creek as this population was concurrently being used as the source for other translocations and resolves closely with the recipient streams (K. Olson, Wisconsin Department of Natural Resources, pers. comm.; Figure 4).

Group A might also be of non-native origin due to the large genetic distances observed within the group and between other groups. These types of patterns might be expected if a highly

450 divergent strain introgressed with a subset of native Wisconsin populations or if multiple
451 hatchery strains introgressed to varying degrees within a subset of populations. Indeed, several of
452 these populations (Parfrey's Glen, North Chipmunk Coulee, Manley, and John Coulee creeks)
453 share affinities with other eastern origin hatchery strains (Figure S1). Therefore, it is plausible
454 that this group represents historical introgression from genetically divergent undocumented
455 hatchery strains which may have been used prior to 1972. The West Branch of Mill Creek also
456 falls within this group and was used as a locally derived broodsource from 1997 to 2001. Thus,
457 stocking of West Branch Mill Creek progeny may have facilitated the spread of non-native
458 alleles during this time period.

459 We further identified two putatively native groups (B & F) based off relatively large
460 genetic distances to hatchery strains and a lack of historical stocking. Interestingly, group B
461 consists primarily of populations from the St. Croix drainage which suggests that this drainage
462 may represent a distinct evolutionary lineage. In contrast, we did not observe any further
463 hydrological structuring in group F which may be due to a higher degree of divergence between
464 the St. Croix drainage and all other sampled drainages or historical introgression of locally
465 derived broodstock. We also identified two groups (D & E) which likely represent native
466 populations that have introgressed with the St. Croix Falls or Ash Creek hatchery strains to a
467 moderate degree (in comparison to group C).

468 While we identified Brook Trout populations that appear to retain Wisconsin's genetic
469 heritage, this does not imply that these populations have been unaffected by hatchery operations.
470 In fact, we suggest that over a century of stocking both exogenous and locally derived hatchery
471 strains has eroded natural population structure. Indeed, genetic data from several other endemic
472 species (Smallmouth Bass, *Micropterus dolomieu*; Rock Bass, *Ambloplites rupestris*; and Johnny

Darter, *Etheostoma nigrum*) supports this claim as these species have been shown to exhibit hydrologic population structure in the Midwest (Westbrook 2012; Euclide et al. 2020). However, there are two main differences between Midwestern Brook Trout and these sympatric species. First, Brook Trout are primarily a cold-water species and are more likely to be affected by anthropogenic disturbance than more generalist warmwater fishes. As such, contemporary Brook Trout population structure has likely been more affected by historical extirpations or reductions in abundance (i.e. greater potential for genetic drift) than warmwater species. Second, the aforementioned warmwater species have not been stocked nearly as intensively as Brook Trout. Indeed, more intensively stocked species (i.e. Walleye, *Sander vitreus*; Muskellunge, *Esox musquinongy*) have been shown to exhibit less hydrological population structure in the Midwest, likely as a result of hatchery introgression (Hammen 2009; Westbrook 2012; Turnquist et al. 2017). These two processes in conjunction may have served to homogenize the regional gene pool or otherwise obscure Brook Trout population structure to levels undetectable by this study. Finally, while hatchery identity estimates were low for putatively native populations, it is important to recognize that these estimates were derived using an eastern-origin hatchery strain as a reference. As such, we would not be able to accurately quantify introgression from locally derived hatchery strains which may have been used from 1888 to 1972 (Fields and Philipp 1998).

*Hatchery introgression and modeling the relationship between introgression and stocking practices*

Estimates of hatchery identity were generally low, but variable. The vast majority of populations with relatively high hatchery identity estimates occurred in the Driftless Region, which was once considered a stronghold for native trout (Threinen and Poff 1963). However, nearly a century of poor land use practices had largely extirpated trout from this area, leading to

widespread stocking through much of the 20th century to support recreational fisheries (Vetrano 2017). Stream conditions in the Driftless Area improved throughout the latter part of the 20th century and soon Brook Trout populations became reestablished, likely with complete or partial hatchery ancestry (Juckem et al. 2008; Vetrano 2017). These populations may have been founded from a single hatchery strain, multiple strains, or a combination of remnant native populations and hatchery strains. Further, it is likely that the recolonization process induced founder effects which reduced genetic diversity in these populations and quickly differentiated them.

Stocking record beta regressions indicated similarities and differences in respect to previous studies. First, the amount of time elapsed since the mean year of stocking was significantly correlated with decreased estimates of hatchery identity. This finding is similar to previous studies of historical hatchery introgression that suggested that this phenomenon may be attributable to either selection against hatchery ancestry or high rates of genetic drift (Perrier et al. 2013; Harbicht et al. 2014; Valiquette et al. 2014; ). Second, several other studies have shown that hatchery introgression is positively correlated with stocking intensity (Almodódovar et al. 2006; Marie et al. 2010; Lamaze et al. 2012; ). However, we did not observe these relationships with any of the life stages examined. The lack of these relationships may be due to a multitude of reasons including reduced competition among native and stocked trout following previous extirpations, varying relative fitness among hatchery strains, and uncertainty regarding the genetic identity of historical hatchery strains.

While our models suggest that historically supplemented populations may become increasingly dissimilar from hatchery strains over time, it should be noted that it is still unclear whether this phenomenon is a result of selection against hatchery ancestry or high rates of

519 genetic drift that allow populations to rapidly differentiate from hatchery sources. We estimated
520 effective population sizes for Midwest and inland Wisconsin populations and found them to be
521 relatively low (medians = 70.6 and 112.5, respectively), but a large amount of uncertainty
522 surrounds these estimates due to the low number of markers used in this study (Tables S3 and
523 S7). We recommend that future studies seeking to identify the causal mechanisms of this
524 phenomenon obtain more precise estimates of genetic drift, quantify rates of gene flow from
525 adjoining populations, and estimate the relative importance of local adaptations and
526 domestication selection. Genomic techniques will likely prove fruitful in these endeavors as
527 increased numbers of loci will provide more precise estimates of effective population sizes and
528 allow for the reconstruction of hatchery-derived haplotypes which can provide insights into the
529 relative importance and scale of natural selection (Waples and Do 2010; Leitwein et al 2019)

530 *Conclusion*

531 Findings of this study suggest that a complex history of habitat degradation and stocking
532 has largely eroded the natural population structure of Midwestern brook trout. Yet, most
533 populations remain highly differentiated, likely due to high rates of genetic drift in populations
534 with small effective sizes. We also observed relatively low estimates of hatchery identity in the
535 majority of our inland Wisconsin study populations and found that wild populations were
536 generally more genetically diverse than eastern hatchery strains. The main exceptions to this
537 trend were populations in the Driftless Region that displayed concurrently high estimates of
538 hatchery identity and low genetic diversity that may be attributed to historical bottlenecks and
539 subsequent introgression. Our results also indicated that introgressed populations may initially
540 resemble their hatchery source and become more divergent through time. The direct mechanisms
541 behind this phenomenon are not well understood, but may reflect background selection against

hatchery ancestry (due to local adaptation, domestication selection, or both) that is maintained via gene flow from adjoining populations. Conversely, we also caution that this phenomenon may be an artifact of genetic drift as the relatively small effective population sizes observed in this study may allow hatchery-founded or introgressed populations to rapidly differentiate from hatchery strains and subsequently lead to underestimates of hatchery identity that are exacerbated with time. Collectively, these findings summarize the genetic effects of over a century of anthropogenic disturbance on an economically important sportfish, exemplify the complexities of inferring population structure of intensively managed species, highlight the importance of incorporating historical observations into contemporary empirical research, and illustrate the benefits of using standardized suites of genetic markers.

**Acknowledgements**

This project would not have been possible without the assistance of innumerable state, federal, provincial, and university personnel. We also thank and recognize the late Dr. Tim King for his coordinated brook trout research efforts that facilitated our metanalysis and contributed to many other brook trout conservation initiatives. We are also appreciative of Barb Lubinski, Mike Eackles, and Robin Johnson for their roles in genotyping eastern origin hatchery strains as well as Dr. Shannon White for reviewing an early draft of this manuscript. Funding was provided by the U.S. Geological Survey; National Park Service; Ontario Ministry of Natural Resources; Wisconsin, Minnesota, and Iowa Departments of Natural Resources; North Carolina Wildlife Resources Commission; Great Lakes Fishery Commission; Minnesota Sea Grant; Iowa's Fish and Wildlife Trust Fund; Maine Agricultural and Forest Experiment Station (University of Maine/USDA – contribution # ); National Fish and Wildlife Foundation; Iowa Chapter of the American Fisheries Society; Trout Unlimited; and Fly Fishers International. Any use of trade,

product, or company name is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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781 **Tables**

782 Table 1. Estimates of allelic richness ($A_r$) rarefied to a sample size of 20, effective number of
783 alleles per locus ($A_e$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), and
784 inbreeding coefficients ($F_{IS}$) for 187 Midwestern Brook Trout populations and 22 eastern origin
785 hatchery strains at several spatial scales which correspond to political or hydrological
786 boundaries. All populations were genotyped at 5 microsatellite loci and group-specific estimates
787 were derived by averaging across the populations within the group ($N$). Superscripts correspond
788 to significant differences ($p < 0.05$) at each scale following pairwise Wilcoxon rank sum tests
789 and multiple test corrections using the false discovery method.

| Spatial Extent | $N$ | $A_r$ | $A_e$ | $H_o$ | $H_e$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| **State** | | | | | | |
| a. Iowa | 13 | $3.94^{c,d}$ | 2.94 | $0.66^{b,e}$ | 0.63 | -0.051 |
| b. Michigan | 10 | 4.66 | 2.84 | $0.56^{a,c,d}$ | $0.58^{d}$ | 0.043 |
| c. Minnesota | 77 | $4.51^{a,d}$ | 3.15 | $0.65^{b,e}$ | $0.64^{e}$ | -0.023 |
| d. Wisconsin | 87 | $4.92^{a,c,e}$ | $3.23^{e}$ | $0.65^{b,e}$ | $0.65^{b,e}$ | -0.002 |
| e. Hatchery | 22 | $3.92^{d}$ | $2.73^{d}$ | $0.55^{a,c,d}$ | $0.55^{c,d}$ | 0.006 |
| **HUC2 Watershed** | | | | | | |
| a. Great Lakes Region | 41 | $5.09^{b,c}$ | $3.21^{b}$ | 0.62 | $0.64^{b}$ | $0.034^{c}$ |
| b. Hatchery | 22 | $3.92^{a,c}$ | $2.73^{a,c}$ | $0.55^{c}$ | $0.55^{a,c}$ | 0.006 |
| c. Upper Mississippi Region | 146 | $4.55^{a,b}$ | $3.14^{b}$ | $0.66^{b}$ | $0.64^{b}$ | $-0.024^{a}$ |
| **HUC4 Watershed** | | | | | | |
| a. Chippewa | 14 | $5.03^{b,i,j}$ | 3.25 | 0.66 | 0.67 | 0.006 |
| b. Hatchery | 22 | $3.92^{a,e,k,l}$ | 2.73 | 0.55 | 0.55 | 0.006 |
| c. Mississippi Headwaters | 3 | 4.93 | 3.40 | 0.67 | 0.67 | -0.015 |
| d. Northeastern Lake Michigan | 1 | 6.66 | 4.70 | 0.78 | 0.75 | -0.033 |
| e. Northwestern Lake Michigan | 16 | $5.32^{b,i,j}$ | 3.40 | 0.68 | 0.67 | -0.005 |
| f. Rock | 1 | 6.01 | 4.60 | 0.76 | 0.77 | 0.015 |
| g. Southern Lake Superior | 3 | 4.45 | 2.68 | 0.52 | 0.57 | 0.078 |
| h. St. Croix River | 10 | 4.74 | 3.30 | 0.66 | 0.65 | -0.013 |
| i. Black-Root | 68 | $4.41^{a,e}$ | 3.11 | 0.66 | 0.63 | $-0.039^{k}$ |
| j. Maquoketa-Plum | 19 | $4.16^{a,e,l}$ | 2.96 | 0.65 | 0.63 | $-0.033^{k}$ |
| k. Western Lake Superior | 21 | $4.94^{b}$ | 3.06 | 0.58 | 0.61 | $0.061^{i,j,l}$ |
| l. Wisconsin River | 31 | $4.74^{b,j}$ | 3.16 | 0.65 | 0.64 | $-0.006^{k}$ |

790

Table 2. Analysis of molecular variance for 188 Midwestern Brook Trout populations and 22 eastern origin hatchery strains. Midwestern and eastern origin hatchery strains were genotyped at 5 microsatellite loci while inland Wisconsin populations and the St. Croix Falls hatchery strain were genotyped at 7 microsatellite loci. Note that HUC4 and HUC6 watersheds are identical for inland Wisconsin populations.

| | Midwest | | | | Inland Wisconsin | | | |
|---|---|---|---|---|---|---|---|---|
| | **d.f.** | **SSQ** | **Variance** | **Variance (%)** | **d.f.** | **SSQ** | **Variance** | **Variance (%)** |
| **HUC2 Watersheds** | | | | | | | | |
| Among groups | 3 | 706.31 | 0.15 | 6.59 | - | - | - | - |
| Among populations within groups | 206 | 4,720.20 | 0.54 | 23.52 | - | - | - | - |
| Within populations | 8089 | 12,993.66 | 1.61 | 69.88 | - | - | - | - |
| **HUC4 Watersheds** | | | | | | | | |
| Among groups | 12 | 1,238.01 | 0.14 | 6.03 | 6 | 311.81 | 0.07 | 2.26 |
| Among populations within groups | 197 | 4,188.50 | 0.50 | 22.34 | 59 | 1,859.19 | 0.64 | 20.75 |
| Within populations | 8089 | 12,993.66 | 1.61 | 71.63 | 2,946 | 6,945.77 | 2.36 | 76.98 |
| **HUC6 Watersheds** | | | | | | | | |
| Among groups | 15 | 1,423.21 | 0.15 | 6.63 | 6 | 311.81 | 0.07 | 2.26 |
| Among populations within groups | 194 | 4,003.30 | 0.49 | 21.75 | 59 | 1,859.19 | 0.64 | 20.75 |
| Within populations | 8089 | 12,993.66 | 1.61 | 71.62 | 2,946 | 6,945.77 | 2.36 | 76.98 |
| **State Boundaries** | | | | | | | | |
| Among groups | 4 | 1,049.17 | 0.17 | 7.33 | - | - | - | - |
| Among populations within groups | 205 | 4,377.35 | 0.50 | 22.13 | - | - | - | - |
| Within populations | 8089 | 12,993.66 | 1.61 | 70.54 | - | - | - | - |
| **Classifications** | | | | | | | | |
| Among groups | - | - | - | - | 2 | 302.21 | 0.16 | 5.08 |
| Among populations within groups | - | - | - | - | 63 | 1,868.79 | 0.60 | 19.29 |
| Within populations | - | - | - | - | 2,946 | 6,945.77 | 2.36 | 75.63 |

Table 3. Hatchery identity estimates for the six groups (A-F) depicted in the inland Wisconsin neighbor-joining tree (Figure 4). These estimates were used in conjunction with the resolved tree topology and stocking records to conclude that groups A and C have been substantially affected by historical hatchery introgression, groups B and F are primarily of native ancestry, and groups D and E are of mixed ancestry.

| Group | Mean hatchery identity | Standard deviation |
|---|---|---|
| A | 22.9% | 16.6% |
| B | 1.5% | 2.0% |
| C | 20.2% | 20.8% |
| D | 16.2% | 6.5% |
| E | 5.4% | 3.7% |
| F | 4.1% | 3.5% |

Table 4. Relative likelihoods for stocking record beta regression models predicting the mean hatchery identity of 38 wild inland Wisconsin Brook Trout populations with documented stocking histories. The global model is an additive model of all variables and no interactions thereof. Years since mean stocking corresponds to the number of years elapsed since the mean year of all population-specific stocking events; number of stocking events corresponds to the total number of stocking events in each population; and fry (length ~ 20mm), fingerlings (length ~ 100mm), and adults (length > 200mm) correspond to the total number of individuals for each life stage stocked into populations.

| **Model** | **Variables** | **$\beta_1 \pm$ SE** | **P-value** | **K** | **AICc** | **ΔAICc** | **Weight** | **LL** | **Pseudo-$R^2$** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Years Since Mean Stocking | -0.05355 ± 0.01337 | < 0.001 | 3 | -85.63 | 0.00 | 0.96 | 46.17 | 0.24 |
| Global | Years Since Mean Stocking | -0.05646 ± 0.01328 | < 0.001 | 7 | -78.44 | 7.19 | 0.03 | 48.09 | 0.29 |
| | Fry | -0.00001 ± 0.00001 | 0.232 | | | | | | |
| | Fingerlings | -0.00002 ± 0.00001 | 0.109 | | | | | | |
| | Adults | -0.00002 ± 0.00002 | 0.196 | | | | | | |
| | Number of Stocking Events | 0.02330 ± 0.01802 | 0.196 | | | | | | |
| Null | None | - | - | 2 | -75.14 | 10.49 | 0.01 | 39.74 | NA |
| 2 | Adults | -0.00001 ± 0.00001 | 0.609 | 3 | -73.07 | 12.56 | 0.00 | 39.89 | 0.01 |
| 3 | # Events | 0.00411 ± 0.00913 | 0.653 | 3 | -72.99 | 12.63 | 0.00 | 39.85 | 0.01 |
| 4 | Fingerlings | 0 ± 0.00001 | 0.942 | 3 | -72.78 | 12.85 | 0.00 | 39.74 | 0.00 |
| 5 | Fry | 0 ± 0.00001 | 0.982 | 3 | -72.78 | 12.85 | 0.00 | 39.74 | 0.00 |

Table S1. Stocking summary statistics used to investigate factors modulating the extent of hatchery introgression in 38 inland Wisconsin Brook Trout populations with documented stocking histories. Introgression estimates are provided as well as the number of stocking events ($N_{events}$); years elapsed since the mean year of all stocking events ($Y_{SinceMean}$); and the total number of fry (length ~ 20mm), fingerlings (length ~ 100mm), and adults (length > 200mm) stocked into each population.

Table S2. F-statistics and the number of alleles observed (*A*) for loci used in Midwest and inland Wisconsin Brook Trout datasets.

Table S3. Measures of genetic diversity estimated from 5 microsatellite loci for 188 wild Midwestern Brook Trout populations and 26 hatchery strains. Sample sizes (N), allelic richness rarefied to sample size of 20 ($A_r$), effective number of alleles per locus ($A_e$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), inbreeding coefficients ($F_{IS}$), effective population sizes ($N_e$), political locations, hydrological locations, and data sources are provided.

Table S4. Pairwise $F_{ST}$ matrix for 188 Midwestern Brook Trout populations and 26 hatchery strains genotyped at five loci. Asterisks denote exact *G* tests which indicated significantly divergent populations ($p < 0.001$). P-values are otherwise reported for non-significant tests.

Table S5. Pairwise $F_{ST}$ matrix for 65 Brook Trout populations from inland Wisconsin and the St. Croix Falls hatchery strain genotyped at seven loci. Asterisks denote exact *G* tests which indicated significantly divergent populations ($p < 0.001$). P-values are otherwise reported for non-significant tests.

Table S6. Pairwise matrix of Nei's genetic distance and classifications for 65 Brook Trout populations from inland Wisconsin and the St. Croix Falls hatchery strain genotyped at seven loci.

Table S7. Measures of genetic diversity and hatchery introgression estimates derived from 7 microsatellite loci for 65 wild Brook Trout population from inland Wisconsin and the St. Croix Falls hatchery strain. Introgression estimates, allelic richness rarefied to sample size of 20 ($A_r$), effective number of alleles per locus ($A_e$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), inbreeding coefficients ($F_{IS}$), and effective population sizes ($N_e$) are provided.

**Figure Captions**

Figure 1. Sampling locations for 188 wild Midwestern Brook Trout populations included in this study. Populations are color coded by HUC4 drainage and grey lines correspond to HUC2 drainage boundaries in the United States.

Figure 2. Neighbor-joining tree of Nei's genetic distance ($D_A$) for 188 wild Midwestern Brook Trout populations and 26 hatchery strains. Branches are color coded by HUC4 drainage. Population names and node statistics have been omitted due to printing constraints. However, a labeled version can be found in the supplementary materials (Figure S1).

Figure 3. Principal coordinate analysis of pairwise estimates of $F_{ST}$ for (a) 65 inland Wisconsin Brook Trout populations and the St. Croix Falls hatchery strain color coded by HUC4 drainage, (b) 65 inland Wisconsin Brook Trout populations and the St. Croix Falls hatchery strain color coded by the classification framework used to simulate a metapopulation of putatively native trout from inland Wisconsin, and (c) 188 Midwestern Brook Trout populations and 22 eastern origin hatchery strains color coded by HUC4 drainage.

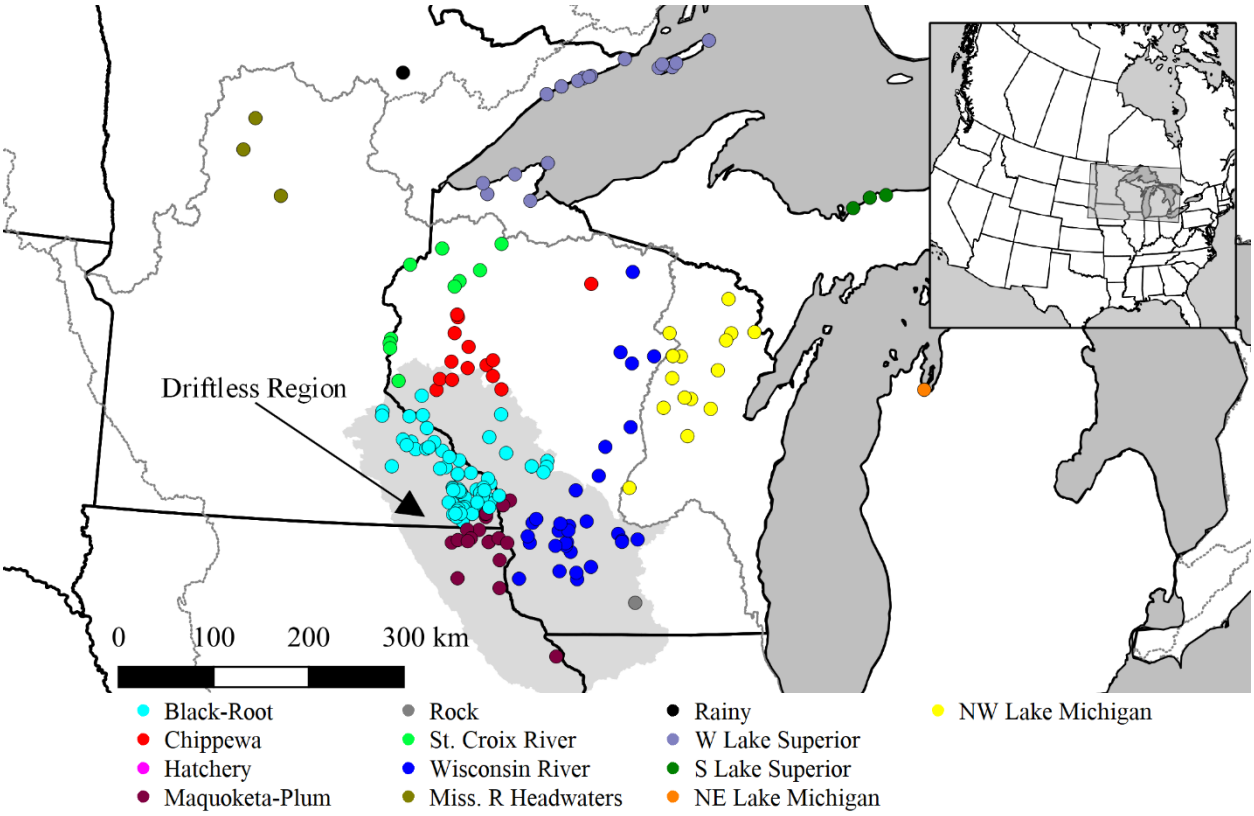
Figure 4. Neighbor-joining tree of Nei's genetic distance ($D_A$) for all 65 wild Brook Trout populations from inland Wisconsin and the St. Croix Falls hatchery strain. Blue branches denote populations in the Wisconsin River watershed, red branches denote populations in the Chippewa River watershed, green branches denote populations in the St. Croix watershed, cyan branches denote populations in the Upper Mississippi-Black-Root watershed, purple branches denote populations in the Upper Mississippi-Maquoketa-Plum watershed, grey branches denote populations in the Rock River watershed, and the pink branch denotes the St. Croix Falls hatchery strain. Groups A-F correspond to inferred groups of varying ancestry. Group C reflects

a St. Croix Falls strain hatchery ancestry, group A reflects a hatchery ancestry from one or more strains which have not yet been characterized, groups B and F reflect native ancestry, and groups D and E reflect native ancestry with low amounts of introgression from the St. Croix Falls strain.

Figure 5. Hatchery identity estimates and geographic locations of 65 wild Brook Trout populations from inland Wisconsin. Black lines denote HUC2 watershed boundaries and grey lines denote HUC4 watershed boundaries.

Figure 6. Relationship between the mean extent of hatchery introgression and the amount of time elapsed since the mean year of stocking. Black dots represent observed values from 38 wild inland Wisconsin Brook Trout populations with documented stocking histories and shaded areas represent 95% prediction intervals.

**Figures**

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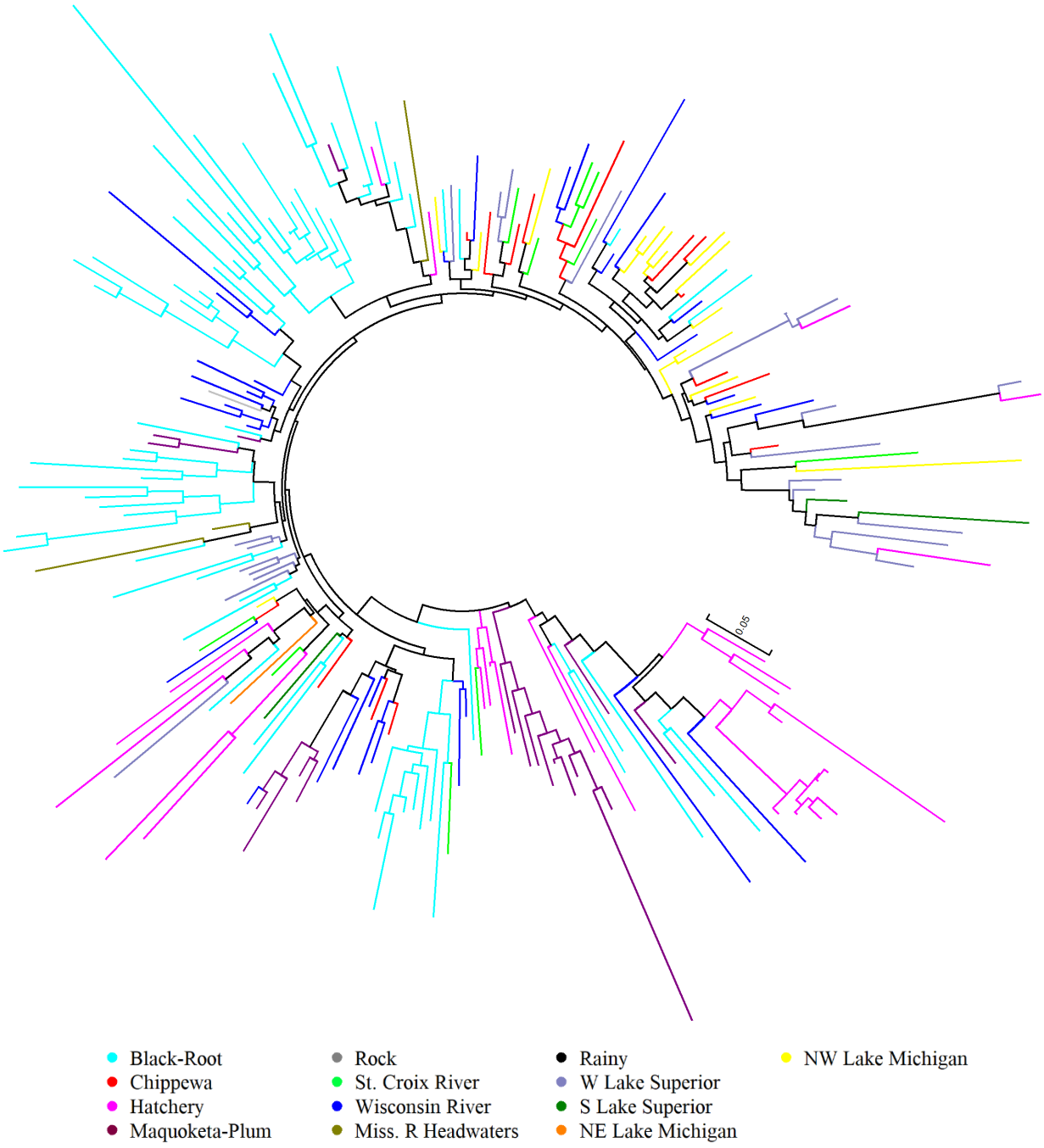


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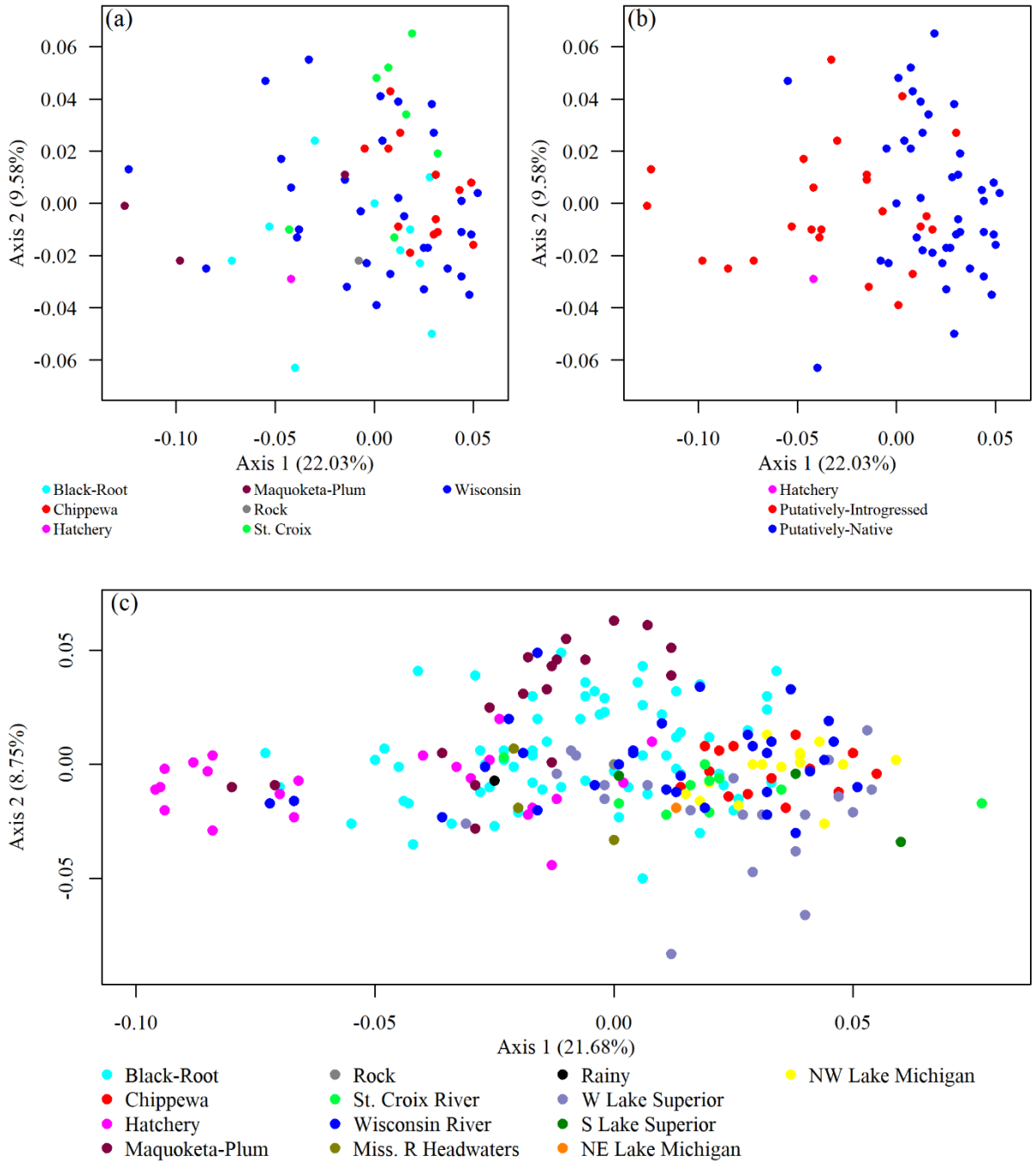


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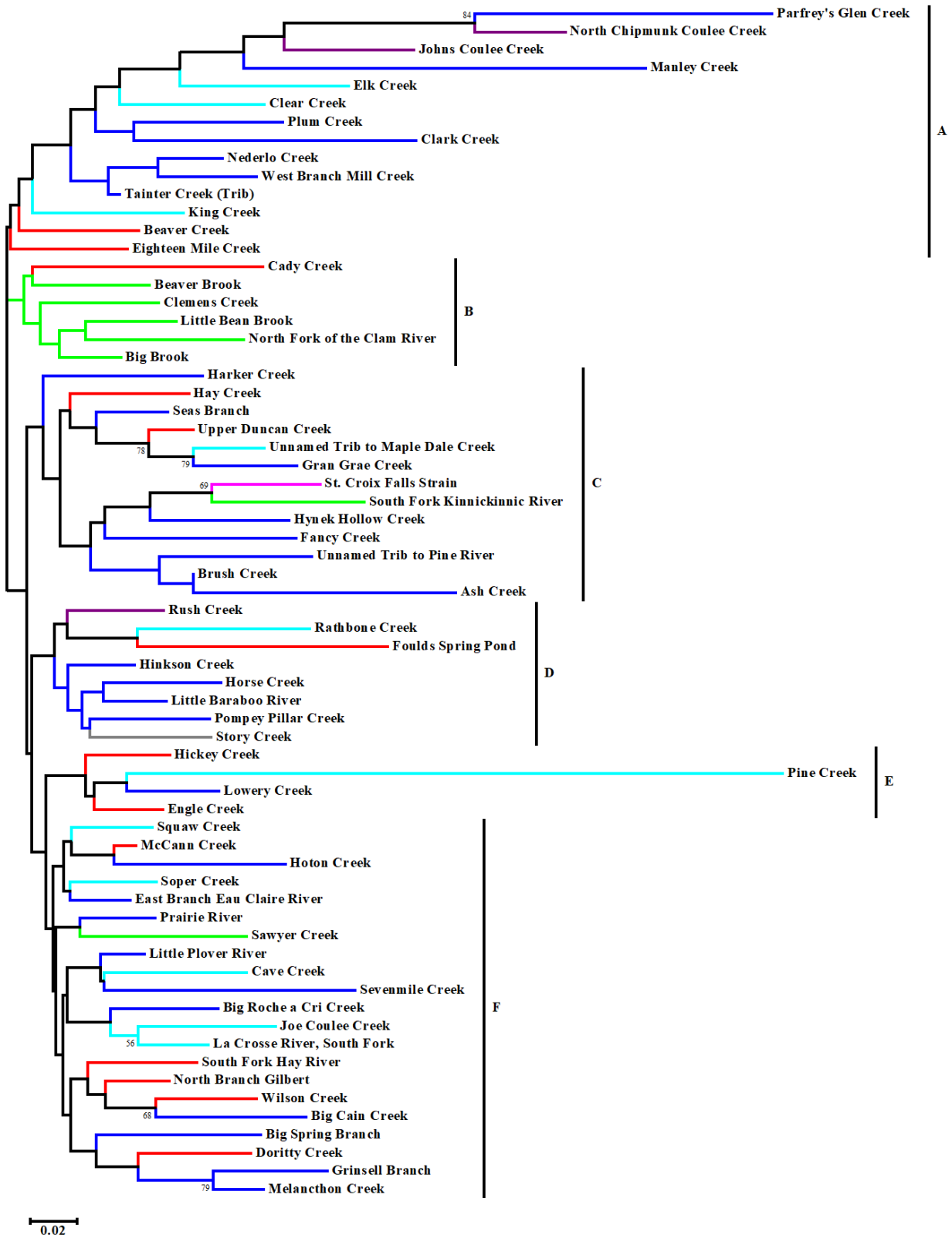
coded by the classification framework used to simulate a metapopulation of putatively native trout from inland Wisconsin, and (c) 188 Midwestern Brook Trout populations and 22 eastern origin hatchery strains color coded by HUC4 drainage.


84
Parfrey's Glen Creek
North Chipmunk Coulee Creek
Johns Coulee Creek
Manley Creek
Elk Creek
Clear Creek
Plum Creek
Clark Creek
Nederlo Creek
West Branch Mill Creek
Tainter Creek (Trib)
King Creek
Beaver Creek
Eighteen Mile Creek
A
Cady Creek
Beaver Brook
Clemens Creek
Little Bean Brook
North Fork of the Clam River
Big Brook
B
Harker Creek
Hay Creek
Seas Branch
Upper Duncan Creek
78
Unnamed Trib to Maple Dale Creek
79
Gran Grae Creek
69
St. Croix Falls Strain
South Fork Kinnickinnic River
Hynek Hollow Creek
Fancy Creek
Unnamed Trib to Pine River
Brush Creek
Ash Creek
C
Rush Creek
Rathbone Creek
Foulds Spring Pond
Hinkson Creek
Horse Creek
Little Baraboo River
Pompey Pillar Creek
Story Creek
D
Hickey Creek
Pine Creek
Lowery Creek
Engle Creek
E
Squaw Creek
McCann Creek
Hoton Creek
Soper Creek
East Branch Eau Claire River
Prairie River
Sawyer Creek
Little Plover River
Cave Creek
Sevenmile Creek
Big Roche a Cri Creek
Joe Coulee Creek
56
La Crosse River, South Fork
South Fork Hay River
North Branch Gilbert
Wilson Creek
68
Big Cain Creek
Big Spring Branch
Doritty Creek
Grinsell Branch
79
Melancthon Creek
F
0.02

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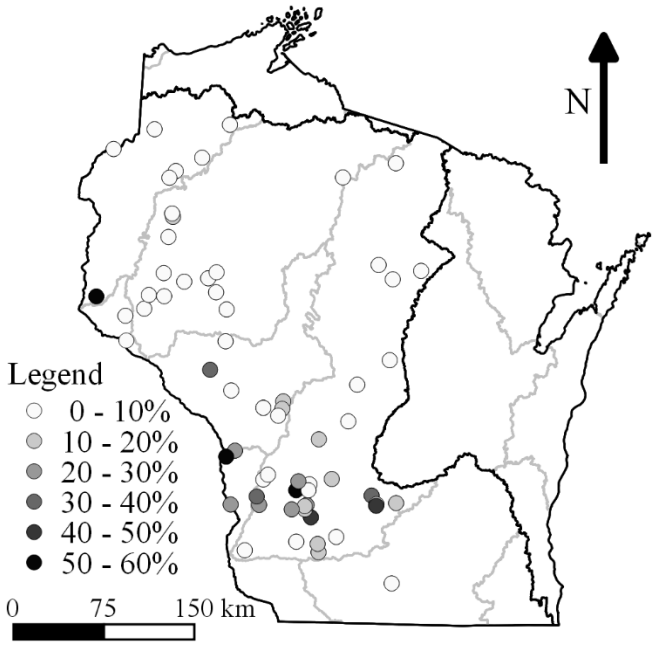


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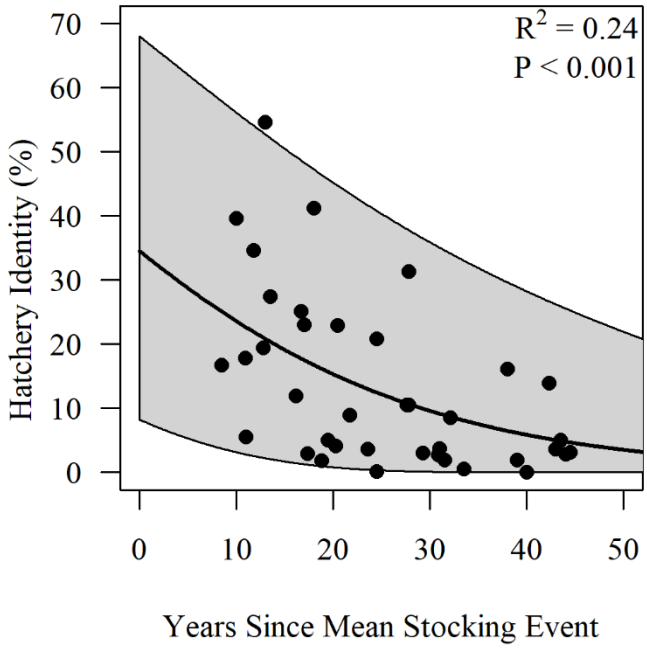


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