GENETIC STRUCTURE OF ROCK BASS AND JOHNNY DARTERS: IMPLICATIONS FOR GAMEFISH MANAGEMENT IN WISCONSIN

by

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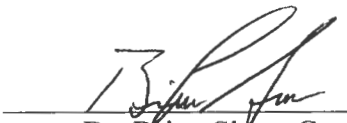

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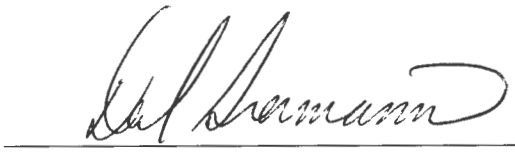

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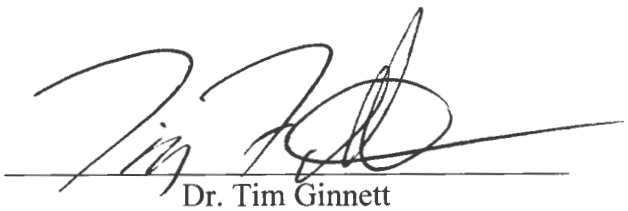

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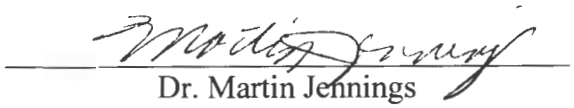

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ABSTRACT

Understanding the patterns of spatial genetic structure within and among populations has become a critical component of fisheries management practices. The hierarchical genetic structure of aquatic organisms is highly influenced by watershed boundaries or other key geological features. Previous genetic research on walleye (*Sander vitreus*) and muskellunge (*Esox masquinongy*) across their native range in Wisconsin showed sampled populations in the Upper Chippewa River watershed were more similar to fish from the neighboring Upper Wisconsin River watershed than they were to fish in the lower reaches of the Chippewa River watershed. The discordance is likely the result of glacial processes or widespread, cross-boundary stocking. The underlying cause is important in implementing stock-based management of coolwater gamefish in Wisconsin. The objective of this study was to determine if the previously observed genetic structures for walleye and muskellunge populations in Wisconsin are likely the result of zoogeographic processes or anthropogenic events. Three primary sub-objectives were: (1) to determine if rock bass exhibit genetic structure among populations in northern Wisconsin, (2) to determine if johnny darters exhibit genetic structure among populations in northern Wisconsin, and (3) to determine if the genetic structures of rock bass and johnny darter are consistent with the previously identified genetic structures of walleye and muskellunge. The genetic structures were evaluated by sampling 22 rock bass populations and 16 johnny darter populations in the five major watersheds in northern Wisconsin. Genetic diversity was assessed with 10 microsatellite markers for rock bass and 14 for johnny darters. A modified genetic stock identification (GSI) method using a Bayesian hierarchical process for determining genetic structure across a

landscape combined with analysis of molecular variance (AMOVA) was employed to delineate genetic structure. Analysis of genetic structures of rock bass and johnny darters revealed 14 and 16 unique genetic units, respectively. Because of limitations in managing a large number of genetic units, putative management groups were identified using AMOVA and a minimum ratio of among group variance (V_a) to within-group variance (V_b) of one. This approach identified nine genetic units for rock bass and eight for johnny darters. The rock bass and johnny darter genetic structures were inconsistent with the previously identified walleye and muskellunge genetic structures, but were consistent with watershed boundaries. The current genetic structures of walleye and muskellunge are most likely the result of past cross-watershed boundary stockings rather than natural biogeography. Two management alternatives should be considered in the future management of walleye and muskellunge in northern Wisconsin. The first option is to manage based on the contemporary genetics and risk altering communities downstream. The second alternative is to manage based on contemporary watershed boundaries and risk altering populations that have already been altered through past anthropogenic events.

ACKNOWLEDGEMENTS

Special thanks go out to the Wisconsin Department of Natural Resources and Sport Fish Restoration Act for funding this research. I would also like to thank my graduate advisor, Dr. Brian Sloss for all his guidance through the process, patience as I evolved not only in the lab but also in the field, and help in teaching me that fish are not as bad as I initially thought. I would like to acknowledge and profusely thank Andrea Musch for all her guidance, help, support, and swimming lessons. Thanks to Ryan Franckowiak for pushing me in the lab and in reading so I could become a better scientist. I would not have been able to finish this project without all the equipment and facilities supplied by the Wisconsin Cooperative Fishery Research Unit. I would also like to Dr. Michael Bozek for being a mentor for the past two years. Thanks go out to Dr. Martin Jennings for his insights in my sampling design and help in sample collection as well as being a member of my committee. I would also like to thank various employees of the Wisconsin Department of Natural Resources for collecting samples and providing me information about rock bass and johnny darter populations. Thanks to Dr. Dan Isermann and Dr. Tim Ginnett for being a part of my committee and for their insight and help. Thanks to my field technicians Brandon Gilbertson and Adam Grunwald. Special thanks to all the graduate students and undergraduates whose patience, encouragement, support and knowledge helped me to gain perspective in science and evolve as a better person, also for becoming my family and home away from home. Finally I would like to thank my family for their understanding and patience when I didn't make it home and for all their support as I worked my way through this research. To my Mom and Dad for being

supportive of my need to go where ever the wind blows me. To my sister whose life advice was always welcome when days were hard and homesickness overwhelming.

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INTRODUCTION

Understanding the patterns of spatial genetic structure within and among populations has become a critical component of fisheries management practices. Genetic structure can be defined as the distribution of genetic diversity across a landscape or the non-random spatial distribution of genotypes resulting from different processes (Vekemans and Hardy 2004). Species are seldom entirely panmictic and are typically composed of subpopulations or stocks that are at least partially reproductively isolated and differentiated from one another (Shaklee and Currens 2003). Just as species are arranged into hierarchies of subpopulations, populations, metapopulations, etc., genetic variation is often hierarchically structured (Heithaus and Laushman 1997). Genetic diversity is most similar within populations and between geographically proximate populations; as geographical distances between populations increase the degree of genetic divergence also increases (Vekemans and Hardy 2004). Understanding and incorporating this genetic structure can improve the efficacy of resource management practices. For example, restrictions in temporal and spatial distribution along with partial reproductive isolation provide a basis for local adaptation through natural selection and are the foundation of the stock concept (Shaklee and Currens 2003).

The stock concept is a central theme in the management of nearly all fish and shellfish species (Shaklee and Currens 2003). The concept is based on the premise that productivity and evolutionary potential of a species is dependent on maintaining the abundance and diversity of its component stocks (Shaklee and Currens 2003). A genetic stock is defined as a population(s) of fish that occur in proximity to one another and share sufficient genetic similarities to assume a common ancestor or sufficient migration to

allow adaptations to spread (Bryan and Larkin 1972; Shaklee and Currens 2003). Members of a stock have similar biological and/or ecological attributes such as growth rate, population dynamics, habitat selection, and food habits, that collectively warrant their designation as unique stocks (Dizon et al. 1992; Carvalho and Hauser 1994). Therefore, under the stock concept, effective management of a species is dependent on understanding the number, distribution, and characteristics of all stocks so genetic and ecological integrity, diversity, and abundance can be maintained (Dizon et al. 1992).

The Wisconsin Department of Natural Resources (WDNR) has identified several fish management goals important for maintaining populations of gamefish, such as walleye (*Sander vitreus*) and muskellunge (*Esox masquinongy*). These goals include maintaining the genetic integrity of naturally reproducing populations (Hewett and Simonson 1998; Simonson 2008). Genetic integrity can be defined as the relative stability of genetic diversity within a population over time and is correlated with the fitness of a population (Thelen and Allendorf 2001; Wang et al. 2002; Allendorf and Luikart 2007). Conservation of locally adapted populations is important to maximize the adaptive and evolutionary potential of a species (Lande and Shannon 1996; Hilborn et al. 2003). Because of the importance of genetic diversity, the conservation of genetic integrity is a common goal of science-based management. The maintenance and protection of genetic diversity within Wisconsin gamefish species requires knowledge of the levels of diversity present in populations and its distribution or structure among populations on the landscape; in short, a stock-based management system.

Previously, management units based primarily on watershed boundaries were identified and currently serve as the basis of an approximate stock-based management

program. Fields et al. (1997) used multiple genetic marker systems and multiple species to delineate management zones for fish species in the upper Midwest. This study identified six genetic management zones for walleye and five for muskellunge in northern Wisconsin (Figure 1; Fields et al. 1997). Despite poor resolution, the observed patterns of diversity were mostly consistent with watershed boundaries within northern Wisconsin (Fields et al. 1997). This finding was logical and, as such, these zones were prescribed but considered tentative or preliminary because the study had relatively small sample sizes, a low number of populations, and low genetic variability at the available genetic markers (Fields et al. 1997). Hammen (2009) examined the contemporary genetic structure of walleye in the Ceded Territory of Wisconsin (i.e., northern third of Wisconsin; Staggs et al. 1990) using a more intensive sampling strategy and highly polymorphic, microsatellite DNA loci. This study showed the genetic structure of walleye differed from contemporary watershed management zones. A concurrent study on muskellunge, resolved a genetic structure that deviated from contemporary watershed management zones in a similar manner (Spude 2010). Although both studies found general concordance of genetic structure and watershed boundaries within and among the species, an obvious discordance existed between resolved genetic structure and watershed boundaries in the headwater regions of the Upper Chippewa River watershed (Figures 2 and 3). Populations of both species in this region were consistently more similar to populations in the headwaters of the adjacent Upper Wisconsin River watershed. Given the large number of populations of gamefish in this region and the associated intensive management activities on these populations, this discordance is a challenge to effective stock-based management of these and other coolwater species.

Understanding the extent and identifying the fundamental cause(s) of the discordance between these resolved genetic structures and contemporary watershed boundaries remains important to fully implement a stock-based management program. At least two possibilities merit evaluation: 1) the discordance is because of past stocking events using sources/hatcheries that were more geographically proximate (i.e., convenient) as opposed to sources from the same genetic management unit, and 2) geological events, likely glacial, that may have impacted the colonization and establishment of native fishes in this region resulting in a pattern of diversity that captures the geologic history as opposed to the current watershed boundaries. These two possibilities have different implications for management decisions. One management decision would rely on contemporary genetic diversity to delineate management units regardless of watershed boundaries. Management based on such genetic units would have to consider the implications of downstream migration into populations that belong in a different genetic unit. Another possible decision would be to delineate management units based solely on contemporary watershed boundaries. Risks with this approach include potentially homogenizing diversity within a watershed despite the presence of native heterogeneity.

Estimating the genetic structure of species that are not stocked or popular bait fish could serve as a surrogate for estimating the natural genetic structure of fish species in northern Wisconsin. This approach would minimize or even eliminate the potential anthropogenic influences that may be driving key aspects of genetic structure in the two previous gamefish genetic studies. Caro and O'Doherty (1999) described surrogate species as a substitute, proxy, or focal organism used in place of another. The use and

practicality of surrogates is highly debated (Favreau et al. 2006). Some conservationists claim the concept to be effective, efficient, or often the only practical way to continue with research when little data is available (Favreau et al. 2006). Others have demonstrated that the use of surrogates rarely correlates with many other species or taxa (Favreau et al. 2006). In a few cases, surrogate species provided better coverage than random threatened species and can provide helpful information as long as the objectives of the use of surrogate species are clearly defined (Caro and O'Doherty 1999; Andelman and Fagan 2000).

The use of surrogate species to estimate gamefish genetic structure in northern Wisconsin is dependent on the availability of species that share a similar zoogeographic history. The recession of the Wisconsonian glaciation (~12,000 years ago) produced the watery landscape currently seen in northern Wisconsin (Pielou 1991). Several walleye studies have shown that glacial refugia are key elements in shaping the genetic structure of this species (Billington et al. 1992; Stepien and Faber 1998; McParland et al. 1999; Strange and Stepien 2007). Assuming the majority of coolwater fish species recolonized Wisconsin under a similar geographic and temporal model, these studies can be used to infer recolonization of walleye and other fish species in this region. As the glaciers receded, melt-water provided corridors between waterbodies for walleye, muskellunge and other fish species (Knowles 2001).

Headwater capture is another geologic process that might have influenced the genetic structure of aquatic species in northern Wisconsin. Headwater capture, also known as river vicariance theory, is a geomorphological process through which stream sections are displaced from one catchment to another (Burridge et al. 2007). As a result,

aquatic populations that genetically diverged from neighbor populations due, in part, to geographical separation (genetic drift) are subsequently found in the same drainage and/or river system. In this situation, apparent conflicts in the distribution of diversity or genetic structure are observed. In fact, prime evidence of headwater capture events are observed genetic relationships that are reflective of historical rather than contemporary catchment boundaries (Burridge et al. 2007). Headwater capture can occur through several means, including glaciation, flooding, mountain uplift, and continental drift. Headwater capture can promote range expansion and vicariant isolation at the same time, similar to glaciations, but over shorter geologic time scales than glaciation (Burridge et al. 2007). In the case of walleye or muskellunge, waterways that are contemporarily contiguous could have historically been part of different stream systems. If a geological disturbance caused a change in the waterway, all fish located in that waterway would be diverted into a new direction, possibly into a different watershed. Genetically, this process can leave a signature similar to the discordance observed in the Wisconsin walleye and muskellunge genetic structures.

In addition to zoogeographic considerations, the choice of logical surrogates should focus on species that are ubiquitous and sympatric with both walleye and muskellunge. Furthermore, the surrogate species should be relatively easy to sample in large numbers. Rock bass (*Ambloplites rupestris*) and johnny darters (*Etheostoma nigrum*) are both native species that are not stocked or used as bait fish, so anthropogenic impacts on the genetic structure of these fish throughout their sympatric range with walleye and muskellunge should be minimal.

The objective of this study was to determine if the previously observed genetic structures for walleye and muskellunge populations in Wisconsin are likely the result of zoogeographic processes or anthropogenic events. Three primary sub-objectives were developed: (1) to determine if rock bass exhibit genetic structure among populations in northern Wisconsin, (2) to determine if johnny darters exhibit genetic structure among populations in northern Wisconsin, and (3) to determine if the genetic structures of rock bass and johnny darter are consistent with the previously identified genetic structures of walleye and muskellunge.

METHODS

Experimental Design

Four primary considerations were addressed in designing this study: 1) the species to use as surrogates; 2) the number, location, and overall distribution of populations to be sampled; 3) the number of samples collected from each population, and 4) the number and type of molecular markers to use. Three criteria were used for selecting surrogates: 1) species had to be native to the study area of northern Wisconsin, 2) species could not have been stocked, translocated, or used in the bait industry, and 3) species had to be sympatric with walleye and muskellunge. As described previously, rock bass and johnny darters were selected as surrogates for this study. Neither species is thought to have been commonly used or targeted for use as bait. A review of WDNR propagation records from 1998-2009 (David Giehtbrock, WDNR unpublished data, personal communication) showed only two lakes in Wisconsin with documented rock bass stockings/translocations. Those lakes were not considered for inclusion in this study. The same review showed no evidence of intentional translocation or stocking of johnny darters.

The number and distribution of sampled populations partially followed the design of three previous studies focused on walleye and muskellunge genetic structure (Hammen 2009; Murphy 2009, and Spude 2010). Since the previous studies of walleye and muskellunge were restricted to the northern third of Wisconsin this study was also restricted to the same region (Figures 2 and 3). Because the major discordance between genetic structure and watersheds were observed in the Upper Chippewa River/Upper Wisconsin River headwaters, the primary target region of this study consisted of sub-watersheds in and around those two headwaters. Five sub-watersheds on each side of the shared border of the two watersheds were chosen as target areas (10 total sub-watersheds;

Figure 4). Two to three other sub-watersheds were randomly chosen in each of the five major watersheds (Lake Superior, St. Croix River, Chippewa River, Wisconsin River, and Lake Michigan) for a total of thirteen randomly chosen sub-watersheds (Figure 4). Within the target sub-watersheds, preference was given to lakes previously sampled in the walleye and muskellunge studies assuming they contained healthy populations of either or both surrogate species. If no previously studied lakes were available in a given sub-watershed or the population size of either species was limiting, the selection of lakes was based on: (a) lakes listed by WDNR as having rock bass and/or johnny darters, and (b) lakes with public access boat landings.

The minimum sample size of a population was dependent on the choice of molecular markers for this study. Two species-specific suites of microsatellite markers, specifically designed for this study, were chosen to predict genetic diversity and structure. Microsatellites were chosen for several reasons including their high level of polymorphism (Bernatchez and Duchesne 2000), the ability to assay their diversity via PCR (Shaklee and Currens 2003), and the use of microsatellite markers in the previous studies on muskellunge and walleye. The numbers of loci and sample size to collect are dependent on each other and collectively relate to the expected levels of differentiation between putative populations. A minimum of 10 and a maximum of 14 microsatellite markers are commonly referenced as adequate for similar studies (Ruzzante 1998; Hedrick 1999); however, this cannot be fully determined until after data have been collected. A targeted minimum of 50 individuals of each species was sampled per lake as recommended by Ruzzante (1998). The genetic data was analyzed using a series of tests

that hierarchically examined diversity and its partitioning into various groups; a process commonly referred to as genetic stock identification (GSI; Shaklee and Currens 2003).

Sample Collection

Samples were collected via a combination of fyke netting, shoreline seining, and electrofishing during the spring and summer of 2010 and 2011. Rock bass were sampled non-lethally with genetic samples consisting of an anal fin clip stored in individually labeled tubes and preserved with 95% non-denatured ethanol. When possible, measures of weight (g), total length (mm) and sex were taken. Johnny darters were lethally sampled by preserving whole fish in 95% non-denatured ethanol because fin clips sufficient for repeated extractions would have likely been lethal to the majority of sampled fish. Sampling techniques followed the Molecular Conservation Genetics Laboratory's (MCGL) standard operating protocol and received UWSP IACUC approval.

DNA Extraction

Genomic DNA from individual tissue samples were extracted using Promega Wizard® Genomic DNA purification kit[1] (Promega Corp., Madison, WI) following a standard operating procedure for a 96-well plate extraction. The extracted DNA was quantified using a Nanodrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). All DNA samples were normalized to a final concentration of 20 ng/µL before microsatellite amplification.

[1] Use of trade and product names throughout does not constitute endorsement by the U.S. Government, the Wisconsin Department of Natural Resources, or the University of Wisconsin-Stevens Point.

Genetic Analysis

A suite of 12 rock bass microsatellite loci and 14 johnny darter loci, developed in-house using the methods of Glenn and Schable (2005), were used to characterize the genetic diversity of the two species (Tables 1 and 2). Samples for each species were PCR amplified using 5 multiplex reactions (per species) with fluorescently labeled primers (Table 3 and 4). Amplicons were size fractionated on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc., Foster City, CA) with an in-lane standard (Geneflo™ 625, Chimerx, Inc., Milwaukee, WI) for accurate allele sizing. Allele sizes were determined using GeneMapper® software (Applied Biosystems) and multilocus genotypes were compiled using Microsoft Office Excel© 2010 v14.0.6 (Microsoft Corporation, Redmond, WA). Because some samples would not yield complete genotypes despite multiple efforts, an individual had to have a minimum of seven and ten successfully genotyped loci to be included for rock bass and johnny darters, respectively. For quality assurance and control, 10% of samples (randomly selected) were re-genotyped to estimate global and locus-specific error rates.

Statistical Analysis

Genetic diversity.—Genetic diversity was determined using several different measures including population specific allele frequencies, mean number of alleles per locus (A), allelic richness (A_r), observed heterozygosity (H_o), and expected heterozygosity (H_e). Microsatellite Toolkit v3.1.1 (Park 2001) was used to calculate allele frequencies, H_o, H_e, and A. Differences in sample size are expected to impact estimates of allelic diversity (A) (Leberg 2002; Kalinowski 2004), therefore, a rarefaction method implemented in HP-RARE v1.0 (Kalinowski 2004) was used to calculate A_r and

the number of rarefacted private alleles per sampled population (alleles only found in a single population).

Hardy-Weinberg equilibrium and linkage disequilibrium.—A genetic stock identification (GSI) procedure was used to determine the genetic structure of each target species across northern Wisconsin. The first step of GSI was to determine if all populations conformed to Hardy-Weinberg equilibrium (HWE; Shaklee and Currens 2003). Hardy-Weinberg equilibrium is a critical factor in allowing allele frequencies to be used to explain the genetic composition of the population(s) with deviations being used to infer improper sampling techniques (Allendorf and Luikart 2007) or the presence of genetic admixture (Wahlund effect; Wahlund 1928). Estimates of HWE were performed using an exact test with a Markov Chain Monte Carlo (MCMC) method of 50,000 dememorization steps, 500 batches, and 50,000 iterations per batch (Guo and Thompson 1992; Raymond and Rousset 1995b) as implemented in GENEPOP 4.0 (Rousset 2008). Alpha ($\alpha_o = 0.05$) was adjusted with a sequential Bonferroni correction to account for multiple tests (Rice 1989). A common problem with HWE tests and highly polymorphic loci (such as microsatellites) is a high Type-I error rate because of the cumulative effect of rare expected genotypes (Pamilo and Varvio-Aho 1984). To correct this problem, all significant HWE tests from the MCMC tests were re-tested following the pooling of all genotypes with a frequency of <1% (Hedrick 1999). Re-testing was performed using a chi-square goodness of fit test in Microsoft Office Excel® 2010 (Microsoft Corporation, Redmond, WA) and a sequential Bonferroni correction ($\alpha_o = 0.05$; Rice 1989). Any populations that deviated significantly from HWE after

corrections were tested for null alleles, sequence stutter, or typographic/data entry errors using the program MICRO-CHECKER v.2.2.3 (Oosterhout et al. 2004).

The next step in performing GSI was to assess the independence of the sampled loci also known as linkage or gametic disequilibrium (Raymond and Rousset 1995a). When a locus has allele frequencies that are not independent of another locus, the two loci are in linkage disequilibrium and thus violate Mendel's law of independent assortment. The result is one of the two loci cannot be used in tests of genetic differentiation among populations such as those used in GSI (Shaklee and Currens 2003; Allendorf and Luikart 2007). GENEPOP 4.0 (Rousset 2008) was used to test for linkage disequilibrium using Fisher's exact tests with MCMC as described above. A sequential Bonferroni correction was used to correct for multiple tests (Rice 1989).

Population-specific admixture analysis.—A confounding factor to the prediction of genetic structure is the presence of admixed populations in a dataset. A Bayesian-based clustering method was employed to test each sampled population for the presence of admixture. Admixture analysis was performed in the program STRUCTURE (Pritchard et al. 2000) using a burn-in of 50,000 cycles followed by 50,000 MCMC repetitions with K (the number of potential genetic units) ranging from 1-4 with three iterations to predict the number of genetic units contained in the sample. The ΔK method from Evanno et al. (2005) was used in conjunction with the slope of the lnP(K) as described by Pritchard et al. (2000) and Evanno et al. (2005) to predict the most likely value of K, which requires finding the breakpoint in the slope of the distribution of lnP(D) for different K values tested. LnP(D) is an estimate of the posterior probability of the data for a given K. The asymptotic value of the posterior probabilities is assumed to

be associated with the correct K value in a given sample (Pritchard et al. 2000). Evanno et al. (2005) showed the ΔK method detects the top level of population structure when several hierarchical levels exist.

Basic Genetic Structure.—The first step in estimating the genetic structure of each species was to construct a graphical representation of overall genetic divergence/structure among populations from which predictions of genetic groups could be constructed. An unrooted neighbor-joining (NJ) tree (Takezaki and Nei 1996) was constructured using Cavalli-Sforza and Edwards chord distance (D_c; 1969) in PowerMarker v3.25 (Liu and Muse 2005). Confidence in the topology was estimated using 10,000 bootstrap pseudoreplicates from PowerMarker v3.25 and a majority rule consensus tree constructed using CONSENSE from the PHYLIP package (Felsenstein 2005).

Analysis of molecular variance (AMOVA) was used to determine the significance of the groups predicted from the NJ tree (Excoffier et al. 1992). AMOVA calculates the total molecular variance for all sampled individuals and partitions the total variance into among group variance (V_a), among populations within groups variance (V_b), and within population variance (V_c). Similar to ANOVA, AMOVA determines the significance of each partition. All AMOVA tests were performed with 10,000 permutations and 5,000 pseudoreplicates (Lowe et al. 2004) using Arlequin v3.5.1.3 (Excoffier et al. 2005). Ideally, the ultimate genetic structure scenario would show significant among group variance (V_a) while simultaneously recovering non-significant within group variance (V_b). However, this occurs only when the number of groups approaches the number of sampled populations so the ratio of V_a/V_b was also calculated for all hypothetical groups

with the supposition that a biologically-relevant structure should, at a minimum, have a V_a/V_b ratio ≥ 1 (Spude 2010).

A final test of divergence using Wright's (1931) fixation index (F_{ST}) was used to test for the presence of additional structure within and among any and all final groups after the AMOVA test. The F_{ST} analog, theta (θ), as recommended by Weir and Cockerham (1984) for highly polymorphic genetic markers, was used to estimate the fixation index. Pairwise population θ values were estimated in FSTAT v2.9.3.2 (Goudet 2001) and significance (deviation from zero) was tested with 5,000 bootstrap pseudoreplicates. All groups that were identified with AMOVA and showed no significant θ were determined to be genetically stable, likely biologically significant, and candidates to be considered distinct populations and/or stocks. Because F_{ST} and its analogs have been shown to deviate from linearity at moderate to high values, especially with highly polymorphic microsatellite loci (Hedrick 2005), the divergence estimator D_{est} (Jost 2008) was estimated. Pairwise population D_{est} values were estimated in SMOGD v1.2.5 (Crawford 2010). The resulting matrix was tested for linearity versus the θ matrix using a Mantel test with 9,999 iterations in GenAlEx v6.4 (Peakall and Smouse 2006).

Bayesian-admixture based genetic stock identification.—A modified GSI approach was also used to estimate genetic structure of the rock bass and johnny darter populations. An iterative genetic structure prediction approach modified from that used by Coulon et al. (2008) was performed using the Bayesian admixture/structure prediction method in STRUCTURE v2.3.3 (Pritchard et al. 2000). A series of steps was conducted to identify the largest rate of change for K (ΔK) with the smallest variance and largest probability. The iteration with the highest probability within the most likely K value is

then used to determine population groupings. Individuals within each population are assigned a probability of belonging to a particular group (*q*) with all samples from that population resulting in a mean *q* value (*Q*) representing a measure of overall genetic composition for each population. The method of Coulon et al. (2008) uses an iterative approach with an *a priori* threshold value of *Q* used to assign a population into a group. For this study, the threshold for inclusion into a group was 75% (i.e., >75% of each population's genetic material assigned to a particular group). Populations with mean *Q* $\leq$75% were assumed to be independent of the other populations within a given iteration and, subsequently, their own gene pool. Similar to the previous description of STRUCTURE-based admixture detection, three iterations of K ranging from 1-23 (rock bass) or 1-17 (johnny darters) were performed with an initial burn-in period of 50,000 cycles followed by 50,000 MCMC repetitions. The most likely value of K for a given group was predicted as described previously using the Pritchard et al. (2000) and Evanno et al. (2005) approach. After each step of the process, new groups (consisting of sub-groups) of each previously predicted group were constructed. Finally, an AMOVA, as described previously, was used to test the significance of the smallest number of presumed groupings.

Genetic and geographic distances.—Populations are expected to diverge across a landscape through time because of limited connectivity and subsequent gene flow (Manel et al. 2003). This isolation by distance (IBD) process can provide insight into the patterns of divergence and, in some cases, the processes impacting this pattern. For example, conformance to IBD suggests natural patterns of diversity whereas violation of IBD could mean a disruption because of anthropogenic effects. The resolved genetic

structures of both species were tested for IBD using a Mantel test comparing a genetic distance matrix (pairwise D_{est} as described previously) to a geographic distance matrix (Manel et al. 2003). Pairwise geographic distances were calculated between centers of each lake using the Point Distance feature in ArcGIS v9.3.1 (Environmental Systems Resource Institute, Redlands, CA). The Mantel test was performed using GenAlEx v6.4 (Peakall and Smouse 2006) with 9,999 iterations.

Contemporary genetic management units.—The observed genetic structures of both species were tested against the contemporary (watershed) genetic management units. An AMOVA was performed with *a priori* groups of populations based on their watershed membership. Analyses were conducted in Arlequin v3.1 (Excoffier et al. 2005) using the methods previously described.

Species comparisons. —The purpose of this project is to determine if rock bass and johnny darter genetic structures were consistent with walleye and muskellunge genetic structure. Studies that involve quantitatively comparing genetic differences between species are currently lacking. No studies could be found describing methods that could be used to evaluate the genetic differences between walleye, muskellunge, rock bass and johnny darters. To determine if genetic structures were consistent across species, a comparative approach through geographical representation was used. Rock bass and johnny darter genetic structures determined from the previous sections were viewed visually by maps created in ArcGIS v9.3.1 (Environmental Systems Resource Institute, Redlands, CA). Sampled sub-watersheds were color coded to match the genetic units they grouped into. Then to determine if these genetic structures were consistent with walleye and muskellunge GPS points, color coded for genetic units, of sampled

walleye and muskellunge populations were compared with rock bass or johnny darter genetic structure maps individually. Maps were compared visually to determine similarities.

RESULTS

In total, 1,092 rock bass were sampled from 22 of the 23 sub-watersheds targeted for this study (Table 5; Figure 5). Sample sizes ranged from 28 to 72 with an average of 49.6 individuals (Table 6). Likewise, 784 johnny darters were collected from 16 of the 23 targeted sub-watersheds (Table 7; Figure 6). Other sampled sub-watersheds for johnny darters yielded insufficient sample sizes to be used in the current study. Sample sizes ranged from 30 to 58 with an average of 49 individuals (Table 8). One targeted sub-watershed located in the Wisconsin River watershed was removed from the study because of a lack of lakes with sufficient rock bass or johnny darter populations.

Genetic Diversity

All rock bass samples were initially analyzed at 12 microsatellite loci (Table 1). Two loci (PanD02 and PanA74) were removed from the study due to inconsistencies in amplification. Data from the remaining 10 loci were used for subsequent analyses. For johnny darters, 14 microsatellite loci were amplified in 5 multiplex reactions (Table 4).

Genetic diversity varied considerably among the sampled populations for both rock bass and johnny darters. Rock bass had relatively low levels of diversity across all populations with a mean H_o of 0.4071, a mean H_e of 0.4000, and an observed A of 3.12 (Table 6). The variability in allelic richness was high with the largest difference between Cranberry Lake (CRA) and Lake Noquebay (NOQ) at locus AruA46 (2.00 and 8.48, respectively; Table 6). Rock bass private allelic richness followed similar patterns as the other genetic diversity characteristics (Table 6).

Johnny darters had comparatively higher levels of genetic diversity with a mean H_o of 0.6340, mean H_e of 0.6404, and observed A of 9.08 (Table 8). Allelic richness was

also higher in johnny darter than rock bass (Table 8). Total population values for private allelic richness ranged from 0.69 (BIG) to 6.76 (HMO; Table 8).

Genetic Stock Identification

Hardy-Weinberg equilibrium and linkage disequilibrium.—Following sequential Bonferroni correction, 3.4% (7 of 206) of rock bass locus by population comparisons departed significantly from HWE. Loci PniB86 and PanB80 each showed two significant differences from HWE expectations (PniB86: SAW and NOQ and Pan B80: WSL and SPE), while AruA31, AruA55 and AruA63 were significant for one population each (PM, BN, and MCK, respectively). When rare genotypes were pooled per Hedrick (2005), AruA55 in BN was the only locus by population comparison that deviated from HWE expectations. Because all remaining locus by population comparisons conformed to HWE expectations (99.5%), it was concluded that the rock bass samples, as a whole, conformed to HWE. The sampled rock bass also conformed to linkage equilibrium at all 990 tests following sequential Bonferroni correction. None of the sampled populations for rock bass showed evidence of admixture.

A higher number of johnny darter locus by population comparisons (following Bonferroni correction, 11.76%; 26 of 221) were significantly different from HWE expectations. After rare genotypes were pooled per Hedrick (2005) no significant locus by population tests were observed. Johnny darters also conformed to linkage equilibrium at all 1,456 tests following sequential Bonferroni correction. None of the sampled populations for johnny darters showed evidence for admixture.

Basic genetic structure.—The rock bass D_c unrooted NJ tree resolved a consistent geographic split between the Chippewa River and Wisconsin River watersheds for rock

bass with a few exceptions (Figure 7). All sampled populations located within the Wisconsin River watershed resolved to one group (Group A) and sampled populations from the Chippewa River watershed grouped with the inclusion of two populations from the Lake Superior watershed (GAL and LYN) and one population from the Lake Michigan watershed (SPE) (Group B). Other populations sampled from the Lake Michigan, St. Croix, and Lake Superior watersheds grouped although with low bootstrap values (Group C). Confidence levels of internal nodes on the unrooted NJ tree were generally low, resolving under the 60% moderate support level with the exception of NOQ and SAW with 68.79% support and LCO and LHC with 68.66% support. The results of AMOVA on the three putative rock bass groups (A, B, and C) showed significant among group variance (10.98% variance; $p < 0.0001$) and within group variance (13.80%; $p < 0.0001$) (Table 9a) suggesting further substructure existed.

The johnny darter D_c unrooted NJ tree resolved similar results as observed in rock bass. Sampled johnny darter populations showed geographic splits roughly similar to watershed boundaries. Populations sampled within the Wisconsin River watershed all grouped with the inclusion of BUT and NOQ from the Lake Michigan watershed (Figure 8.) This group, without NOQ, had 89.86% bootstrap support. Populations sampled in the Chippewa River watershed grouped with the inclusion of LYN from Lake Superior watershed. Populations sampled in the Lake Superior and St. Croix watersheds were also clustered. Overall, the resolution of internal nodes on the johnny darter unrooted NJ tree was high. These three groups were used as the initial putative groups for AMOVA tests. Significant among group variance (9.87%; $p < 0.0001$) and within group variance

(13.46%; $p < 0.0001$) was detected, suggesting further structure within the three groups (Table 10a).

Pairwise F_{ST} (θ) comparisons for all populations in both rock bass and johnny darters were significantly different from zero consistent with the presence of significant genetic structure and a high number of genetic units (Tables 11 and 12). Mantel tests for both rock bass and johnny darter samples examining the linearity of F_{ST} (θ) versus Jost's D_{est} (2008) showed significant linearity for both species (rock bass $r^2 = 0.7832$, $p < 0.001$; Figure 9; and johnny darter $r^2 = 0.8527$, $p < 0.001$; Figure 10). However, an apparent loss of linearity was observed as F_{ST} increased in the pairwise rock bass comparisons (Figure 9). Therefore, D_{est} (instead of F_{ST}) was used for all further tests for both species as a genetic distance measure. Pairwise D_{est} values for rock bass ranged from 0.00 (BIG and WSL) to 0.39 (HMO and WSL; Table 11). Johnny darter D_{est} values ranged from 0.03 (BN and BIG) to 0.78 (LYN and MCK; Table 12).

Bayesian genetic stock identification.—The Bayesian GSI method showed significant genetic structuring among the sampled rock bass and johnny darter populations. For rock bass, two groups were initially identified with three populations that failed to assign to a group with $\geq$ 75% probability (Figure 11). Subgroup A was consistent with populations sampled in the Wisconsin River watershed with the inclusion of Half Moon Lake (HMO) and McKenzie Lake (MCK) located in the St. Croix River watershed. Similar to the basic genetic structure, subgroup B consisted of all populations from the Upper Chippewa River watershed with the inclusion of four Great Lakes drainage populations. The final groupings resulted in 14 genetic units identified from the 22 originally sampled rock bass populations (Figure 11). Using these as *a priori* groups

for AMOVA, significant among group variance (15.54%, $p < 0.0001$) and within group variance (8.09%, $p < 0.0001$) was detected with a V_a/V_b ratio of 1.921 (Table 9b).

Managing a large number of genetic units is impractical; subsequently, the next logical step was to determine if a smaller number of genetic units could be identified that would meet the objective of conserving genetic diversity among groups of populations. Each step of the iterative process was tested for significance with AMOVA and a V_a/V_b ratio ≥ 1. The genetic structure of rock bass, as suggested by the results of the Coulon et al. (2008) method described previously, assigned the 22 populations into 14 groups. Following the first iteration that utilized data from all 22 populations, two groups were resolved. All populations located within the Wisconsin River watershed and including two populations located in the St. Croix River watershed (HMO and MCK) assigned to a Group 1(Figure 11). All populations located in the Chippewa River watershed, two populations located in the Lake Superior watershed (LYN and GAL), and two populations located in the Lake Michigan watershed assigned to Group 2 (Figure 11). A few populations (NOQ, SOL, NEB) failed to assign to either group with $\geq$ 75% *Q*-value and were therefore treated as isolated, unique groups. This five genetic group iteration (Group 1, Group 2, NOQ, SOL, and NEB) failed to recover an AMOVA V_a/V_b ratio greater than 1 suggesting further structuring ($V_a = 5.38\%$, $p < 0.0001$; $V_b = 17.77\%$, $p < 0.0001$; $V_a/V_b = 0.303$). The Wisconsin River watershed and St. Croix populations that initially grouped as Group 1 were subsequently separated into two subgroups, Wisconsin River watershed populations and St. Croix River watershed populations (Figure 11). Now with six potential groups, subgroups 1a, 1b, Group 2 and the three independent populations, AMOVA once again failed to report a V_a/V_b ratio greater than 1 ($V_a =$

9.84%, $p < 0.0001$; $V_b = 14.15\%$, $p < 0.0001$; $V_a/V_b = 0.695$). Group 2 could not be further divided using the Bayesian approach, however, two populations located in the Lake Michigan watershed, SPE and SAW, were distinctly different from the other populations in the group. These two populations were placed in their own group and AMOVA was reassessed with seven groups, subgroups 1a, 1b, subgroups 2a, 2b and three independent populations. This grouping once again failed to report a V_a/V_b ratio greater than one, suggesting the presence of further genetic structuring ($V_a = 9.48$, $p < 0.0001$; $V_b = 13.93$, $p < 0.0001$; $V_a/V_b = 0.681$). Continuing with the iterative processes, STRUCTURE separated subgroup 1b into individual groups, subgroup 1b1 and subgroup 1b2 and subgroup 2b was also separated into individual groups, subgroup 2b1 and subgroup 2b2. Using these nine groups as a priori groupings for AMOVA, significant among group variance (12.11%, $p < 0.0001$) and within group variation (11.72%, $p < 0.0001$) was detected and a V_a/V_b ratio of 1.033 was achieved (Table 9c).

The Bayesian GSI method initially predicted eight groups for johnny darters. One population (PRC) failed to assign to a group with > 75% probability, however, it most closely resembled populations in subgroup B (Figure 12). Subgroup A was consistent with populations located within the Wisconsin River watershed, and subgroup B was consistent with the Chippewa River watershed showing a separation across watershed boundaries. In total, 16 stable genetic units corresponding to the sampled populations were identified (Figure 12); this was not testable via AMOVA because of a lack of within group variation. Using a 14 group scenario (TOM and PM grouped and NEB and MCK grouped) a significant among group variance (13.55%, $p < 0.0001$) and within group variance (7.25%, $p < 0.0001$) was detected with a V_a/V_b ratio of 1.869 (Table 10b)

Similar to rock bass, the genetic structure of johnny darters, as suggested by the results of the Coulon et al (2008) method described previously, assigned the 16 populations into unique genetic units. Following the first iteration that utilized all 16 populations eight genetic units were determined with one population (PRC) failing to resolve with $\geq$ 75% probability to a group, but most closely resembling populations in Group 5 (Figure 12). Group 1 consisted of populations located in the Wisconsin River watershed. Groups 2 and 3 were individual populations both located in the Chippewa River watershed. Group 4 consisted of populations located in two watersheds, the Wisconsin River watershed and Lake Michigan watershed. Group 5 consisted of populations located in the Chippewa River watershed and included PRC which it most likely resembled. Group 6 was formed with two groups from the St. Croix River watershed and one from the Lake Superior watershed. Groups 7 and 8 were individual populations; one from the Lake Michigan watershed and one from the Lake Superior watershed. The eight group scenario (leaving PRC in Group 5) from the first step of the Bayesian GSI is most likely the smallest number of genetic units for johnny darters (Figure 12). AMOVA testing showed significant among group variance (13.36%, $p < 0.0001$) and within group variance (8.79%, $p < 0.0001$) and also provided a V_a/V_b ratio greater than one (1.52; Table 10c).

Genetic and geographic distances.—Significant positive relationships existed between pairwise genetic distances (D_{est}) and geographic distances for both species. The geographic distance between sampled rock bass and johnny darter populations ranged from 9.02 km (LYN and BIG) to 355.37 km (NOQ and HMO; Table 11). The Mantel test showed a significant relationship between genetic and geographic distance in rock

bass ($R^2 = 0.126$; $p = 0.012$; Figure 13) and johnny darters ($R^2 = 0.145$; $p = 0.005$; Figure 14).

Contemporary genetic management units.—Rock bass and johnny darter populations were grouped according to the watershed in which they are located for AMOVA testing. Five groups were created consisting of samples located in the Lake Superior, St. Croix River, Chippewa River, Wisconsin River, and Lake Michigan watersheds. For rock bass, significant among group variance (9.34%, $p < 0.0001$) and within group variance (14.11%, $p < 0.0001$) was observed with and an overall V_a/V_b of 0.662 (Table 9d). A similar pattern was found for johnny darters with a significant among group variance (9.03%, $p < 0.0001$) and within group variance (13.19%, $p < 0.0001$) resulting in a V_a/V_b ratio of 0.685 (Table 10d).

Species comparisons.—Maps were created in ArcGIS to visualize the predicted genetic structures of rock bass and johnny darters. Since it is infeasible to manage based on individual lakes, the smallest number of genetic units discovered (nine for rock bass and eight for johnny darters) were used to color code the sub-watersheds that were sampled (Figure 15 and 16). The nine rock bass genetic units show a separation between most of the major watersheds in northern Wisconsin. All sampled populations located in the Wisconsin River watershed distinctly group together with no overlap with sampled populations in any other watershed. The sampled populations in the Chippewa River watershed group together except for one population that resolved as its own genetic unit. This group includes two populations from the Lake Superior watershed which are located on the border of the Chippewa River watershed. All other sampled populations in the other watersheds were determined to be individual genetic units.

The sampled johnny darter populations separated into eight distinct genetic units. The genetic units showed a distinct separation between most of the watersheds. The populations sampled in the Wisconsin River watershed grouped together with the inclusion of one population located in the Lake Michigan watershed, but did not include any populations from the Chippewa River watershed. The sampled populations in the Chippewa River watershed separated into two genetic units but did not include populations from any other watershed.

The patterns of genetic structure observed in rock bass and johnny darter were not consistent with the previous studies of walleye and muskellunge. The walleye and muskellunge studies determined three genetic units in northern Wisconsin with one genetic unit over lapping two major watersheds, the Wisconsin River watershed and the Chippewa River watershed. The current study did not find any genetic units that crossed this boundary (Figure 15 and 16).

DISCUSSION

The goal of this study was to determine if the previously observed patterns of genetic structure within walleye and muskellunge were the likely result of zoogeographic processes or anthropogenic events. By using the genetic structure of two native coolwater fishes that have not been stocked as surrogate patterns of natural genetic diversity in Wisconsin, this study showed distinct differences between the previously resolved walleye and muskellunge structure and the johnny darter and rock bass genetic structure. The most stringent results of the modified GSI resolved 14 unique rock bass gene pools (from 22 sampled populations) and the johnny darter populations all resolved as unique gene pools (n = 16 populations). The smallest number of potential genetic groups for rock bass and johnny darters revealed genetic structure consistent with contemporary watershed management zones. Through close consideration and evaluation of these two surrogate genetic structure patterns and subsequent comparison to the previously resolved walleye and muskellunge genetic structures, a better understanding of what processes have resulted in the observed genetic structures arises. These findings were inconsistent with the resolved genetic structures of walleye and muskellunge, suggesting past stocking events altered the genetic structures.

Genetic Structure of Rock Bass and Johnny Darters

Significant genetic differences occurred among the sampled rock bass populations consistent with expectations based on geographic proximity and contemporary watershed boundaries. Populations located in the Chippewa River watershed consistently showed greater similarities to each other rather than when compared to other populations in the

other watersheds regardless of the analytical method. The ultimate resolution of the remaining (13) populations as unique gene pools failed to account for the observed geographical similarity throughout the hierarchical analysis. For example, the populations from the Upper Wisconsin River consistently resolved separately from the Upper Chippewa River populations and showed significant similarity in the AMOVA analyses. Only when the full iterative approach (i.e., significant among group variance and non-significant within group variance) was employed did the individual populations resolve as unique.

The johnny darter populations resolved a similar, high degree of genetic structure with all sampled populations representing unique gene pools. When the entirety of the analytical process was considered, geographical structure was apparent at early phases of the analysis consistent with watershed influence on the genetic structure. The primary pattern observed in the data was a discrete separation of the Upper Chippewa River populations from the Upper Wisconsin River populations.

The high degree of genetic structure was consistent with other microsatellite studies of fish species throughout the Upper Midwest including walleye (Hammen 2009), muskellunge (Spude 2010), lake sturgeon (*Acipenser fulvescens*; Welsh et al. 2008) , smallmouth bass (*Micropterus dolomieu*; Stepien et al. 2006), and white sucker (*Catostomus commersoni*; Lafontaine and Dodson 1997). Stepien et al. (2006) found little or no gene flow between separated lakes or between river drainages in smallmouth bass populations in the Great Lakes region. They suggested the degree of differentiation reflects patterns of colonization and populations have been separated by geographical barriers among lakes and the basins in which they are located, constricting gene flow

between populations. On a small scale, genetic divergence was linked to low migration and a tendency for behavioral site fidelity (Stepien et al. 2006). Within lakes, migration was higher between sites and genetic divergence was tied to geographic distances. All of these factors are likely occurring in rock bass and johnny darter populations used in this study and the original walleye and muskellunge populations studied by Hammen (2009) and Spude (2010), respectively. Of special consideration is the lack of natural migration between isolated populations of coolwater species resulting, essentially, in aquatic 'islands' on the geographical landscape.

The hierarchical genetic structure observed in Wisconsin's rock bass and johnny darter populations are the likely result of numerous factors including life history, landscape connectivity, and anthropogenic changes to the landscape. Since rock bass and johnny darters are not translocated for stocking or bait, anthropogenic factors not related to the landscape (i.e., direct management activities) are likely minimized or eliminated from consideration as an influence. The life histories of rock bass and johnny darters are conducive to the high level of observed genetic differentiation. Life history traits such as generation time, life span and mobility have an impact of the genetic structure of a species (Stepien et al. 2007). Certain life history traits can have an impact not only on within population genetic diversity but also between population genetic diversity. Rock bass and johnny darters have been classified as sedentary species that remain in limited areas (Becker 1983) indicating the potential for genetic structuring through the lack of migration among populations. Migration between populations helps to maintain genetic diversity within connected populations allowing for potential adaptive traits to be shared through populations. With a lack of migration, such as with rock bass and johnny darters,

populations are subject to genetic drift, a nonselective random process that changes allele frequencies. Genetic diversity is most similar within populations and as geographical distance between populations increases the degree of genetic divergence also increases (Vekemans and Hardy 2004). The distance between sampled populations of rock bass and johnny darters would be consistent with the degree of structuring since migration is lacking between populations. The lesser migratory probability of rock bass and johnny darters, coupled with shorter generation times likely enhances measures of genetic divergence among populations compared to the heavily managed and comparatively long-lived walleye and muskellunge.

Reproductive and parental care specificity may promote genetic divergence among spawning groups (Stepien et al. 2006). Rock bass, much like smallmouth bass, a related species, are territorial nest builders that are relatively less fecund when compared to other freshwater fish species, annually producing 2,000-11,000 eggs (Becker 1983). Females spawn with more than one male and the largest individuals of both sexes are the most reproductively successful due to high mate selectivity (Wiegmann and Baylis 1995). Genetic studies for smallmouth bass spawning success showed 5.4% of all spawning males produce 55% of the total number of fall young of the year (Gross and Kapuscinski 1997). Johnny darters mate in much the same way. Males are territorial breeders annually producing 30-200 eggs (Becker 1983). This mating system influences the effective population size (N_e) of a population. The effective population size is defined as the size of an idealized population with the same level of genetic drift as the population in question and determines the rate of genetic drift (Allendorf and Luikart 2007). Effective population size can be altered by unequal sex ratios, nonrandom number of progeny and

fluctuation in population size (Allendorf and Luikart 2007). When the effective population size of a population decreases, genetic drift increases causing differentiation between populations. Skewed sex ratios limit the evenness of the genetic contribution of individuals to the next generation and therefore decrease N_e (Allendorf and Luikart 2007). Low fecundity and low progeny survival generally results as a greater proportion of the progeny are coming from a smaller number of parents reducing the effective size of the population (Allendorf and Luikart 2007).

Given the high degree of concordance between rock bass and johnny darter genetic structure and contemporary watershed boundaries, recent geologic events (e.g., Wisconsonian glaciation) were likely critical determinants in the distribution and dynamics of these two species. Geologic events during the Wisconsonian glaciation have been considered critical in the diversification and zoogeography of Wisconsin fishes (Wiley and Mayden 1985; Mandrak and Crossman 1992; Roe et al. 2008). Knowles (2001) showed that glaciation played an important active role in diversification of populations. During glacial periods, populations were displaced to allopatric refuges and regional structuring of genetic variation can be seen (Knowles 2001). Migration of fish species was likely higher during or immediately after the Wisconsonian glaciation (Mandrak and Crossman 1992) because of retreats and expansions of glacial lobes. During the last glaciation, approximately 12,000 years ago, Wisconsin was divided into two regions, one covered by a continental ice sheet and the other, an area free of ice, known as the driftless region (Martin 1965). The area of concern for muskellunge and walleye is in the northern region of Wisconsin and can be characterized as a highland region (Martin 1965). It is thought that the ice sheet made several partial or complete

withdrawals from the highland region, and during the Wisconsonian glaciation a large area of the highland was left unglaciated (Martin 1965). The retreat and expansion also influenced the availability of a species' suitable habitat and caused major range changes (Hewitt 1996). As the glacial lobes retreated, more suitable habitats opened up creating range expansion for many species, including the majority of fish species in Wisconsin's waters.

Recolonization of aquatic habitats as the glaciers receded has been shown in numerous species to largely influence the spatial distribution of genetic diversity (Underhill 1986; Billington et al. 1992; Mandrak and Crossman 1992). Recolonization of Wisconsin's coolwater fish community likely occurred relatively quickly because the landscape was riddled with numerous (since lost) glacial melt water-fed bodies of water and connections between these waters were numerous (Clayton and Moran 1982; Underhill 1986). Past studies have shown that glacial refugia are key elements in shaping the genetic structure of walleye (Billington et al. 1992; Stepien and Faber 1998; Strange and Stepien 2007). These dispersal routes shifted, receded, and eventually were eliminated, cutting off migration between populations.

Species Comparisons

Rock bass and johnny darter genetic structures were not consistent with the genetic structures resolved for walleye and muskellunge. Although the 22 sampled rock bass populations resolved into 14 distinct genetic units, hierarchical structure was consistent with current watershed management units. A distinct separation between the Chippewa River watershed and the Wisconsin River watershed was observed for both

species. No populations from either watershed were genetically similar to populations in the other watershed. Such similarities were clearly observed in the previous walleye and muskellunge studies (Hammen 2009; Spude 2010). The neighbor joining tree with three initial groupings showed some lakes located in the Lake Superior watershed grouped with lakes in the Upper Chippewa River watershed, but had clear separation between the Chippewa and Wisconsin River watersheds (Figure 7). Big Lake located in the Upper Chippewa River watershed was sampled for both rock bass and muskellunge. This lake was determined to have a genetic similarity to the Upper Wisconsin River watershed for muskellunge, but distinctly grouped with the Upper Chippewa River watershed for rock bass. Isolation by distance (IBD) patterns for rock bass showed populations within close geographic proximity to each other, but across watershed boundaries, in the targeted sample area were more genetically differentiated than populations in the same watershed in close proximity (Table 11, Figure 13). For example, Plum Lake (PM) and White Sand Lake (WSL) were located 23.23 km apart in opposite watersheds and had a D_{est} value of 0.088. Tomahawk Lake (TOM) and Plum Lake (PM) were located 22.43 km apart in the same watershed and had a D_{est} value of 0.014 (Table 11). Although only a single comparison, it illustrates a nearly 6-fold increased level of divergence across watersheds versus a within watershed comparison.

Similar pattern of watershed concordance, in contrast to the walleye and muskellunge studies, was observed in the sampled johnny darter populations. All 16 populations resolved as distinct individual populations (i.e., gene pools); however, the hierarchical structure was consistent with watershed boundaries. The neighbor joining tree also resolved three distinct groups with discrete separations between the Wisconsin

River watershed and the Chippewa River watershed (Figure 8). The initial eight groups from the Bayesian GSI showed populations in the Wisconsin watershed grouped together and populations sampled in the Chippewa River watershed were distinctly different. Isolation by distance patterns for johnny darters showed populations within close geographic proximity to each other, but across watershed boundaries, in the targeted sample area were more genetically differentiated than populations in the same watershed in close proximity similarly to rock bass (Table 12, Figure 14). Lakes PM and WSL, located in different watersheds but within 23.23km of each other, had a D_{est} value of 0.18 and lakes TOM and PM, located in the same watershed and 22.43km apart, had a D_{est} value of 0.07 (Table 12).

The disjunct seen in walleye and muskellunge is most likely the result of past stocking events. Given the genetic divergence of the previously studied walleye and muskellunge populations and the introgressive behavior of fish, alteration of the gene pool through cross watershed stocking is a plausible scenario (Campton and Johnston 1985; Cagigas et al. 1999; Franckowiak et al. 2009). Previous stocking events did not always adhere to genetic or watershed boundaries. Historical stockings were often from the most geographically proximate hatchery and not based on management units. The Governor Tommy Thompson Hatchery and Art Oehmcke Hatchery have served as the main hatcheries for muskellunge and walleye in the northern third of Wisconsin. Two populations of muskellunge from the previous study (Spude 2010), located in the Wisconsin River watershed, were determined to be more similar to populations located within the Chippewa River watershed. These two populations had extensive stocking records that showed stocking had taken place across watershed boundaries (Spude 2010).

It is likely that cross boundary stocking also occurred from the Wisconsin River watershed to the Chippewa River watershed altering the genetic structure.

Despite the likely impact of cross-watershed stocking on the genetic structure of walleye and muskellunge, this current study has some potential limitations that may limit it's applicability in interpreting all coolwater fish genetic structure. A possible limitation to this present study was choosing two surrogate species whose life histories and mobility are not more reflective of walleye and muskellunge. Rock bass and johnny darters have limited mobility compared with walleye and muskellunge, which could have affected dispersal patterns during colonization of the area. Perhaps the previously identified walleye and muskellunge genetic structures represent different paths of recolonization or times of dispersal during the last glaciations because of their ability to disperse farther distances than rock bass and johnny darters in shorter amount of time. Johnny darters in single lakes are sometimes limited by barriers that would be irrelevant to walleye and muskellunge (Meixler et al. 2009). Some migration routes may have been more conducive for larger fish and were barriers to smaller fish. Rock bass and johnny darter populations may have been separated for a longer period of time than walleye and muskellunge because of these differential migration impacts. Lack of migration and increase in genetic drift for rock bass and johnny darter populations may have caused changes in genetic structure to occur before walleye and muskellunge populations were separated.

Another possible limitation of this study is that lakes sampled for rock bass and johnny darters were not the same as the lakes sampled for walleye and muskellunge. The current study design specifically used sub-watersheds instead of the lake specific

identifications. Therefore, the genetic structure could have been missed or differ between the putative surrogates used in this study and the two coolwater species of interest. By not sampling all the walleye and muskellunge lakes in the Upper Chippewa River watershed that showed genetic similarities with the Upper Wisconsin River watershed, the nature of the genetic structure for rock bass and johnny darters could not be known and rock bass and johnny darters could in fact show similar disjuncts. When designing this study, the assumption was made that if the genetic structure of walleye and muskellunge was natural then it would be wide spread in the area. Sampling was based on sub-watersheds for a broad sampling distribution of the area and to get a range of the disjunct genetic structure of walleye and muskellunge. Also, other than the target area, sub-watersheds were randomly chosen. The walleye and muskellunge sampled lakes were based on WDNR survey lakes and tended to group together because of the close proximity of lakes to each other in headwaters of the Chippewa River and Wisconsin River watersheds. When possible, previously sampled lakes were sampled but not all lakes showing disjunct patterns in past studies overlapped with a sub-watershed for this study.

Management Implications

The Wisconsin DNR explicitly aims to manage walleye and muskellunge in ways consistent with conserving the genetic integrity of self-sustained populations (Hewett and Simonson 1998; Simonson 2008). The current management units for walleye and muskellunge are based largely on watershed boundaries that likely reflect post-glacial dispersal patterns and should contain genetically similar populations as reflected by this

study. To achieve these goals, sound biologically relevant management units need to be followed. Contemporary watershed based management units are currently inconsistent with the genetic structures of walleye and muskellunge; however, they are consistent with the higher level genetic structures of rock bass and johnny darters observed in this study. A key assumption of this study was that rock bass and johnny darter genetic structure would represent a natural, 'model' genetic structure unaltered by anthropogenic events. Based on this assumption, it is a logical conclusion that the lack of a strong watershed to genetic relationship in walleye and muskellunge is most likely the result of past stocking events that did not adhere to current watershed boundaries.

This study has resulted in two possible management actions: 1) manage walleye and muskellunge based on current watershed boundaries or 2) manage based on the contemporary genetic units determined in the previous studies. Continued reliance on the current management units may not adequately protect the genetic integrity of individual populations described in the previous studies since stocking has likely altered the genetic structures. Managing on a unique gene pool basis is inefficient and infeasible. Management units should be developed with the aim to minimize the genetic risks while also considering the limitations of the management agency to manage a large number of units. Limiting the number of genetic units would help improve efficiency by managing a lower number of stocks and decreasing financial needs associated with management.

Continued management based on watershed boundaries is consistent with the genetic structures observed in rock bass and johnny darters. Managing by watershed boundaries would allow management units to remain the same. The number of lakes in the Upper Chippewa River watershed that have been altered is unknown and this

management decision would eliminate the need to examine the genetic structure of every lake in the watershed. Based on the walleye and muskellunge data, the primary discordance is mostly isolated in the headwaters of the Upper Chippewa River; consistent with populations in and around the Lac du Flambeau River system. However, continuing to manage based on watershed boundaries does pose some risk to the current genetic structures of walleye and muskellunge. Supplemental stocking of lakes that are more genetically similar to a different watershed risks losing not only genetic diversity but also similar biological and life history traits. Populations may be exposed to genetic outbreeding. Genetic outbreeding and total gene pool replacement has been documented in several species including walleye (Campton and Johnston 1985; Allendorf and Leary 1988; Sheridan 1995; Franckowiak et al. 2009). A study conducted on Escanaba Lake, WI, by Franckowiak et al. (2009), looked at the temporal stability of the genetic diversity of walleye and found almost total replacement of the original pool through supplemental stocking. Genetic variation was comparable to the genetic variation in other populations of walleye and genetic diversity did not decrease as would be expected with substantial stocking.

The other management option would be to manage based on the contemporary genetic units that were discovered in the walleye and muskellunge studies. This option would preserve and prioritize the current genetic diversity and, ideally, integrity implying that past historic structure cannot or should not be restored. This option assumes that genetic integrity has already been altered by past stocking events, making the preservation of what is on the landscape now crucial to keep from possibly losing genetic diversity by further altering populations. This management option would most likely

conserve demographic characteristics that may be crucial to the fitness of the altered populations and the common focus of primary management activities or monitoring.

Managing based solely on genetics, ironically, also poses potential genetic risks. This option would require the knowledge of which specific populations belong to the different genetic units. If populations are continually stocked across watershed boundaries in accordance with their genetic unit, downstream populations connected through waterways or flooding events would be at more-or-less continual risk from stray, stocked individuals of a different genetic unit. Managing based on current genetic units should be carefully managed so as to not alter the genetic integrity of downstream populations. If these lakes have maintained unique gene pools, management should be directed to reduce potential disruption. Supplemental stocking should only be applied to these lakes when necessary and within their respective genetic units. However, managing these lakes individually across watershed boundaries could become cumbersome in determining which lakes have been altered. Time and financial costs, as is always the case, will be considered by the responsible agencies in determining their optimal approach to this challenge. The data presented herein will ideally provide a scientific foundation for their informed decision making.

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Table 1. Suite of microsatellite loci developed in-house for rock bass with description of primer sequence, repeat motif, number of alleles (A), and allele range in numbers of base pairs.

Locus ID	Primer Sequence (5'-3')	Repeat Motif	A	Range (bp)
AruA31	F:*HEX*-TTGAATGCGCTTAACAGTGTG R:TTCACCCCAACTGAACTTCC	$(AC)_{10}$	5	117-131
AruA46	F:*HEX*-TTTCCACTCTGCTCTCTGACTG R:TCCACATCGCTGTGAATCAG	$(CA)_{16}$	14	196-226
AruA55	F:*HEX*-CCTCAGACATGACACACAAGC R:GCAAATCTCAGCAGGTAGCC	$(AC)_{21}$	6	165-181
AruA63	F:GCATACGTTCAGACACCAAGG R:*HEX*-CTCAGGAGCTGCAGGACAG	$(AC)_{12}$	4	154-162
AruB29	F:*NED*-CTGCTTTTGCAGCCTACTCC R:ATCAGCTGGGGAGATTGTTG	$(CA)_{11}$	5	127-151
AruB38	F:*6FAM*-TGATTGCCAAGATTGACAGC R:TGTTGTTCGCCTATCTGTCC	$(AG)_{28}$	14	128-158
AruC39	F:*6FAM*-GAGCCTGAACTGTCGGGTAG R:GATAGCAAAACATGCATGGAG	$(AC)_{18}$	7	185-209
AruC58	F:*NED*-TGTTGACACCCATTCACTGC R:GGAGGGAGAGACAAGAGCTTC	$(TGTT)_{7}$	3	110-122
PanA74	F:GCCAAGTCAATAATGACACTC R:*6FAM*-AGCATAGAACCCACAATGTC	$(GT)_{11}$	10	137-163
PanB80	F:GGCCTAAATTGGTTTGTGAG R:*NED*-ATGTGGGGATCTTCAACAAC	$(AC)_{9}$	14	156-198
PanD02*	F:GGTGATTGAAGTCATTGTAGC R:*HEX*-CCTGTTTCGGAGTATAACTG	$(GT)_{14}$	8	97-111
PniB86*	F:*6FAM*-TTTGGTGTCTGTCAGCTATG R:TTAAGGAGCCTTGCTACATC	$(GT)_{11}$	10	130-154

*Developed in-house for white and black crappie.

Table 2. Suite of microsatellite loci developed in-house for johnny darters with description of primer sequence, repeat motif, number of alleles (A), and allele range in numbers of base pairs.

Locus ID	Primer Sequence (5'-3')	Repeat Motif	A	Range (bp)
EniA15	F:HEX-TCATATTGGCAAGTGGATGG	$(CA)_{26}$	20	225-273
	R:GCAGTAAAGCCAGGAAGTGC			
EniA33	F:NED-AGCTGAACAATGCTGACCTG	$(TG)_{20}$	17	111-149
	R:AGACAGCTTCACAGCCATGC			
EniA93	F:HEX-TTGCACATATGGACGGACAC	$(AC)_{8}$	7	95-127
	R:GCTAATGACTGCCACCAACC			
EniB46	F:HEX-TGTATGTGGTGCATGTGTGG	$(TG)_{10}$	14	187-221
	R:GGACTTGCAATTGTGTTGATG			
EniC02	F:NED-CTGCTCCCGGATTGTTCTG	$(TG)_{17}$	8	238-262
	R:TGATGCTCGCATGAGTCAAC			
EniC42	F:6FAM-GCTGGATGGTGTCTAGATGG	$(GT)_{13}$	11	124-148
	R:GGAGTGAGCTGATGGTGTCC			
EniC74	F:6FAM-ACTGAGCCCTGAAGAAGTCG	$(TC)_{23}$	25	193-245
	R:GGTGCCAGTGAATGAGCAG			
EniD53	F:6FAM-TCTACATGCCGATTGTCCAC	$(GAAA)_{22}$	21	204-294
	R:TACTCAGGCTGGGACCAGAG			
EniE12	F:NED-TGTGCAAGCTGCAGATCAAG	$(TG)_{26}$	17	197-239
	R:CTGGAATAAGATGCAGCTACAATG			
EniE23	F:6FAM-TCGGTCTCCTTTACCGTCAG	$(TC)_{15}$	18	222-292
	R:GAAAGGTGCTGGTCAGGAAG			
EniE71	F:6FAM-CACAAGGAATAGCTGGCTGTC	$(GACA)_{7}$	12	192-244
	R:GACTACCTCGTGGCTCATGG			
EniF02	F:HEX-AGTGCAGTGTGGGAACTGG	$(AGAC)_{11}$	15	151-207
	R:GGACGTCTGACTTGCTCTCC			
Ebl4[1]	F:6FAM-TGTGACTGATATTTTGCTGCTG	$(TATC)_{7}GT(TCTA)_{7}$	23	164-260
	R:TGCATATCAAGATTCCCATTTG			
Esc26b[2]	F:HEX-CAATGCGCCACATTGAGAAGG	$(TAGA)_{27}$	33	151-287
	R:GCACAACATATGTCGTTAAGCTCC			

1. Locus developed by Beneteau et al. (2007).
2. Locus developed by Gabel et al. (2008).

Table 3. Rock bass PCR reaction recipes, fluorescent labels, and thermocycler temperature profiles for all multiplexes. Multiplex refers to the temperature profiles listed as footnotes, 10x Buffer refers to 10x ThermoFisher PCR Buffer B without $MgCl_2$, dNTPs refers to final of deoxynucleotides at equal concentrations, and $MgCl_2$ refers to the concentration of 25mM magnesium chloride solution. All reactions contained 0.5U of *Taq* DNA polymerase in 10 µL volumes and 40 ng DNA/reactions.

Locus	Multiplex	10X Buffer (Conc.)	dNTP (Conc.)	$MgCl_2$ (Conc.)	Forward Primer (Conc.)	Reverse Primer (Conc.)	Label
AruA31	1	1.00x	0.06mM	1.3mM	0.18µM	0.18µM	Hex
AruB38					0.15µM	0.15µM	6Fam
AruC39					0.10µM	0.10µM	6Fam
AruA46	2	1.00x	0.06mM	1.7mM	0.18µM	0.18µM	Hex
PniB86					0.28µM	0.28µM	6Fam
AruC58					0.09µM	0.09µM	NED
AruA55	3	1.00x	0.06mM	1.3mM	0.15µM	0.15µM	Hex
AruB29					0.22µM	0.22µM	NED
AruA63	4	1.00x	0.06mM	1.25mM	0.12µM	0.12µM	Hex
AruB80					0.22µM	0.22µM	NED
PanA74	5	1.00x	0.06mM	1.25mM	0.24µM	0.24µM	6Fam
PanD02	6	1.00x	0.06mM	1.25mM	0.22µM	0.22µM	Hex

1. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

2. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 52°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

3. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

4. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 58°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

5. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

6. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 55°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

Table 4. Johnny darter PCR reaction recipes, fluorescent labels, and thermocycler temperature profiles for all multiplexes. Multiplex refers to the temperature profiles listed as footnotes, 10x Buffer refers to 10x ThermoFisher PCR Buffer B without $MgCl_2$, dNTPs refers to final concentration of deoxynucleotides at equal concentrations, and $MgCl_2$ refers to the concentration of 25mM magnesium chloride solution. All reactions contained 0.5U of *Taq* DNA polymerase in 10 µL volumes and 40 ng DNA/reactions.

Locus	Multiplex	10X Buffer (Conc.)	dNTP (Conc.)	$MgCl_2$ (Conc.)	Forward Primer (Conc.)	Reverse Primer (Conc.)	Label
EniA93	1	1.00x	0.06mM	1.70mM	0.15µM	0.15µM	Hex
EniC02					0.20µM	0.20µM	NED
Ebl4					0.20µM	0.20µM	6Fam
EniA33	2	1.00x	0.06mM	1.9mM	0.13µM	0.13µM	NED
Esc26b					0.15µM	0.15µM	Hex
EniE23					0.13µM	0.13µM	6Fam
EniA15	3	1.00x	0.06mM	1.7mM	0.24µM	0.24µM	Hex
EniC74					0.10µM	0.10µM	6Fam
EniB46	4	1.00x	0.06mM	1.7mM	0.10µM	0.10µM	Hex
EniC42					0.09µM	0.09µM	6Fam
EniE12					0.34µM	0.34µM	NED
EniF02	5	1.00x	0.06mM	2.2mM	0.09µM	0.09µM	Hex
EniD53					0.20µM	0.20µM	Hex
EniE71					0.22µM	0.22µM	6Fam

1. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 55°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

2. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 55°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

3. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

4. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

5. 94°C for 2 min., 35 cycles each at 94°C for 30 s., 57°C annealing for 30 s., then 72°C for 30 s., and a final elongation at 72°C for 10 min.

Table 5. Populations sampled for rock bass during the summer of 2010 and 2011. Included are abbreviations for each population, county, Water Body Identification Code (WBIC), latitude, longitude and current management unit.

Lake	Abbreviation	County	WBIC	Latitude	Longitude	Management Unit
Bearskin Lake	BEA	Oneida	1523600	45.731	-89.685	Wisconsin
Big Lake	BIG	Vilas	2963800	46.154	-89.767	Chippewa
Butternut Lake	BN	Price/Ashland	2283300	45.983	-90.515	Chippewa
Cranberry Lake	CRA	Price	221700	45.621	-90.350	Chippewa
Deerskin Lake	DEE	Vilas	1601300	45.975	-89.171	Wisconsin
Half Moon Lake	HMO	Polk	2621100	45.498	-92.438	St. Croix
Lac Courte Oreilles	LCO	Sawyer	2390800	45.892	-91.438	Chippewa
Lake Galilee	GAL	Ashland	2935500	46.287	-90.602	Lake Superior
Lake Nebagamon	NEB	Bayfield	2858400	46.513	-91.703	Lake Superior
Lake Noquebay	NOQ	Marinette	525900	45.249	-87.924	Lake Michigan
Long/Herde Lake Chain	LHC	Chippewa	2351400	45.241	-91.416	Chippewa
Lynx Lake	LYN	Vilas	2954500	46.195	-89.666	Lake Superior
McKenzie Lake	MCK	Washburn	2706800	45.936	-92.046	St. Croix
Pelican Lake	PEL	Oneida	157990	45.503	-89.202	Wisconsin
Pike/Round Chain	PRC	Price	2268300/2267800	45.928	-90.067	Chippewa
Plum Lake	PM	Vilas	1592400	46.004	-89.514	Wisconsin
Sawyer Lake	SAW	Langlade	198100	45.247	-88.757	Lake Michigan
Solberg Lake	SOL	Price	2242500	45.749	-90.369	Chippewa
Somo Lake	SOM	Lincoln	1547700	45.516	-89.868	Wisconsin
Spread Eagles Chain	SPE	Florence	702100	45.901	-88.145	Lake Michigan
Tomahawk Lake	TOM	Oneida	1542700	45.830	-89.661	Wisconsin
White Sand Lake	WSL	Vilas	2321100	46.008	-89.827	Chippewa

Table 6. Rock bass diversity statistics (10 loci) for all sampled populations including expected heterozygosity (H_e) and standard deviation (H_e SD), observed heterozygosity (H_o) and standard deviation (H_o SD), mean number of alleles per locus (A) and standard deviation (A SD), mean allelic richness (A_r) and total private alleles (PA). Full population names are in Table 5.

Population	Sample size	H_e	H_e SD	H_o	H_o SD	A	A SD	A_r	PA
TOM	48	0.4888	0.0371	0.5000	0.0228	3.10	1.73	2.96	1.74
PM	49	0.4897	0.0327	0.5143	0.0226	3.70	1.64	3.32	2.24
BEA	49	0.4666	0.0470	0.4612	0.0226	2.90	0.99	2.81	1.87
SOL	72	0.4416	0.0551	0.4345	0.0186	3.50	1.65	3.34	1.05
WSL	72	0.3178	0.0558	0.3132	0.0173	2.50	0.97	2.32	0.00
PEL	62	0.4329	0.0536	0.4210	0.0198	3.40	1.43	3.18	2.03
LYN	38	0.3703	0.0652	0.3831	0.0250	3.00	1.49	2.90	0.00
LCO	58	0.4242	0.0510	0.4138	0.0205	4.10	2.77	3.45	1.48
BIG	69	0.3614	0.0664	0.3695	0.0184	4.10	2.38	3.30	0.85
SAW	41	0.4330	0.0804	0.4360	0.0245	2.90	1.29	2.87	2.02
SOM	40	0.3454	0.0667	0.3545	0.0240	3.10	1.97	2.95	0.07
CRA	31	0.3742	0.0534	0.4273	0.0283	2.30	0.67	2.30	0.00
DEE	50	0.2847	0.0821	0.3123	0.0208	2.30	1.16	2.15	0.00
NOQ	50	0.4946	0.0729	0.5096	0.0224	4.20	2.57	3.94	3.03
SPE	43	0.3801	0.0706	0.3767	0.0234	2.90	1.52	2.76	0.90
BN	54	0.4465	0.0736	0.4344	0.0214	3.20	1.69	2.96	0.52
GAL	46	0.4552	0.0769	0.4630	0.0232	3.00	1.33	2.95	0.27
PRC	28	0.3792	0.0656	0.3964	0.0292	3.40	1.71	3.40	1.02
LHC	54	0.4108	0.0366	0.4211	0.0213	3.00	1.49	2.85	0.03
MCK	38	0.3281	0.0687	0.3142	0.0239	3.00	1.25	2.90	0.95
NEB	50	0.3658	0.0849	0.3832	0.0219	2.70	1.16	2.56	1.00
HMO	50	0.3090	0.0803	0.3171	0.0209	2.40	1.51	2.29	0.23
Mean	49.64	0.4000	0.0626	0.4071	0.0224	3.12	1.56	2.93	0.97

Table 7. Populations sampled for johnny darters during the summer of 2010 and 2011. Included are abbreviations for each population, county, Water Body Identification Code (WBIC), latitude, longitude and current management unit.

Lake	Abbreviation	County	WBIC	Latitude	Longitude	Management Unit
Bearskin Lake	BEA	Oneida	1523600	45.731	-89.685	Wisconsin
Big Lake	BIG	Vilas	2963800	46.154	-89.767	Chippewa
Butternut Lake	BUT	Forest	692400	45.923	-88.986	Lake Michigan
Butternut Lake	BN	Price/Ashland	2283300	45.983	-90.515	Chippewa
Half Moon Lake	HMO	Polk	2621100	45.498	-92.438	St. Croix
Lac Courte Oreilles	LCO	Sawyer	2390800	45.892	-91.438	Chippewa
Lake Nebagamon	NEB	Bayfield	2858400	46.513	-91.703	Lake Superior
Lake Noquebay	NOQ	Marinette	525900	45.249	-87.924	Lake Michigan
Lynx Lake	LYN	Vilas	2954500	46.195	-89.666	Lake Superior
McKenzie Lake	MCK	Washburn	2706800	45.936	-92.046	St. Croix
Moen Chain	MCK	Oneida	1573800	45.660	-89.304	Wisconsin
Pike/Round Chain	PRC	Price	2268300/2267800	45.928	-90.067	Chippewa
Plum Lake	PM	Vilas	1592400	46.004	-89.514	Wisconsin
Solberg Lake	SOL	Price	2242500	45.749	-90.369	Chippewa
Tomahawk Lake	TOM	Oneida	1542700	45.830	-89.661	Wisconsin
White Sand Lake	WSL	Vilas	2321100	46.008	-89.827	Chippewa

Table 8. Johnny darter diversity statistics (14 loci) for all sampled populations including expected heterozygosity (H_e) and standard deviation (H_e SD), observed heterozygosity (H_o) and standard deviation (H_o SD), mean number of alleles per locus (A) and standard deviation (A SD), mean allelic richness (A_r) and total private alleles. Full population names are in Table 7.

Population	Sample size	H_e	H_e SD	H_o	H_o SD	A	A SD	A_r	PA
LYN	44	0.4203	0.0871	0.4300	0.0201	4.21	2.99	4.03	2.03
SOL	54	0.5838	0.0692	0.5807	0.0179	5.64	2.76	5.30	2.35
BUT	53	0.5598	0.0670	0.5704	0.0183	6.57	2.98	5.67	3.40
NEB	30	0.6680	0.0566	0.6822	0.0229	8.00	4.17	7.89	1.61
BEA	54	0.6835	0.0511	0.6716	0.0172	8.14	3.96	7.49	0.61
MC	44	0.6293	0.0620	0.6249	0.0197	8.50	4.77	7.74	1.60
BIG	58	0.6233	0.0680	0.6182	0.0170	8.64	3.84	7.48	0.69
MCK	44	0.6459	0.0655	0.6349	0.0196	9.71	4.63	8.59	3.43
BN	43	0.6656	0.0585	0.6544	0.0195	9.71	5.09	8.50	2.15
PM	49	0.6967	0.0452	0.6759	0.0179	9.86	5.65	8.55	3.29
PRC	48	0.6476	0.0616	0.6154	0.0189	10.14	5.87	8.81	5.37
NOQ	49	0.6748	0.0535	0.6730	0.0180	10.14	4.61	8.71	4.75
HMO	50	0.6976	0.0410	0.6864	0.0176	10.64	3.88	9.06	6.76
TOM	50	0.6426	0.0585	0.6330	0.0183	10.79	5.63	9.28	1.27
WSL	56	0.6404	0.0632	0.6352	0.0172	11.29	6.11	9.18	2.97
LCO	58	0.7673	0.0348	0.7576	0.0152	13.36	6.58	10.70	7.06
Mean	49	0.6404	0.0589	0.6340	0.0185	9.08	4.59	7.94	3.08

Table 9. Analysis of molecular variance (AMOVA) groupings for rock bass with sum of squares (SS), percent of variation, p-values, and V_a/V_b ratios.

Grouping Scenario	Source of Variation	SS	% of Variation	p-value	V_a/V_b
(a) Three-group AMOVA from NJ Tree					
Group A: LHC, SOL, LYN, LCO, BIG, PRC, CRA, BN, WSL, GAL, SPE	Among Groups (V_a)	465.887	10.98	<0.0001	0.797
Group B: PEL, BEA, SOM, DEE, TOM, PM	Among Populations within Groups (V_b)	722.470	13.80	<0.0001	
Group C: MCK, NEB, NOQ, SAW, HMO	Within Populations (V_c)	4296.306	75.22	<0.0001	
(b) 14-group AMOVA					
Group 1: WSL, LYN, BIG, CRA, BN, GAL, PRC, LHC, LCO	Among Groups (V_a)	1005.893	15.54	< 0.0001	1.921
Group 2-14 (all individual groups): TOM, PM, DEE, PEL, BEA, SOM, HMO, MCK, NEB, NOQ, SOL, SAW, SPE	Among Populations within Groups (V_b)	182.464	8.09	< 0.0001	
	Within Populations (V_c)	4296.306	76.37	< 0.0001	
(c) Nine-group AMOVA from Bayesian GSI					
Group 1: TOM, PM, BEA, SOM, PEL, DEE	Among Groups (V_a)	769.36	12.11	< 0.0001	1.033
Group 2: BIG, BN, PRC, LCO, WSL, LYN, CRA, GAL, LHC	Among Populations within Groups (V_b)	419	11.72	< 0.0001	
Group 3-9 (all individual groups): HMO, MCK, SAW, SPE, NOQ, NEB, SOL	Within Populations (V_c)	4296.31	76.18	< 0.0001	
(d) Contemporary Management Units AMOVA					
Wisconsin River: TOM, PM, BEA, SOM, DEE, PEL	Among Groups (V_a)	534.386	9.34	< 0.0001	0.662
Chippewa River: BIG, WSL, LCO, PRC, SOL, CRA, LHC, BN	Among Populations within Groups (V_b)	653.971	14.11	< 0.0001	
Lake Superior: NEB, GAL, LYN	Within Populations (V_c)	4296.306	76.56	< 0.0001	
St. Croix: HMO, MCK					
Lake Michigan: NOQ, SPE, SAW					

Table 10. Analysis of molecular variance (AMOVA) groupings for johnny darters, sum of squares (SS), percent of variation, p-values, and V_a/V_b ratios.

Grouping Scenario	Source of Variation	SS	% of Variation	p-value	V_a/V_b
(a) Three-group AMOVA					
Group A: TOM, PM, BEA, NOQ, BUT, MC	Among Groups (V_a)	704.229	9.87	< 0.0001	0.733
Group B: BIG, WSL, BN, LYN, SOL, LCO, PRC	Among Populations within Groups (V_b)	1043.605	13.46	< 0.0001	
Group C: NEB, MCK, HMO	Within Populations (V_c)	6828.294	76.68	< 0.0001	
(b) 14-group AMOVA					
Group 1: TOM, PM	Among Groups (V_a)	1670.398	13.55	< 0.0001	1.869
Group 2: NEB, MCK	Among Populations within Groups (V_b)	77.436	7.25	< 0.0001	
Group 3-14 (all individual populations): BEA, MC, LCO, PRC, SOL, WSL, BN, BIG, NOQ, BUT, LYN, HMO	Within Populations (V_c)	6828.294	79.2	< 0.0001	
(c) Eight-group AMOVA					
Group 1: TOM, PM, BEA	Among Groups (V_a)	1320.028	13.36	< 0.0001	1.52
Group 2: BIG, WSL, BN	Among Populations within Groups (V_b)	363.423	8.79	< 0.0001	
Group 3: MC, BUT	Within Populations (V_c)	6409.682	77.84	< 0.0001	
Group 4: NEB, MCK, HMO					
Group 5-8 (all individual populations): NOQ, LYN, LCO, SOL					
(d) Contemporary Management Units					
Wisconsin: TOM, PM, BEA, MC	Among Groups (V_a)	887.275	9.03	< 0.0001	0.685
Chippewa: BIG, WSL,BN, LCO, SOL, PRC	Among Populations within Groups (V_b)	860.559	13.19	< 0.0001	
Lake Superior: LYN, NEB	Within Populations (V_c)	6828.294	77.79	< 0.0001	
St. Croix: MCK, HMO					
Lake Michigan: BUT NOQ					

Table 11. Rock bass population pairwise F_{ST} values from FSTAT v2.9.3.2 (below diagonal) and significance values (above diagonal). Full population names are in Table 5.

	TOM	PM	BEA	SOL	WSL	PEL	LYN	LCO	BIG	SAW	SOM	CRA
TOM	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PM	0.04	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
BEA	0.07	0.10	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SOL	0.15	0.10	0.20	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
WSL	0.24	0.22	0.25	0.13	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PEL	0.15	0.11	0.17	0.13	0.24	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LYN	0.14	0.15	0.17	0.13	0.11	0.21	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LCO	0.18	0.15	0.18	0.08	0.04	0.20	0.09	***	<0.0001	<0.0001	<0.0001	<0.0001
BIG	0.22	0.18	0.22	0.09	0.03	0.22	0.13	0.04	***	<0.0001	<0.0001	<0.0001
SAW	0.28	0.23	0.33	0.18	0.33	0.29	0.26	0.24	0.28	***	<0.0001	<0.0001
SOM	0.17	0.21	0.17	0.16	0.30	0.16	0.26	0.22	0.25	0.36	***	<0.0001
CRA	0.24	0.18	0.24	0.17	0.12	0.24	0.15	0.10	0.14	0.26	0.37	***
DEE	0.28	0.20	0.27	0.30	0.37	0.23	0.26	0.29	0.35	0.37	0.42	0.32
NOQ	0.14	0.09	0.19	0.05	0.19	0.13	0.15	0.12	0.15	0.11	0.18	0.18
SPE	0.23	0.18	0.25	0.10	0.04	0.22	0.15	0.05	0.04	0.25	0.32	0.07
BN	0.18	0.14	0.20	0.11	0.14	0.19	0.13	0.08	0.08	0.21	0.24	0.16
GAL	0.15	0.11	0.20	0.06	0.14	0.20	0.12	0.05	0.12	0.19	0.25	0.13
PRC	0.23	0.18	0.24	0.10	0.07	0.19	0.10	0.03	0.08	0.23	0.28	0.09
LHC	0.24	0.20	0.25	0.11	0.14	0.25	0.17	0.06	0.09	0.21	0.26	0.19
MCK	0.22	0.19	0.33	0.16	0.41	0.30	0.37	0.30	0.34	0.29	0.28	0.42
NEB	0.22	0.21	0.30	0.15	0.33	0.32	0.31	0.21	0.25	0.25	0.29	0.36
HMO	0.38	0.31	0.45	0.33	0.52	0.36	0.47	0.43	0.45	0.32	0.49	0.45

Table 11. Continued.

	DEE	NOQ	SPE	BN	GAL	PRC	LHC	MCK	NEB	HMO
TOM	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PM	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
BEA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SOL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
WSL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PEL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LYN	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LCO	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
BIG	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SAW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SOM	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CRA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DEE	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NOQ	0.26	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SPE	0.34	0.14	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
BN	0.26	0.12	0.10	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GAL	0.27	0.09	0.10	0.09	***	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PRC	0.29	0.13	0.07	0.11	0.09	***	<0.0001	<0.0001	<0.0001	<0.0001
LHC	0.34	0.14	0.12	0.09	0.11	0.09	***	<0.0001	<0.0001	<0.0001
MCK	0.51	0.17	0.37	0.28	0.21	0.38	0.30	***	<0.0001	<0.0001
NEB	0.45	0.16	0.28	0.21	0.15	0.30	0.19	0.12	***	<0.0001
HMO	0.49	0.31	0.44	0.34	0.37	0.45	0.40	0.37	0.41	***

Table 12. Johnny darter population pairwise F_{ST} values from FSTAT v2.9.3.2 (below diagonal) and significance values (above diagonal). Full population names are in Table 7.

	TOM	BIG	PM	WSL	SOL	LCO	MC	BEA	LYN	PRC	BN	BUT	NOQ	NEB	MCK	HMO
TOM	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BIG	0.24	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PM	0.06	0.19	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
WSL	0.20	0.08	0.16	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SOL	0.24	0.11	0.20	0.15	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LCO	0.16	0.11	0.11	0.09	0.13	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MC	0.12	0.31	0.11	0.28	0.31	0.21	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BEA	0.08	0.21	0.07	0.18	0.23	0.15	0.11	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LYN	0.36	0.29	0.32	0.30	0.33	0.27	0.40	0.33	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PRC	0.14	0.10	0.11	0.13	0.12	0.11	0.22	0.14	0.33	***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BN	0.22	0.03	0.17	0.06	0.10	0.09	0.28	0.18	0.26	0.10	***	<0.001	<0.001	<0.001	<0.001	<0.001
BUT	0.17	0.33	0.18	0.29	0.35	0.22	0.12	0.16	0.39	0.27	0.30	***	<0.001	<0.001	<0.001	<0.001
NOQ	0.18	0.24	0.16	0.22	0.23	0.18	0.18	0.13	0.40	0.16	0.21	0.24	***	<0.001	<0.001	<0.001
NEB	0.24	0.24	0.18	0.21	0.27	0.16	0.26	0.17	0.41	0.21	0.21	0.30	0.20	***	<0.001	<0.001
MCK	0.22	0.27	0.19	0.25	0.28	0.17	0.24	0.17	0.43	0.21	0.25	0.28	0.19	0.11	***	<0.001
HMO	0.23	0.18	0.20	0.13	0.25	0.11	0.25	0.21	0.34	0.21	0.17	0.26	0.22	0.18	0.19	***

Table 13. Rock bass population pairwise D_{est} values from SMOGD v 1.2.5 (below diagonal) and pairwise geographic distance in km (above diagonal). Full population names are in Table 5.

	TOM	PM	BEA	SOL	WSL	PEL	LYN	LCO	BIG	SAW	SOM	CRA
TOM	***	22.43	11.07	55.97	21.72	50.93	40.61	138.12	36.92	95.74	38.38	57.92
PM	0.01	***	33.02	72.22	23.23	60.70	24.29	149.59	25.65	102.74	60.75	77.17
BEA	0.04	0.03	***	53.52	30.76	45.41	51.53	137.36	47.33	90.28	27.86	52.68
SOL	0.11	0.04	0.11	***	50.96	95.31	73.61	84.24	64.78	138.05	47.15	14.80
WSL	0.11	0.09	0.11	0.04	***	72.34	25.17	126.36	18.19	116.89	53.14	58.34
PEL	0.06	0.05	0.08	0.05	0.07	***	84.92	179.35	84.55	44.96	52.11	89.95
LYN	0.06	0.07	0.08	0.04	0.01	0.08	***	141.13	9.02	126.90	77.02	82.65
LCO	0.10	0.07	0.11	0.02	0.01	0.07	0.02	***	132.56	221.08	129.08	90.40
BIG	0.09	0.06	0.08	0.02	0.00	0.06	0.02	0.01	***	127.77	71.23	74.22
SAW	0.20	0.15	0.28	0.10	0.16	0.24	0.14	0.13	0.14	***	92.00	130.71
SOM	0.06	0.09	0.06	0.06	0.08	0.06	0.07	0.06	0.06	0.21	***	38.71
CRA	0.13	0.10	0.14	0.09	0.03	0.11	0.03	0.04	0.06	0.11	0.18	***
DEE	0.13	0.09	0.14	0.11	0.13	0.07	0.06	0.11	0.11	0.24	0.18	0.14
NOQ	0.10	0.04	0.10	0.02	0.05	0.10	0.05	0.03	0.03	0.06	0.08	0.08
SPE	0.11	0.06	0.13	0.02	0.01	0.07	0.03	0.02	0.01	0.12	0.13	0.03
BN	0.11	0.06	0.12	0.04	0.04	0.09	0.05	0.03	0.02	0.14	0.11	0.08
GAL	0.09	0.08	0.11	0.02	0.06	0.12	0.04	0.02	0.04	0.12	0.10	0.06
PRC	0.14	0.10	0.16	0.03	0.02	0.07	0.03	0.01	0.02	0.12	0.09	0.02
LHC	0.13	0.08	0.16	0.03	0.03	0.10	0.05	0.01	0.02	0.10	0.08	0.07
MCK	0.11	0.09	0.19	0.05	0.16	0.13	0.16	0.12	0.12	0.14	0.12	0.18
NEB	0.12	0.11	0.22	0.06	0.12	0.17	0.16	0.09	0.11	0.14	0.14	0.17
HMO	0.30	0.23	0.38	0.25	0.39	0.21	0.36	0.36	0.32	0.18	0.29	0.25

Table 13. Continued.

	DEE	NOQ	SPE	BN	GAL	PRC	LHC	MCK	NEB	HMO
TOM	41.50	152.11	118.74	68.39	88.32	32.74	149.36	183.83	174.96	218.05
PM	26.82	151.39	107.53	78.15	89.31	44.91	168.31	194.08	177.90	232.85
BEA	48.48	149.72	121.92	69.85	93.58	35.11	143.33	183.47	178.46	214.79
SOL	96.65	201.40	174.60	26.11	61.55	28.18	96.98	130.52	133.12	162.08
WSL	49.91	170.81	130.56	54.92	68.63	22.83	147.89	170.89	156.62	210.02
PEL	52.86	106.14	94.18	114.65	138.74	80.50	173.26	225.67	223.78	251.36
LYN	45.30	173.16	122.89	71.02	72.56	45.86	170.25	183.50	160.62	227.39
LCO	176.06	285.49	256.24	71.46	78.02	105.94	71.86	46.32	71.95	88.14
BIG	50.09	176.84	129.38	62.14	65.77	37.26	161.30	175.31	154.27	218.57
SAW	87.42	67.81	87.57	158.87	183.70	125.30	206.14	267.25	268.36	288.06
SOM	74.75	157.37	141.62	70.94	102.25	44.87	122.40	175.24	180.05	199.26
CRA	99.42	195.82	174.52	40.49	76.25	37.01	91.47	136.53	144.61	162.68
DEE	***	128.06	80.71	104.73	115.45	70.56	190.85	220.79	204.08	258.37
NOQ	0.11	***	74.54	219.54	239.43	184.31	273.93	331.80	327.01	355.37
SPE	0.13	0.03	***	185.18	195.14	150.31	263.81	301.32	283.49	336.56
BN	0.09	0.05	0.04	***	36.09	35.65	104.17	116.14	109.72	156.45
GAL	0.11	0.03	0.04	0.06	***	59.32	130.32	114.70	88.64	166.03
PRC	0.09	0.05	0.02	0.04	0.03	***	125.16	151.35	143.35	187.99
LHC	0.11	0.04	0.04	0.03	0.07	0.02	***	95.80	142.87	85.69
MCK	0.26	0.09	0.12	0.12	0.06	0.17	0.10	***	63.66	62.39
NEB	0.22	0.08	0.11	0.10	0.07	0.11	0.06	0.02	***	125.78
HMO	0.31	0.25	0.29	0.24	0.30	0.33	0.27	0.14	0.26	***

Table 14. Johnny darter population pairwise D_{est} values from SMOGD v1.2.5 (below diagonal) and pairwise geographic distance in km (above diagonal). Full population names are in Table 7.

	TOM	BIG	PM	WSL	SOL	LCO	MC	BEA	LYN	PRC	BN	BUT	NOQ	NEB	MCK	HMO
TOM	***	36.92	22.43	21.72	55.97	138.12	33.49	11.07	40.61	32.74	68.39	54.55	152.11	174.96	183.83	218.05
BIG	0.37	***	25.65	18.19	64.78	132.56	65.49	47.33	9.02	37.26	62.14	67.40	176.84	154.27	175.31	218.57
PM	0.07	0.30	***	23.23	72.22	149.59	41.49	33.02	24.29	44.91	78.15	43.56	151.39	177.90	194.08	232.85
WSL	0.18	0.11	0.18	***	50.96	126.36	54.17	30.76	25.17	22.83	54.92	66.19	170.81	156.62	170.89	210.02
SOL	0.33	0.12	0.28	0.18	***	84.24	83.73	53.52	73.61	28.18	26.11	110.51	201.40	133.12	130.52	162.08
LCO	0.27	0.15	0.22	0.16	0.22	***	167.83	137.36	141.13	105.94	71.46	191.59	285.49	71.95	46.32	88.14
MC	0.10	0.54	0.19	0.41	0.48	0.43	***	30.65	65.71	65.10	100.33	38.30	119.24	208.21	214.02	243.68
BEA	0.09	0.36	0.12	0.22	0.38	0.38	0.17	***	51.53	35.11	69.85	59.20	149.72	178.46	183.47	214.79
LYN	0.57	0.35	0.51	0.40	0.39	0.47	0.60	0.55	***	45.86	71.02	62.51	173.16	160.62	183.50	227.39
PRC	0.16	0.07	0.18	0.12	0.13	0.13	0.31	0.19	0.44	***	35.65	85.68	184.31	143.35	151.35	187.99
BN	0.33	0.03	0.28	0.10	0.11	0.14	0.45	0.33	0.32	0.10	***	120.57	219.54	109.72	116.14	156.45
BUT	0.18	0.52	0.26	0.45	0.48	0.38	0.13	0.23	0.44	0.36	0.47	***	111.29	221.27	236.72	272.59
NOQ	0.26	0.41	0.27	0.34	0.36	0.34	0.31	0.22	0.72	0.26	0.35	0.37	***	327.01	331.80	355.37
NEB	0.39	0.39	0.32	0.33	0.50	0.33	0.49	0.34	0.70	0.34	0.37	0.48	0.37	***	63.66	125.78
MCK	0.30	0.45	0.33	0.35	0.43	0.37	0.33	0.29	0.78	0.32	0.43	0.45	0.22	0.16	***	62.39
HMO	0.36	0.32	0.38	0.21	0.46	0.28	0.46	0.43	0.52	0.39	0.32	0.41	0.48	0.31	0.28	***

Figure 1. Contemporary management units for walleye and muskellunge based on Fields et al. (1997). Bold lines denote management unit boundaries, dashed line represents the split in the Chippewa River watershed for walleye into Upper Chippewa River watershed and Lower Chippewa River watershed, and thin lines represent county borders

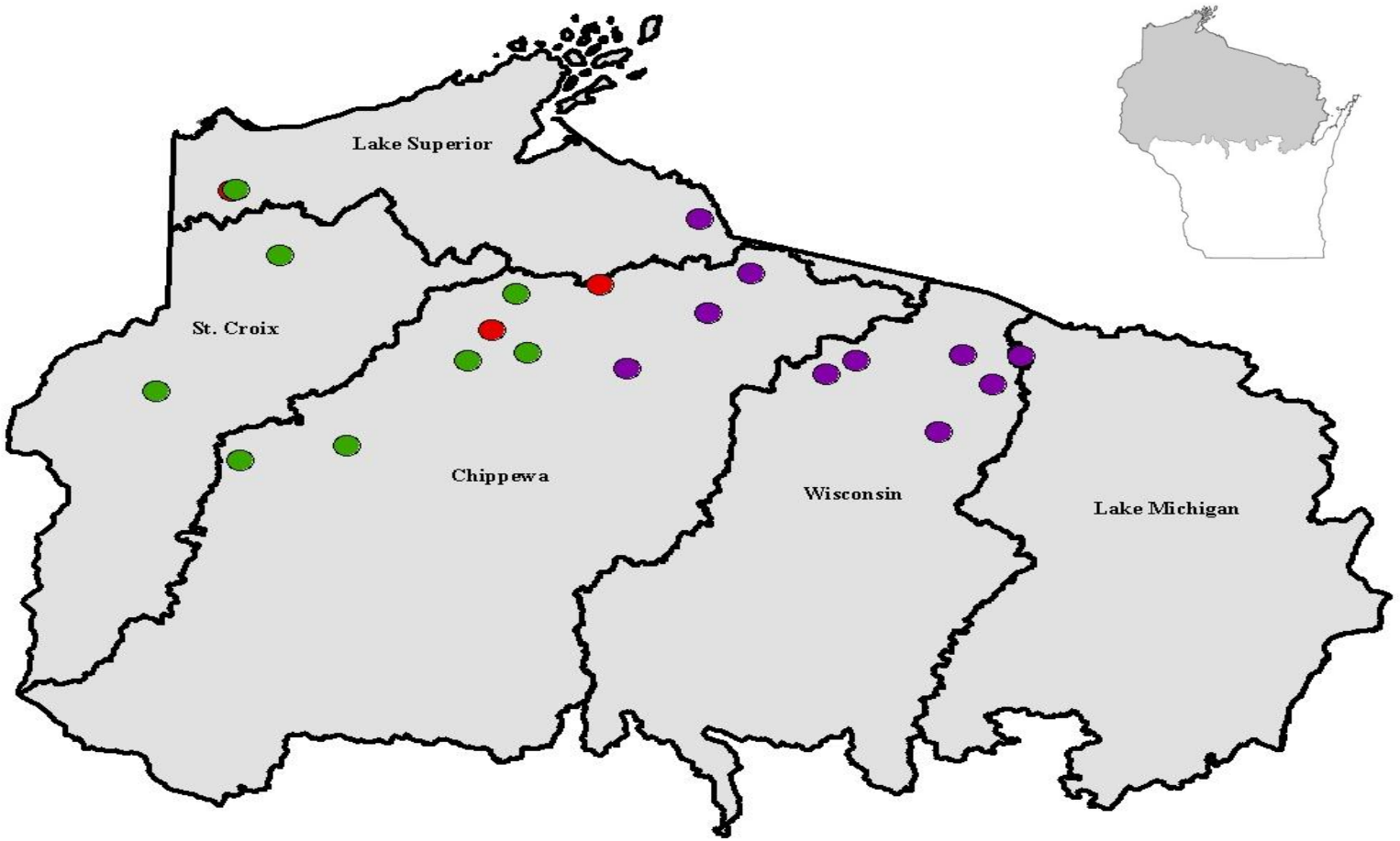


Figure 2. Genetic structure observed in walleye across the five major watersheds in northern Wisconsin based on Hammen (2009) (Upper Wisconsin Unit; Purple, Upper Chippewa Unit; Green, Lake Superior; Red). Genetic discordance was found in walleye populations in the headwaters of the Chippewa River watershed.

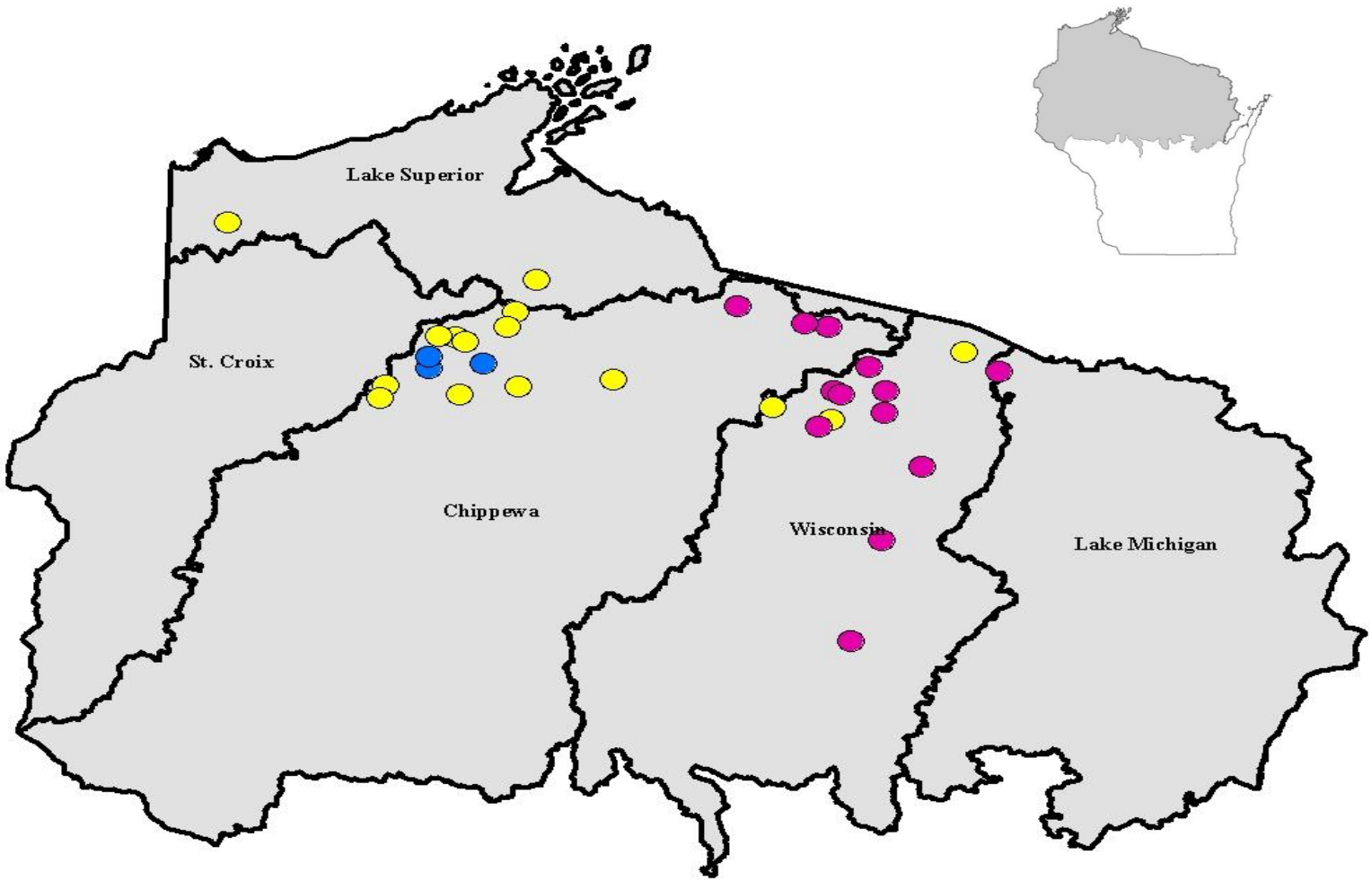


Figure 3. Genetic structure observed in muskellunge across the five major watersheds in northern Wisconsin based on Spude (2010) (Upper Wisconsin Unit; Pink, Upper Chippewa Unit; Yellow, Central Chippewa Unit; Blue). Genetic discordance was found in muskellunge populations in the headwaters of the Chippewa River and Wisconsin River watersheds.

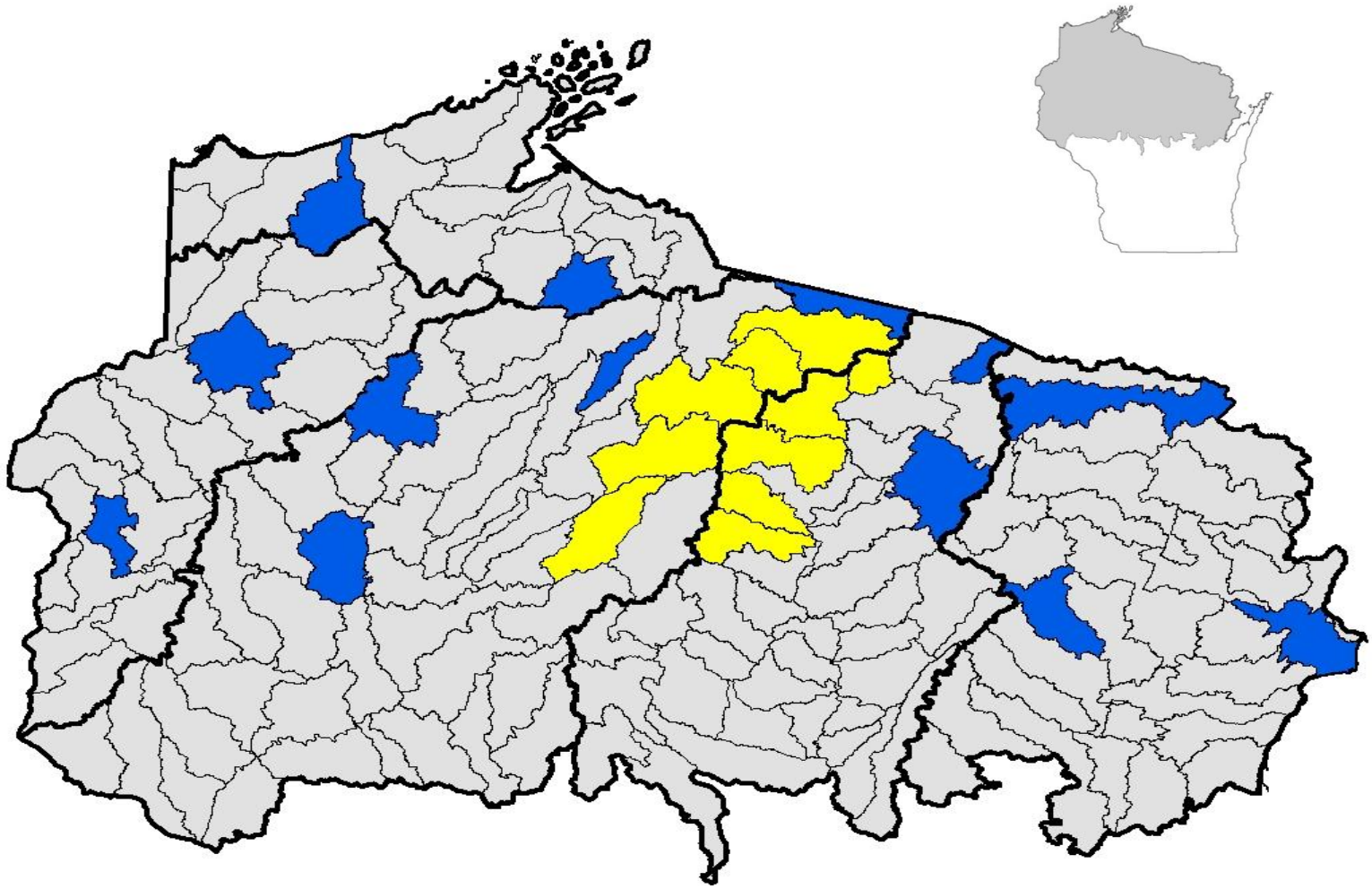


Figure 4. Sub-watersheds selected for sampling in northern Wisconsin during the summer of 2010 and 2011. Yellow sub-watersheds represent target areas. Blue sub-watersheds represent randomly selected areas.

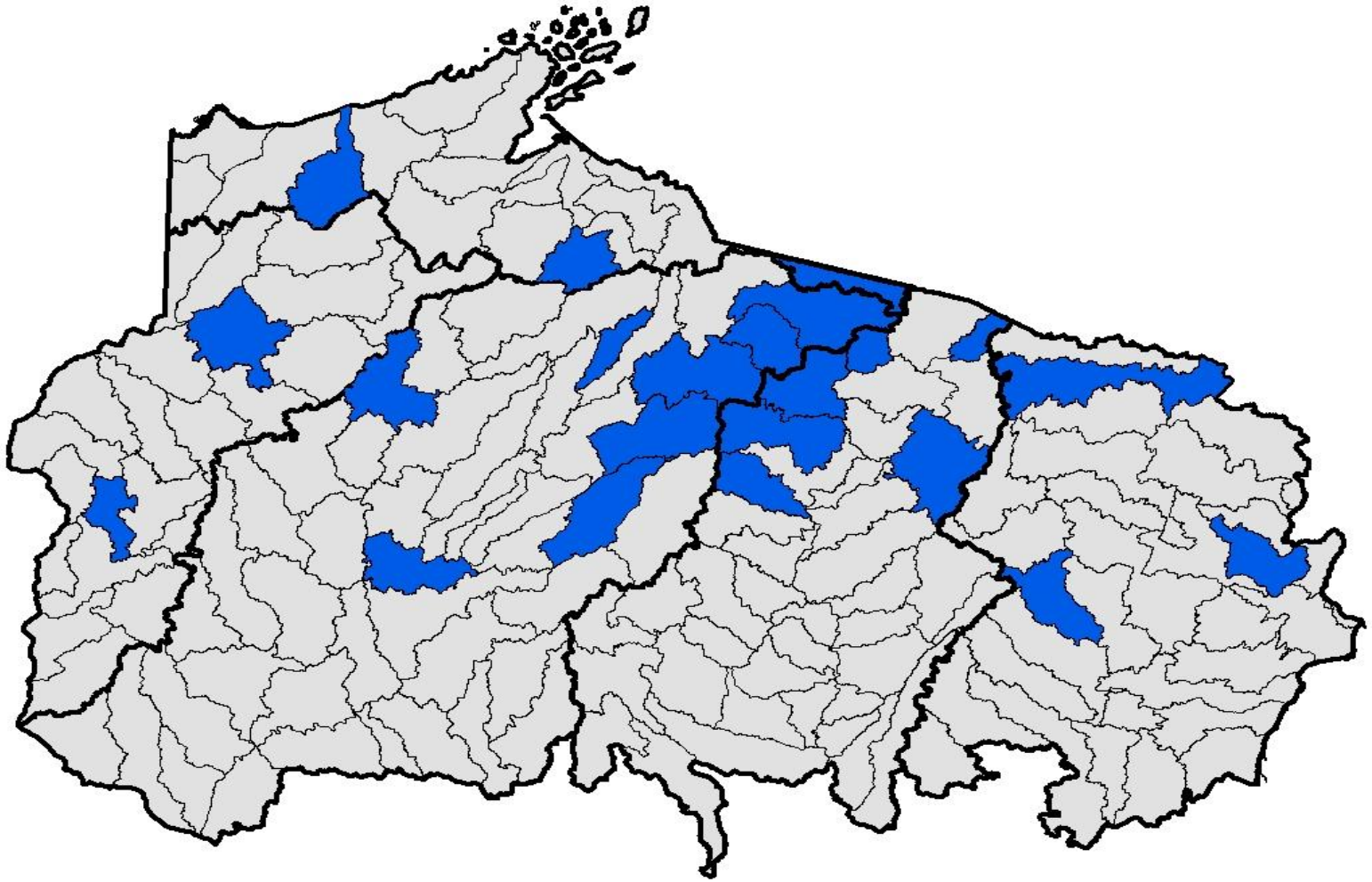


Figure 5. Twenty-two sub-watersheds sampled for rock bass in northern Wisconsin during the summers of 2010 and 2011.

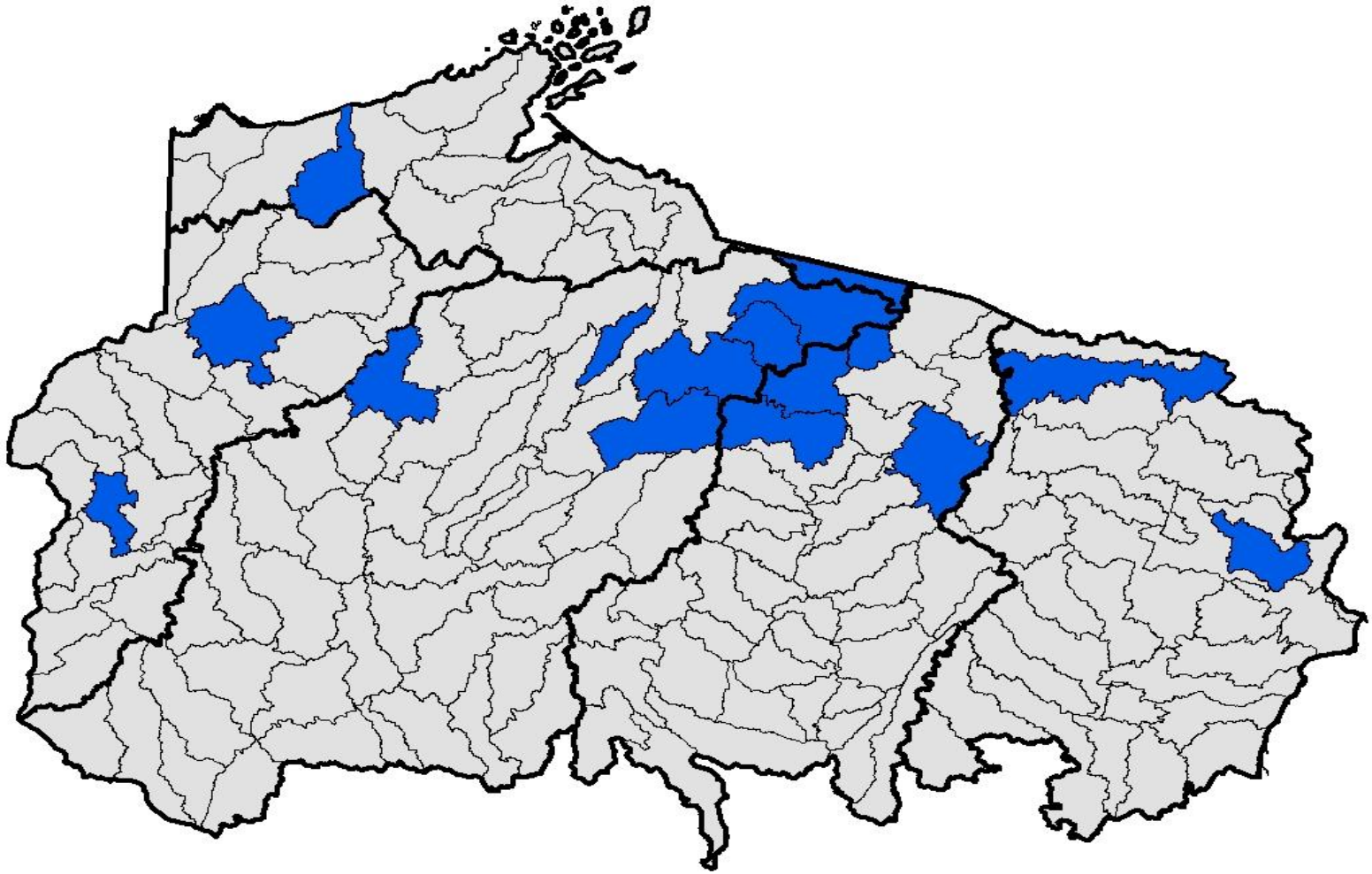


Figure 6. Sixteen sub-watersheds sampled for johnny darters in northern Wisconsin during the summers of 2010 and 2011.

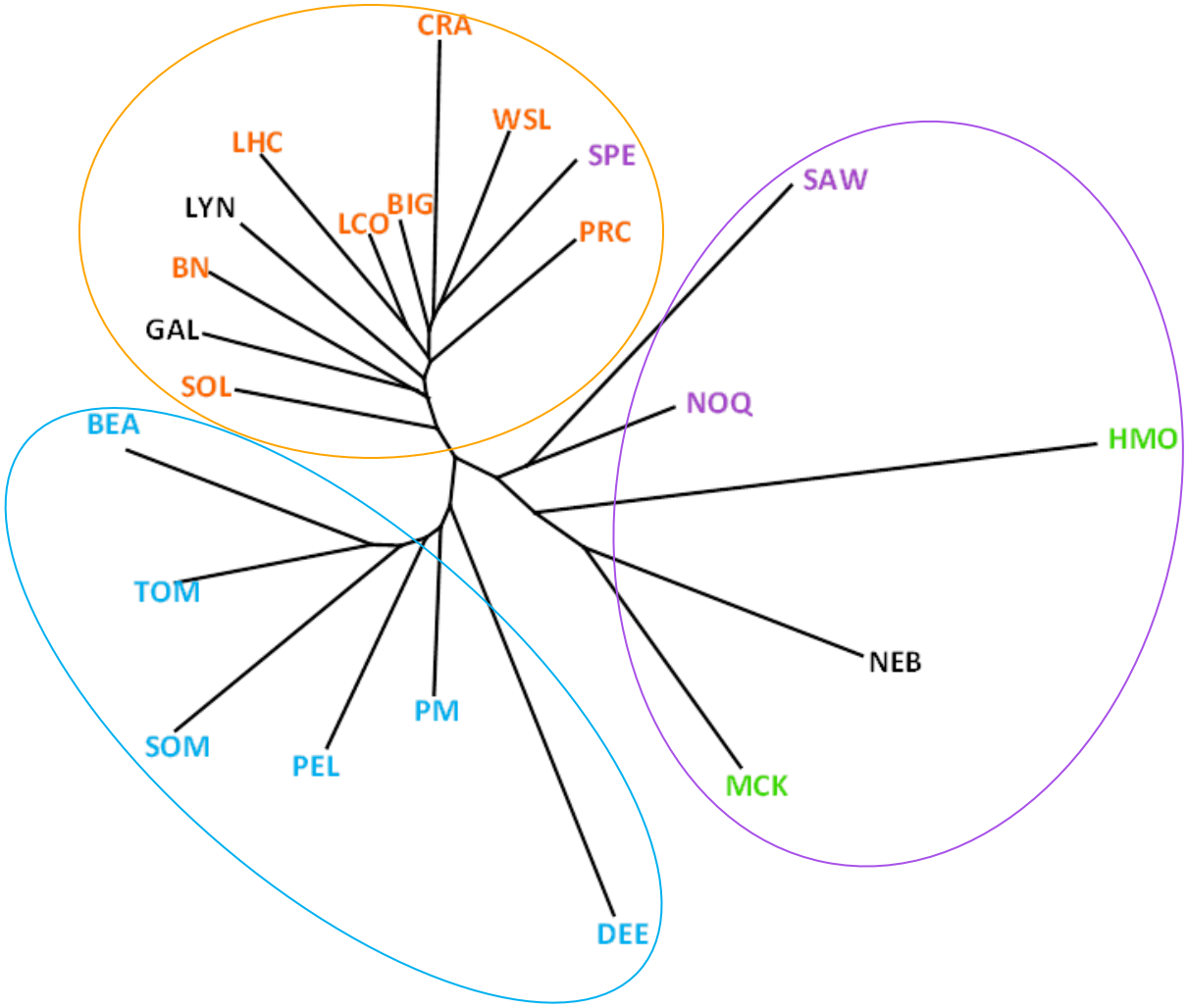


Figure 7. Unrooted neighbor joining tree based on Cavalli-Sforza and Edwards (1969) chord distance (D_c) for rock bass. Full population names are in Table 5. Populations are color coded by watershed location (Blue = Wisconsin; Orange = Chippewa; Black = Lake Superior; Purple = Lake Michigan; Green = St. Croix).

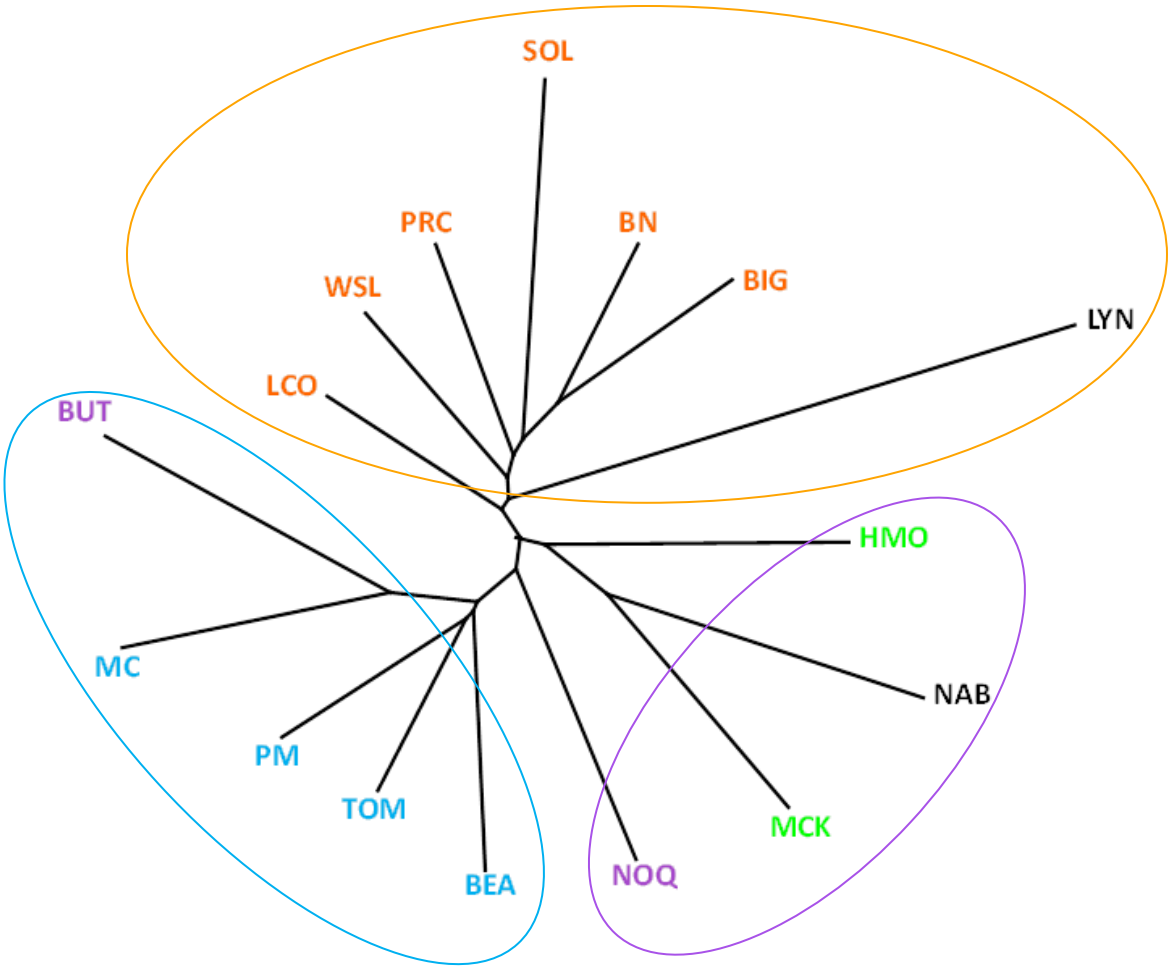


Figure 8. Unrooted neighbor joining tree based on Cavalli-Sforza and Edwards (1969) chord distance (D_c) for johnny darters. Full population names are in Table 7. Populations are color coded by watershed location (Blue = Wisconsin; Orange = Chippewa; Black = Lake Superior; Purple = Lake Michigan; Green = St. Croix).

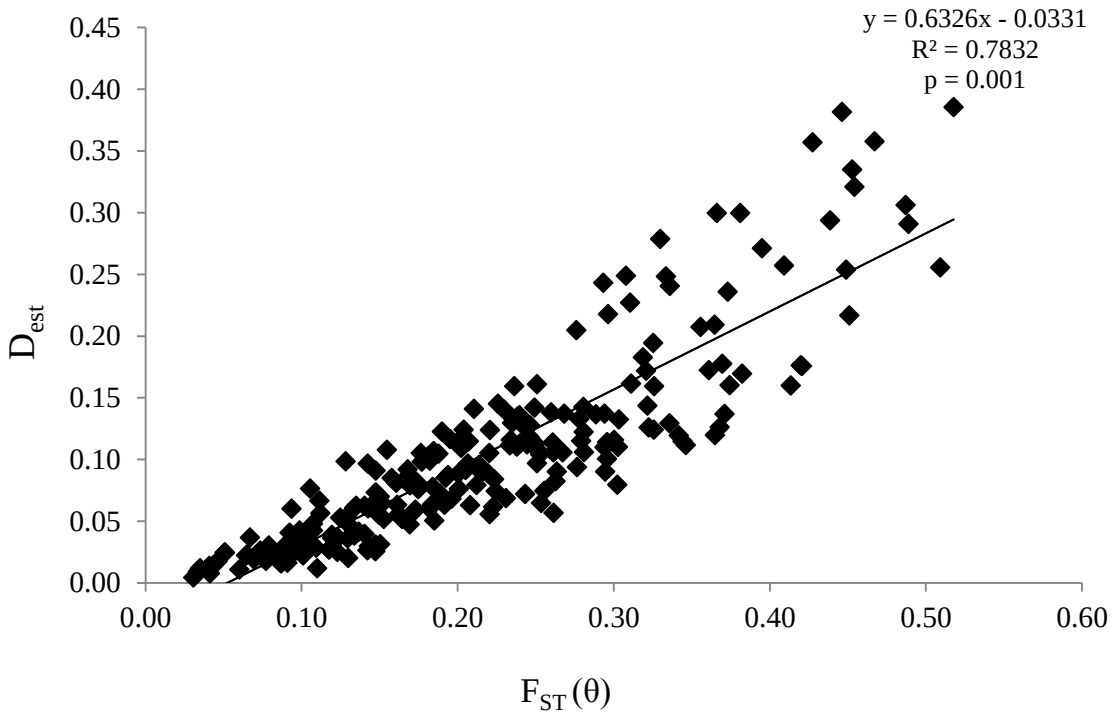


Figure 9. Mantel test comparing pairwise D_{est} values versus F_{ST} (θ) values for rock bass.

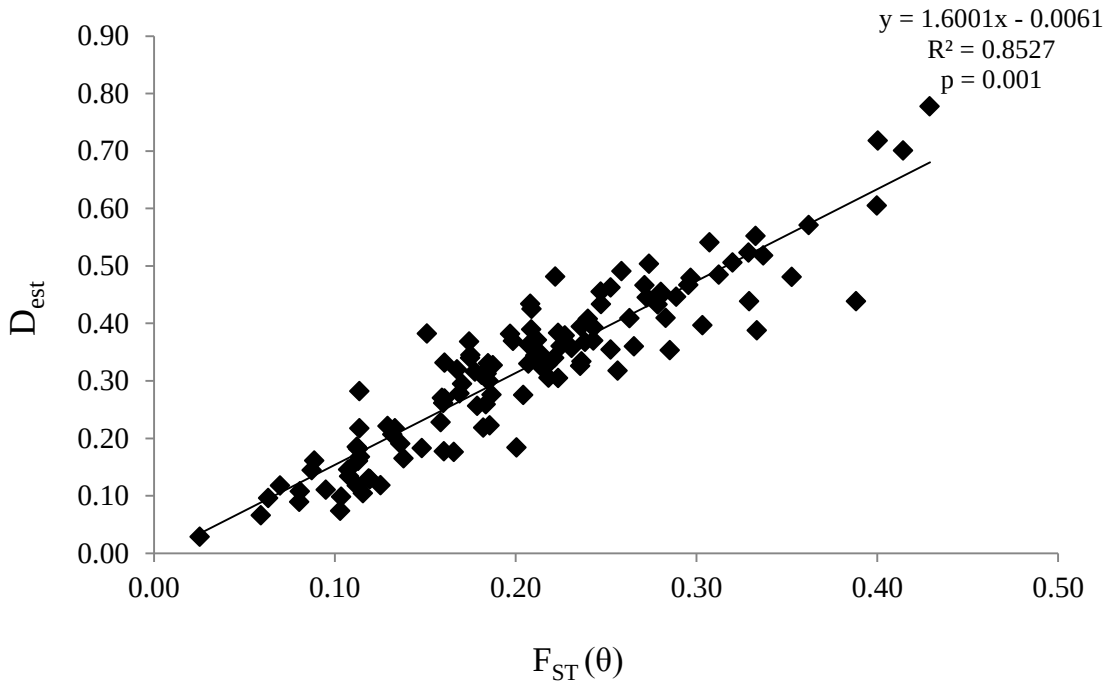


Figure 10. Mantel test comparing pairwise D_{est} values versus F_{ST} (θ) values for johnny darters.

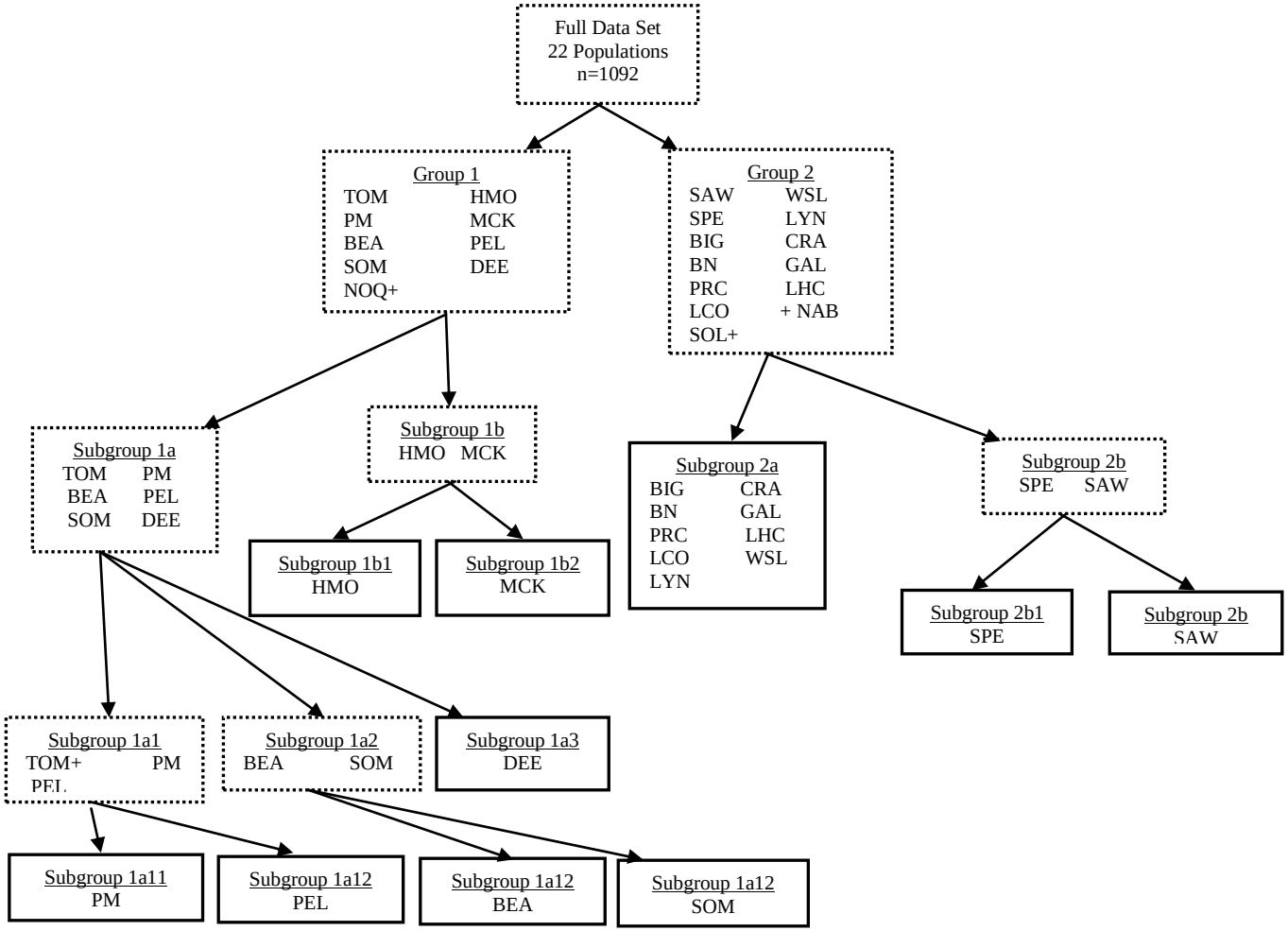


Figure 11. Results from STRUCTURE v2.3.3 following the modified Coulon et al. (2008) method of assignment for rock bass. Dashed boxes represent unreconciled groups and solid boxes represent stable groupings at K=1. Populations with (+) indicate most likely group but failed to assign with ≥75% probability and are assumed to be individual units.

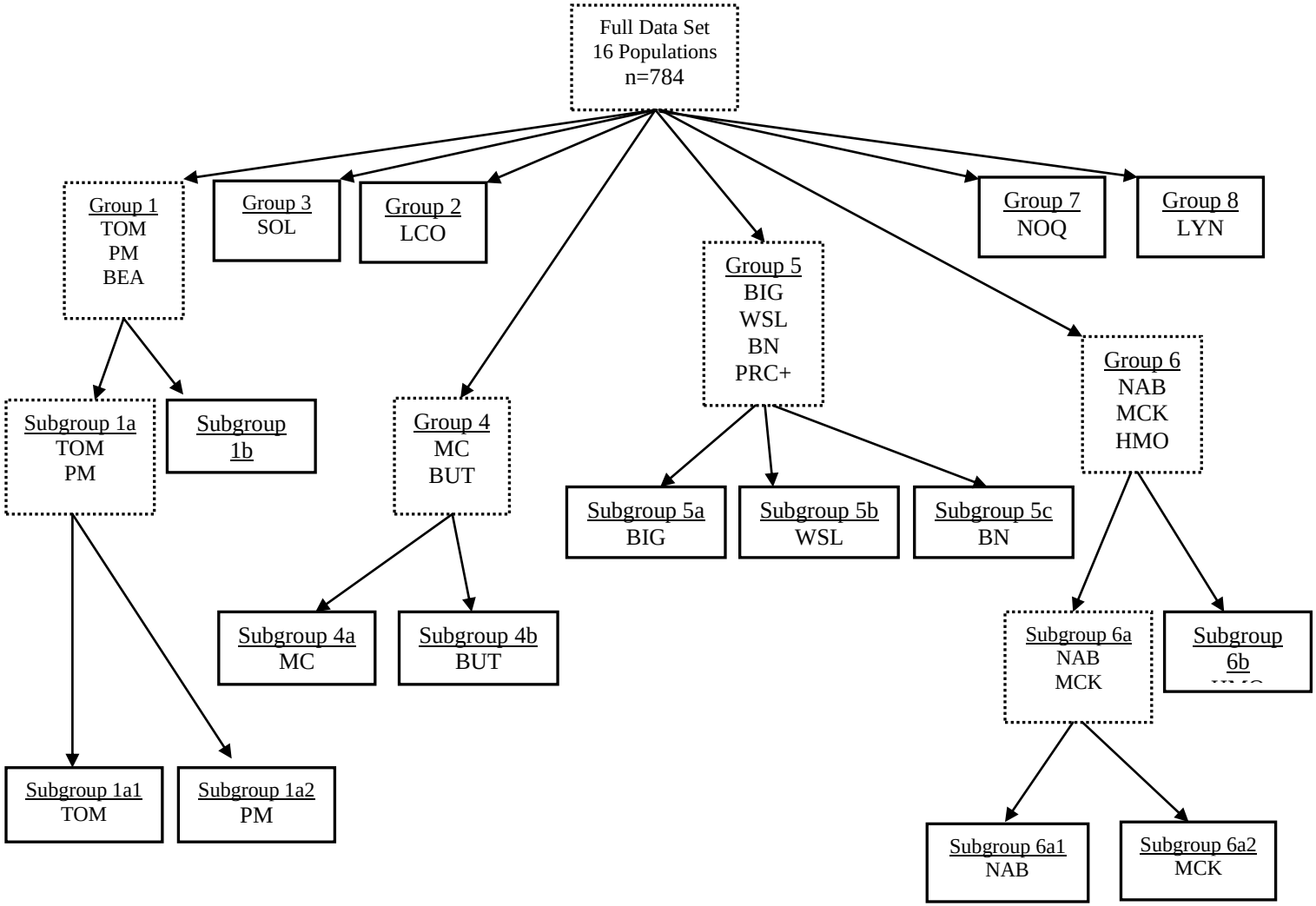


Figure 12. Results from STRUCTURE v2.3.3 following the modified Coulon et al. (2008) method of assignment for johnny darters. Dashed boxes represent unreconciled groups and solid boxes represent stable groupings at K=1. Populations with (+) indicate most likely group but failed to assign with ≥75% probability and are assumed to be individual units.

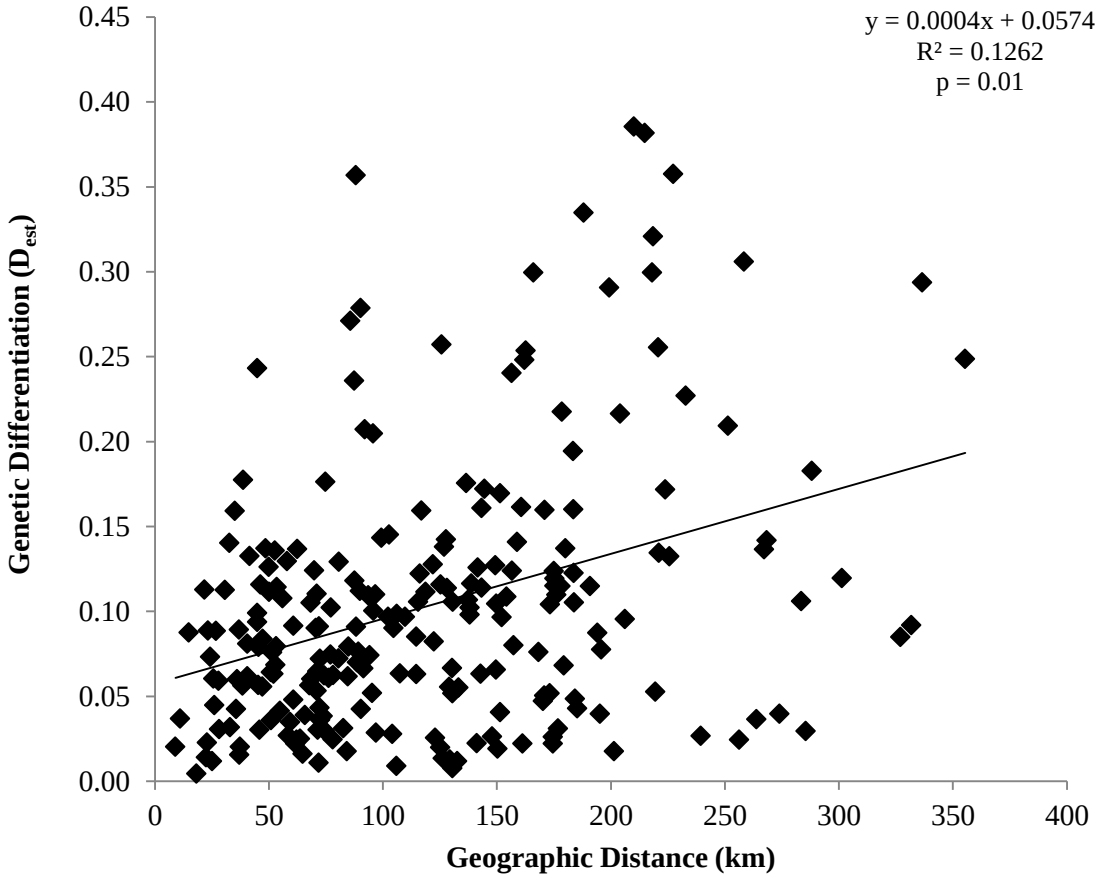


Figure 13. Mantel test comparing geographic distance (km) and genetic differentiation (D_{est}) between all populations of sampled rock bass.

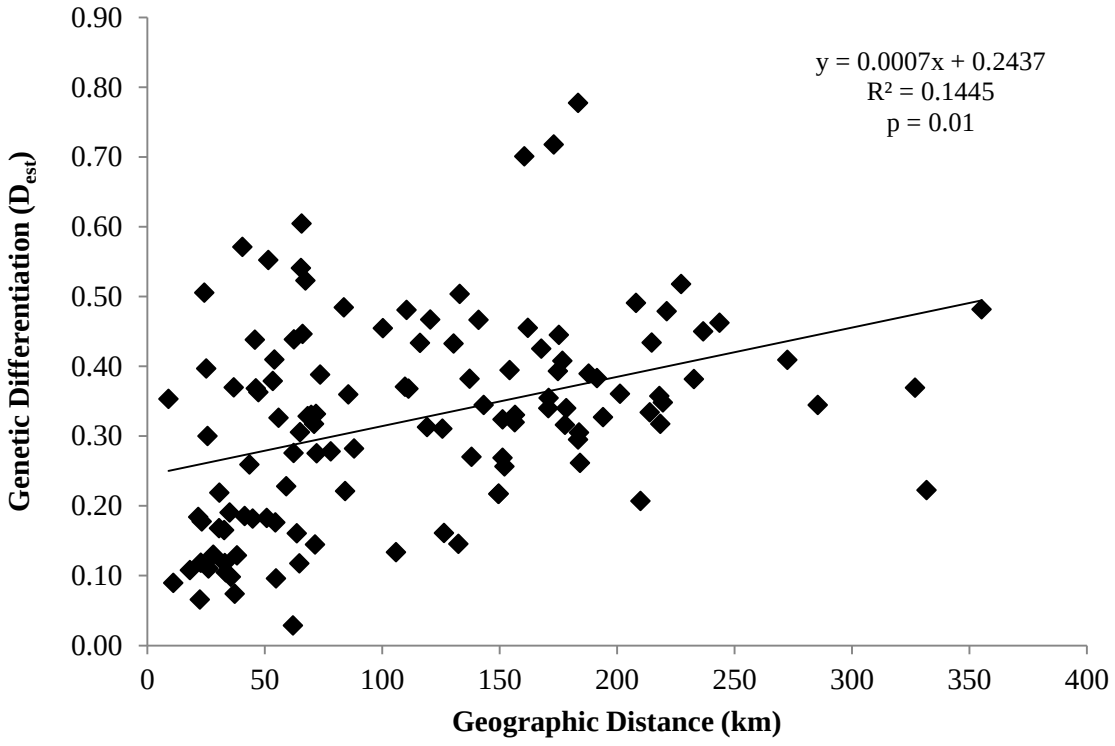
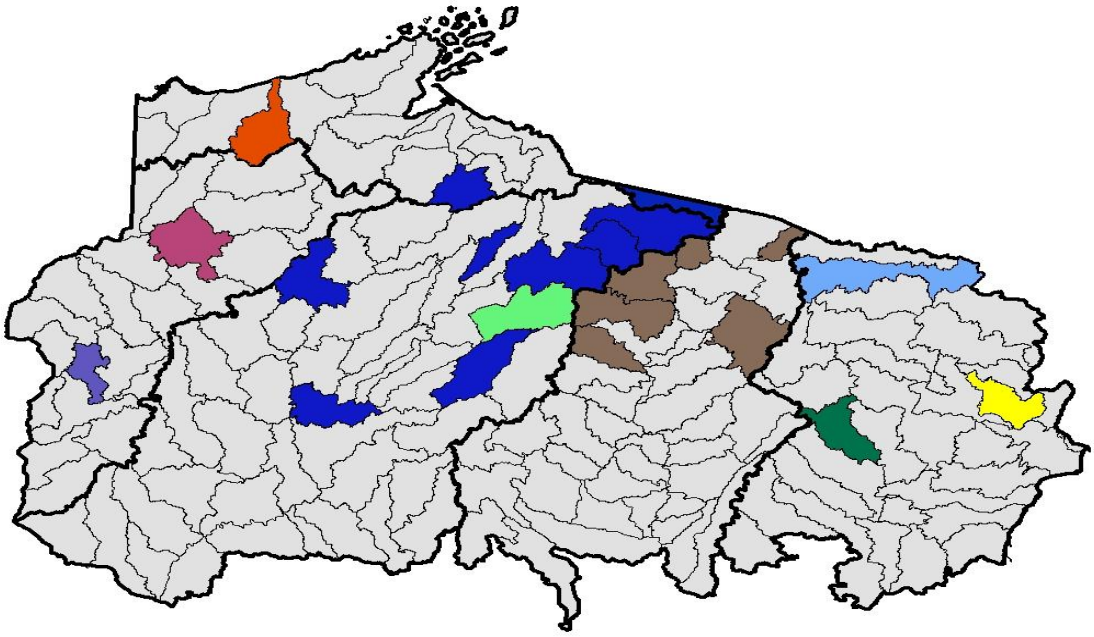
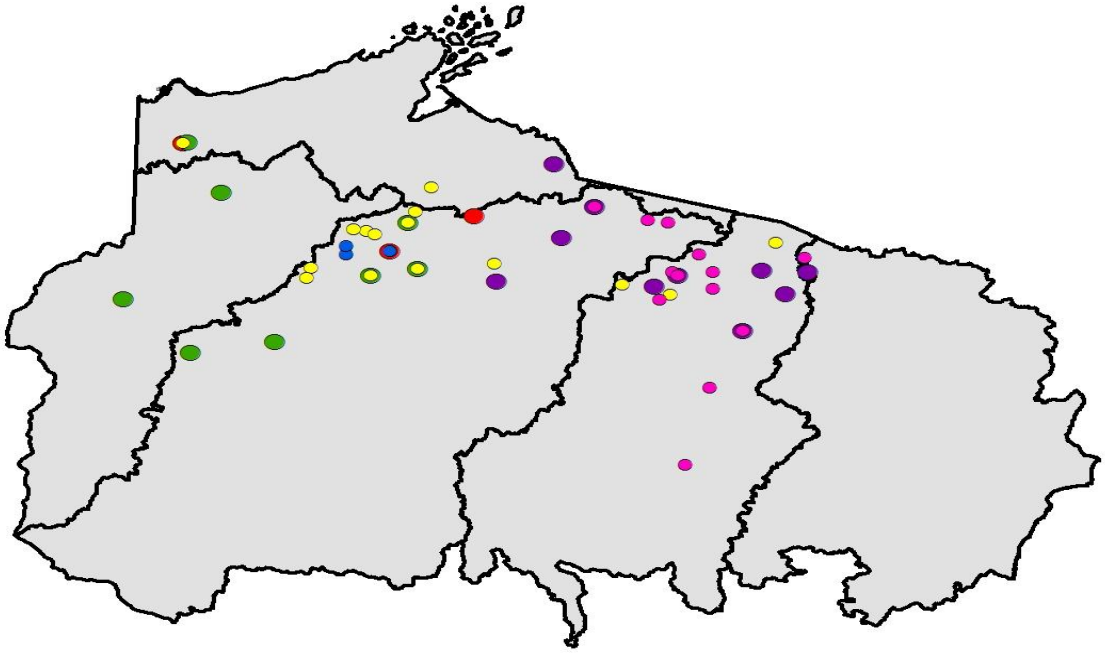


Figure 14. Mantel test comparing geographic distance (km) and genetic differentiation (D_{est}) between all populations sampled for johnny darters.

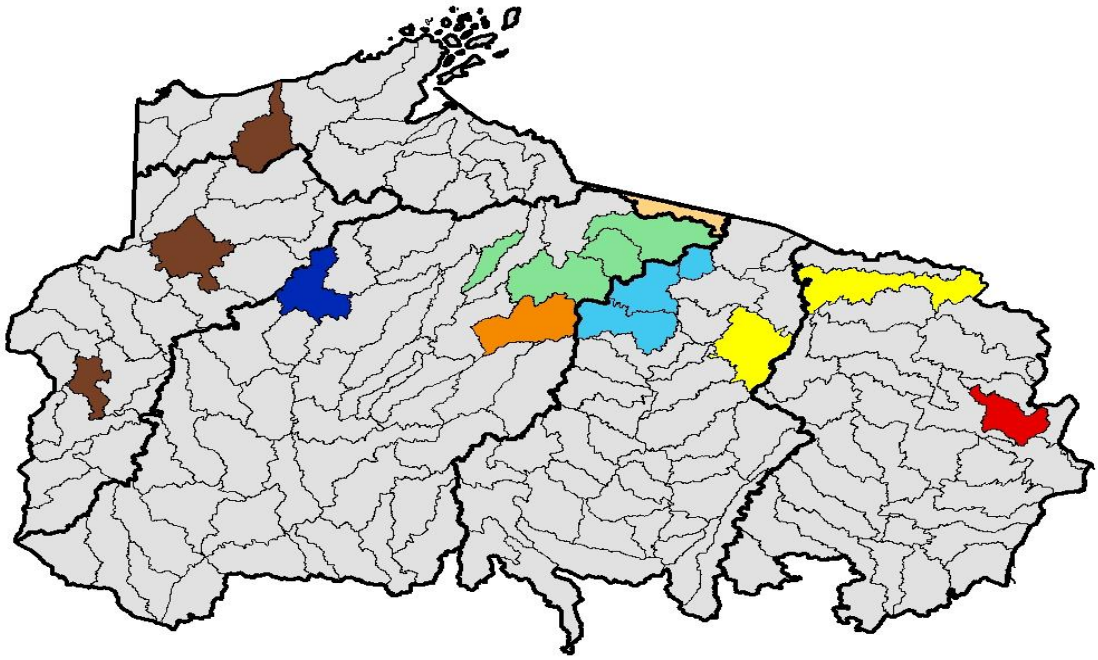


(a)

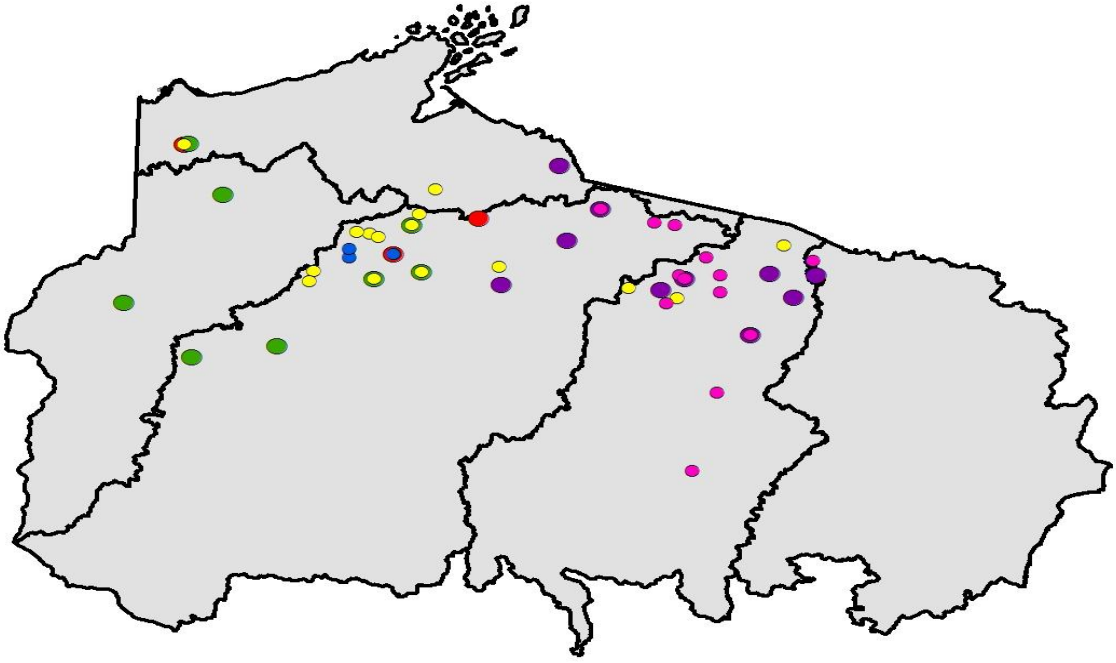


(b)

Figure 15. (a) Nine resolved genetic units for rock bass. All sub-watersheds are color coded to match units in which they grouped. (b) Composite map of walleye and muskellunge genetic units as described in Figure 2 and 3.



(a)



(b)

Figure 16. (a) Eight resolved genetic units for johnny darters. All sub-watersheds are color coded to match units in which they grouped. (b) Composite map of walleye and muskellunge genetic units as described in Figure 2 and 3.