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RESEARCH ARTICLE

# The influence of historical and contemporary landscape variables on the spatial genetic structure of the rainbow darter (*Etheostoma caeruleum*) in tributaries of the upper Mississippi River

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**Abstract** Evaluating spatial genetic patterns is an important method to help inform management efforts for the conservation of native fishes. However, the present study demonstrates that it is vital to consider life history, distribution, and historical processes when interpreting the spatial distribution of genetic diversity. This study examined genetic variation in populations of the Rainbow Darter, *Etheostoma caeruleum*, in tributaries of the upper Mississippi River in northeast Iowa in order to understand the influence of landscape alteration at multiple temporal scales. The diversity and distribution of fishes in this region are influenced by historical geologic and climatic events, and recent, intensive human activities, making this an excellent site for an investigation of this type. Landscape genetic analyses of eight microsatellite loci from 14 localities detected a single genetic population. The amount of genetic diversity observed within localities and drainages was high, but the distribution of genetic variation was almost uniform across the study area. There was no evidence of population subdivision at any spatial scale. Based on what is known about the life history of the Rainbow Darter and the geological history of the region, the best explanation for these results is that historical processes had a more pronounced influence on the observed genetic variation than contemporary impacts. Specifically, the genetic signature supports a conclusion of population expansion into the region following the retreat of glacial advances during the Pleistocene.

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## Introduction

As landscapes become increasingly altered and fragmented due to anthropogenic impacts, understanding the spatial genetic structure of organisms has become an important tool for developing effective management strategies to preserve biodiversity (Storfer et al. 2007; Segelbacher et al. 2010; Sork and Waits 2010). A fundamental goal of landscape genetics is to understand how landscape features and anthropogenic modifications shape the genetic connectivity among populations and spatial patterns of genetic variation (Storfer et al. 2007; Sork and Waits 2010). When populations become fragmented and isolated, the probability of local and regional extinction increases (Hanski 2001; Frankham 2005; Segelbacher et al. 2010). Therefore, landscape genetic studies often seek to identify potential barriers to gene flow and overall connectivity. This information can be valuable to construct or preserve corridors that facilitate gene flow among habitat fragments in order to maintain genetic diversity and overall genetic health of a population (Frankham 2005; Storfer et al. 2007; Storfer et al. 2010; Lowe and Allendorf 2010).

When evaluating the spatial distribution of genetic diversity, it is important to recognize that observed patterns reflect the influence of the landscape at many temporal scales (Storfer et al. 2007; Balkenhol et al. 2009; Zellmer and Knowles 2009). Geologic and climatic processes cause long-term, pervasive changes in the landscape whereas anthropogenic impacts result in intensive, contemporary modifications. In turn, as landscapes and habitats are

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altered, species respond by becoming locally extirpated, migrating to new areas of suitable habitat, or adapting to changing local conditions. These processes can lead to the fragmentation and isolation of populations.

Since landscape structure and species distributions do not remain constant over time, temporal factors and variation should be considered when interpreting the observed spatial pattern of genetic variation for the application of conservation efforts (Storfer et al. 2007; Balkenhol et al. 2009; Apodaca et al. 2012). It is possible that historical processes can have a stronger influence on the observed genetic signature than effects of recent landscape alterations. If this is the case, the interpretation of contemporary impacts on the fragmentation of populations based on genetic data can be difficult to resolve (Balkenhol et al. 2009; Zellmer and Knowles 2009; Epps et al. 2013). In fact, if temporal variation is not recognized, it could lead to erroneous conclusions and potentially mislead conservation efforts.

The upper Mississippi River drainage represents a unique region to examine the spatial distribution of genetic diversity of native fishes for application to conservation efforts. The distribution of the ichthyofauna in this region has been greatly influenced by geologic and climatic events, and recent, intensive human activities. The underlying geology of the upper Mississippi River drainage was shaped by periodic glacial cycles throughout the Quaternary period 2.6 million years ago to 10,500 years ago (Lowe and Walker 1997; Mickelson and Colgan 2004). Advancing and retreating glacial fronts altered the landscape by changing river flow and drainage patterns (Thornbury 1965; Mayden 1988). The distributions of aquatic species were greatly affected by the habitat change, resulting in displacement or extirpation of local populations. Alterations in the landscape also caused changes in dispersal and dispersion opportunities due to the establishment of new drainage connections (Bernatchez and Wilson 1998; Hewitt 2000; Berendzen et al. 2003; Berendzen et al. 2008). Collectively, these events were a major influence in shaping the contemporary assemblage of fishes found in the upper Mississippi River drainage (Berendzen et al. 2010).

Glacial activity during the Quaternary period also contributed to the formation of rich, fertile soils that characterize the modern landscape in the upper Mississippi River drainage (Anderson 1998). Beginning in the early 1800s, pioneers settled the region and converted the landscape from prairie and wetlands to agriculture. Today, the landscape has been dramatically altered by intensive agricultural practices and urbanization. Rivers and streams in this region have been heavily impacted by these practices resulting in extensive modification to habitat structure, water quality and flow regime (Menzel 1983; Karr et al. 1985; Allan 2004). The substantial loss and degradation of the aquatic habitat has caused the decline and fragmentation of populations imperiling many native fishes in the region (Howe 1984; Karr et al. 1985; Jelks et al. 2008).

*Etheostoma caeruleum*, the Rainbow Darter, is a native freshwater fish that has a broad distribution in eastern North America (Ray et al. 2006). However, in tributaries of the upper Mississippi River in Iowa it has a patchy, discontinuous distribution, which represents the northwestern edge of its range (Fig. 1). The Rainbow Darter is a small ($\sim$50–80 mm), sexually dimorphic fish with brightly colored males. They are benthic and typically inhabit clear, cool small to moderate size rivers and streams with swift to moderate currents (Becker 1983). The habitat of the Rainbow Darter typically comprises shallow gravel and rubble riffles, but they can also be found in nearby pools (Vogt and Coon 1990; Heithaus and Laushman 1997). Although the Rainbow Darter is not recognized as imperiled in the state of Iowa, it has been identified as a sentinel species for measuring the effects of anthropogenic impacts (Tonnis 2006; Haponski et al. 2009). Therefore, understanding the spatial distribution of genetic diversity of the Rainbow Darter can be used to guide management efforts to protect native fishes and other aquatic species in the region.

The objective of this study is to use landscape genetics to understand the roles that contemporary and historical landscapes and processes played in shaping the spatial genetic structure of the Rainbow Darter in tributaries of the upper Mississippi River in northeast Iowa. There are multiple hypotheses that can explain the spatial genetic structure of the Rainbow Darter in the region. One hypothesis predicts that geologic and climatic processes predominantly shaped the observed genetic signature. Under this hypothesis, the Rainbow Darter would have colonized the region following glacial retreat. This scenario would be supported by the observation of a homogeneous spatial genetic pattern across the entire region, uniform migration rates over various temporal scales, and evidence of population expansion. A second hypothesis predicts that contemporary modifications to the landscape primarily shaped the observed genetic signature. According to this hypothesis, local populations will be highly fragmented and isolated due to human activities. This scenario would be supported by the observation of substantial population genetic substructure, reduced genetic diversity within populations, and reduced gene flow associated with fragmentation of populations consistent with anthropogenic impacts. A third hypothesis predicts that a combination of historical and contemporary effects shaped the observed genetic signature. This would be supported by the observation of genetic patterns expected from both processes. Based on the results, the utility of these data for application

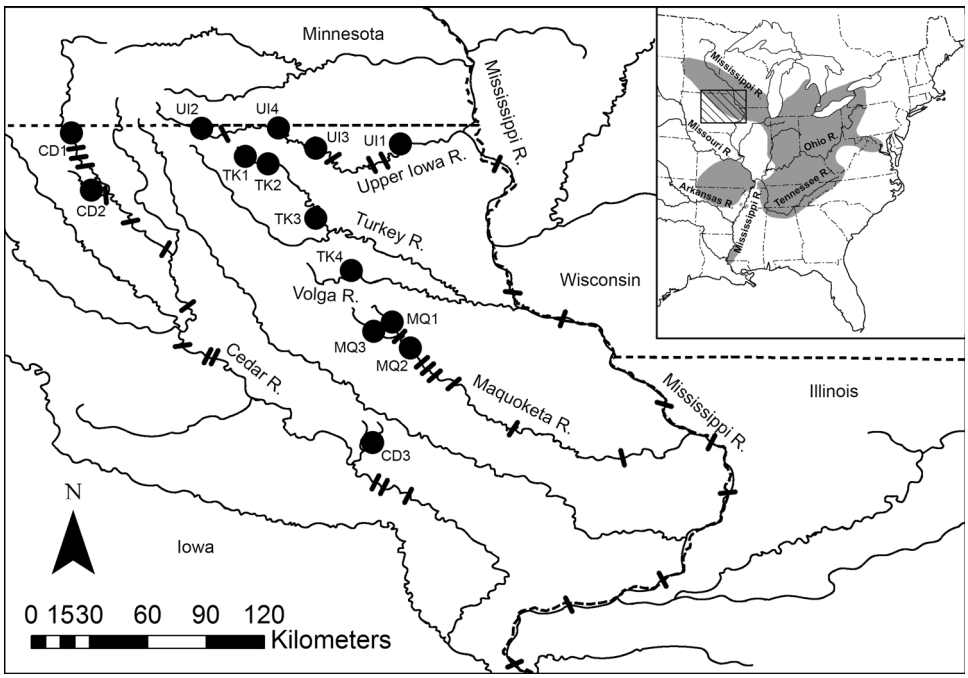


**Fig. 1** Map of tributaries of the upper Mississippi River in northeast Iowa. Sampling localities indicated with a *closed circle*; codes are cross-referenced with Table 1. Dams relavant to the study are indicated with a *dashed line*. The *inset* depicts eastern North America with the estimated range-wide distribution of *Etheostoma caeruleum* and the major rivers of the Mississippi River drainage. The study region is indicated by the *hatched box*

to conservation efforts on aquatic organisms in the region will be evaluated.

## Methods

### Sample collection and genotyping

Tissue samples from 271 individuals of *E. caeruleum* were collected from fourteen localities representing four major drainages in which they occur in northeastern Iowa (Fig. 1; Table 1). An effort was made to obtain individuals from all areas Rainbow Darters were historically found in Iowa. Historical localities not included in this study may be a result of local extirpation or the inability to locate or access populations and suitable habitat. An effort was also made to obtain individuals above and below dams and impoundments distributed within each river system in order to adequately represent landscape heterogeneity (Fig. 1). Fish were collected using a combination of standard backpack electroshocking and seining techniques; all necessary permits were obtained. Individuals were held in aerated buckets prior to tissue sampling. Non-lethal fin clips were taken from the right pectoral fin and immediately placed in 95 % ethanol for storage. Following tissue sampling, individuals were released into the stream and specific habitat where they were obtained.

Genomic DNA was extracted from the fin clips using a standard guanidine thiocyanate extraction protocol. Ten tetranucleotide microsatellite markers developed for *E. caeruleum* (Eca6EPA, Eca10EPA, Eca14EPA, Eca22EPA, Eca24EPA, Eca36EPA, Eca44EPA, Eca46EPA, Eca48EPA and Eca71EPA; Tonnis 2006) were used to genotype all individuals. A PCR method incorporating amplified and fluorescent labels was used to amplify and label all loci (Schuelke 2000; Boutin-Ganache et al. 2001). This reaction is performed with three primers: a sequence-specific forward primer with a M13 (5′-CAC GAC GTT GTA AAA CGA C-3′) tail at its 5′ end, a sequence-specific reverse primer and a universal florescent-labeled M13 (5′-FAM CAC GAC GTT GTA AAA CGA C-3′) primer. The M13 primer was labeled with either HEX or FAM. Nested PCRs were performed in a total volume of 25 µl containing 5–10 ng DNA, 0.25 µM labeled M13 primer and reverse primer, 0.025 µM M13-tailed forward primer, 1X *Taq* salts, 2.5 mM MgCl2, 0.4 µM dNTPs, and 1.25 units of *Taq* DNA polymerase (GoTaq Hot Start Green Master Mix; Promega Corp., Madison, WI, USA). The following thermal profile was used for all nested amplifications: initial denaturation at 94 °C (2 min); 10 cycles of 94 °C (20 s.), 50 °C (20 s.), 72 °C (30 s.); 30 cycles of 94 °C (20 s.), 48 °C (20 s.), 72 °C (30 s.), and a final extension at 72 °C (10 min). PCR products were sent to the DNA Facility of the Iowa State University Office of Biotechnology for genotyping on an ABI 3100 Genetic Analyzer (Applied Biosystems).

Alleles were scored using GENE MARKER version 1.85 (SoftGenetics, LLC, State College, PA, USA). MICROCHECKER version 2.2.3 (van Oosterhout et al. 2004) was used to identify genotyping errors due to null alleles, large allele drop out, and scoring of stutter peaks. Exact tests of conformity to Hardy–Weinberg equilibrium (HWE)

**Table 1** Collection localities and genetic diversity patterns of *Etheostoma caeruleum* in northeast Iowa denoted by river drainage

| Drainage/ID | Lat. (N)/Long. (W) | *N* | $N_A$ (SE) | $N_e$ (SE) | $A_R$ | $N_P$ | $H_o$ (SE) | $H_e$ (SE) | F (SE) |
|---|---|---|---|---|---|---|---|---|---|
| Upper Iowa River | | | | | | | | | |
| UI1 | 43.421666/–91.553500 | 10 | 8.750 (0.491) | 6.17 (0.492) | 6.890 | 4 | 0.897 (0.019) | 0.831 (0.012) | –0.082 (0.035) |
| UI2 | 43.489300/–92.403333 | 8 | 8.125 (0.693) | 5.327 (0.516) | 6.892 | 0 | 0.725 (0.076) | 0.799 (0.021) | 0.104 (0.081) |
| UI3 | 43.40280/–91.915550 | 20 | 10.375 (0.822) | 7.255 (0.739) | 6.730 | 1 | 0.905 (0.031) | 0.851 (0.017) | –0.064 (0.031) |
| UI4 | 43.490790/–92.074530 | 21 | 10.875 (1.156) | 7.691 (0.788) | 6.927 | 0 | 0.942 (0.022) | 0.853 (0.025) | –0.107 (0.025) |
| All UI | | 59 | 14.750 (1.191) | 8.353 (0.839) | 14.339 | 5 | 0.894 (0.022) | 0.0869 (0.017) | –0.030 (0.019) |
| Turkey/Volga River | | | | | | | | | |
| TK1 | 43.368110/–92.216790 | 30 | 11.875 (1.076) | 6.167 (0.450) | 6.326 | 0 | 0.819 (0.046) | 0.831 (0.014) | 0.019 (0.044) |
| TK2 | 43.337150/–92.119710 | 30 | 13.250 (1.146) | 7.836 (1.062) | 7.017 | 1 | 0.902 (0.019) | 0.858 (0.017) | –0.052 (0.012) |
| TK3 | 43.104022/–91.915420 | 25 | 12.250 (1.278) | 8.102 (0.844) | 7.097 | 1 | 0.882 (0.021) | 0.864 (0.019) | –0.023 (0.023) |
| TK4 | 42.879481/–91.763618 | 12 | 8.750 (0.796) | 5.873 (0.540) | 6.581 | 1 | 0.742 (0.054) | 0.817 (0.020) | 0.094 (0.057) |
| All TK | | 97 | 16.375 (1.625) | 8.278 (0.981) | 14.515 | 3 | 0.852 (0.025) | 0.0867 (0.017) | 0.019 (0.014) |
| Maquoketa River | | | | | | | | | |
| MQ1 | 42.658610/–91.587500 | 11 | 8.250 (0.675) | 6.103 (0.566) | 6.549 | 0 | 0.769 (0.040) | 0.826 (0.016) | 0.071 (0.040) |
| MQ2 | 42.547870/–91.510150 | 15 | 10.500 (0.500) | 7.404 (0.470) | 7.142 | 1 | 0.831 (0.032) | 0.861 (0.009) | 0.036 (0.030) |
| MQ3 | 42.619910/–91.666950 | 25 | 11.750 (0.959) | 7.200 (0.813) | 6.827 | 2 | 0.894 (0.031) | 0.845 (0.021) | –0.058 (0.028) |
| All MQ | | 51 | 14.250 (1.031) | 7.784 (0.643) | 14.175 | 3 | 0.849 (0.023) | 0.0864 (0.014) | 0.018 (0.017) |
| Cedar River | | | | | | | | | |
| CD1 | 43.469588/–92.960773 | 30 | 12.250 (0.996) | 8.209 (0.882) | 7.039 | 0 | 0.935 (0.032) | 0.865 (0.018) | –0.080 (0.019) |
| CD2 | 43.223680/–92.876650 | 12 | 10.500 (1.225) | 7.701 (1.075) | 7.335 | 2 | 0.871 (0.042) | 0.843 (0.030) | –0.032 (0.031) |
| CD3 | 42.142530/–91.672300 | 22 | 12.125 (1.381) | 7.202 (0.872) | 6.856 | 2 | 0.881 (0.032) | 0.844 (0.021) | –0.044 (0.032) |
| All CD | | 64 | 15.375 (1.499) | 8.773 (1.141) | 14.736 | 4 | 0.904 (0.025) | 0.868 (0.020) | –0.041 (0.010) |
| All localities | | 270 | 10.688 (0.290) | 7.017 (0.206) | 18.837 | | 0.857 (0.012) | 0.842 (0.005) | –0.016 (0.012) |

*ID* locality identification, cross referenced with Fig. 1, *N* number of individuals collected from each locality, $N_A$ average number of alleles per locus, $N_e$ average number of effective alleles, $A_R$ average allelic richness across all loci, $N_P$ total number of private alleles across all loci, $H_o$ mean observed heterozygosity and standard error (SE), $H_e$ mean expected heterozygosity and standard error (SE), *F* fixation index

expectations and linkage disequilibrium (LD) for all loci were performed on all individuals pooled as one population and individuals pooled into the four drainages using GENEPOP version 4.2 (Rousset 2008). Default parameter values of dememorization were used (10,000), number of batches was increased to 1,000 and number of iterations per batch was increased to 10,000 to minimize standard errors for *P* values. The level of significance was corrected for multiple simultaneous tests by applying the B-Y correction (Benjamini and Yekutieli 2001; Narum 2006).

## Patterns of genetic diversity and population structure

Summary statistics including average number of alleles per locus, number of effective alleles, number of private alleles, average observed and expected heterozygosities, and fixation index were calculated for each locality, individuals pooled by drainage, and all individuals pooled as one population using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). Allelic richness was calculated using FSTAT version 2.9.3 (Goudet 2001). Pairwise $F_{ST}$ values for all sampling localities and localities grouped by drainages were estimated with ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010) with 10,000 permutations. The B-Y correction was applied.

Analysis of isolation by distance (IBD) was conducted among localities to test for correlations between genetic differentiation and geographic distance using a Mantel test (Bohonak 2002). Pairwise $F_{ST}$ values were linearized ($F_{ST}$/1-$F_{ST}$) following Rousset (1997) and two different geographic distance measures were analyzed, river and straight-line distance (Table 2). Straight-line distance reflects the close proximity of the headwaters of the drainages (Fig. 1). It is possible that recent stream capture between headwaters or historical connections may allow the dispersal of individuals between drainages. All distances were geo-referenced in ArcGIS 10 (ESRI, Redlands, CA, USA). A Mantel test was also used to test the correlation between linearized genetic differentiation and number of dams between localities. All Mantel tests were conducted using IBDWS version 3.23 (http://ibdws.sdsu.edu/~ibdws/; Jensen et al. 2005) with 10,000 permutations.

To determine if genetic diversity reflected contemporary patterns of stream connectivity, genetic differentiation

**Table 2** Pairwise $F_{ST}$ estimates of population subdivision below diagonal, pairwise geographic distances in km above diagonal with river distance above and straight-line distance in italics below

| | UI1 | UI2 | UI3 | UI4 | TK1 | TK2 | TK3 | TK4 | MQ1 | MQ2 | MQ3 | CD1 | CD2 | CD 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UI1 | – | 102.2 | 57.6 | 69.0 | 274.9 | 266.0 | 230.0 | 204.3 | 380.3 | 365.9 | 385.8 | 811.6 | 781.1 | 598.3 |
| | | *69.5* | *40.2* | *42.9* | *54.1* | *46.6* | *46.0* | *62.3* | *84.5* | *96.9* | *89.5* | *114.0* | *109.3* | *141.9* |
| UI2 | 0.018 | – | 59.1 | 33.2 | 367.3 | 358.4 | 322.4 | 296.8 | 472.7 | 458.3 | 478.2 | 904.0 | 873.5 | 690.8 |
| | | | *29.0* | *26.7* | *20.3* | *28.8* | *58.4* | *85.3* | *113.9* | *127.3* | *113.7* | *45.1* | *48.1* | *161.1* |
| UI3 | 0.012 | 0.014 | – | 26.0 | 322.8 | 313.8 | 277.8 | 252.2 | 428.2 | 413.7 | 433.7 | 859.5 | 829.0 | 646.2 |
| | | | | *3.8* | *17.6* | *15.2* | *41.3* | *68.7* | *97.2* | *111.1* | *98.7* | *73.9* | *72.2* | *150.1* |
| UI4 | −0.001 | **0.025** | −0.004 | – | 334.2 | 325.2 | 289.2 | 263.6 | 439.5 | 425.1 | 445.0 | 870.8 | 840.3 | 657.6 |
| | | | | | *18.2* | *17.8* | *45.1* | *72.7* | *101.0* | *114.8* | *102.7* | *71.8* | *71.5* | *153.8* |
| TK1 | 0.016 | **0.037** | **0.017** | **0.016** | – | 9.0 | 44.9 | 164.5 | 389.1 | 374.7 | 394.6 | 820.4 | 790.0 | 607.2 |
| | | | | | | *8.8* | *38.0* | *65.4* | *94.2* | *108.2* | *94.7* | *61.3* | *55.7* | *143.1* |
| TK2 | 0.007 | **0.029** | 0.004 | 0.003 | **0.013** | – | 36.0 | 155.5 | 380.2 | 365.8 | 385.7 | 811.5 | 781.0 | 598.2 |
| | | | | | | | *30.5* | *58.3* | *86.8* | *100.8* | *88.0* | *69.9* | *62.7* | *137.5* |
| TK3 | 0.011 | **0.031** | 0.007 | 0.003 | **0.016** | 0.001 | – | 119.5 | 344.2 | 329.8 | 349.7 | 775.5 | 745.0 | 562.3 |
| | | | | | | | | *28.2* | *56.4* | *70.3* | *57.6* | *94.1* | *79.4* | *108.7* |
| TK4 | 0.013 | 0.021 | 0.003 | 0.003 | 0.016 | 0.000 | 0.011 | – | 318.6 | 304.2 | 324.1 | 749.9 | 719.4 | 536.6 |
| | | | | | | | | | *28.8* | *42.5* | *30.4* | *117.4* | *98.2* | *82.2* |
| MQ1 | 0.017 | 0.014 | **0.024** | 0.010 | **0.023** | 0.014 | **0.029** | 0.016 | – | 14.4 | 17.2 | 740.3 | 709.8 | 527.0 |
| | | | | | | | | | | *14.4* | *7.9* | *143.4* | *122.3* | *57.8* |
| MQ2 | 0.018 | **0.033** | **0.023** | **0.013** | **0.031** | 0.014 | **0.013** | 0.015 | 0.001 | – | 19.9 | 725.9 | 695.3 | 512.6 |
| | | | | | | | | | | | *14.9* | *156.3* | *134.4* | *47.0* |
| MQ3 | 0.013 | **0.037** | **0.019** | **0.012** | **0.018** | 0.005 | **0.014** | 0.013 | 0.002 | −0.003 | – | 745.8 | 715.3 | 532.5 |
| | | | | | | | | | | | | *141.4* | *119.1* | *53.4* |
| CD1 | 0.005 | **0.029** | **0.010** | 0.007 | **0.026** | **0.011** | 0.002 | 0.008 | **0.020** | **0.013** | **0.024** | – | 44.4 | 243.5 |
| | | | | | | | | | | | | | *28.4* | *181.4* |
| CD2 | 0.007 | 0.020 | 0.011 | 0.012 | **0.026** | **0.014** | 0.006 | 0.013 | 0.018 | 0.012 | **0.026** | −0.005 | – | 213.0 |
| | | | | | | | | | | | | | | *156.3* |
| CD3 | **0.015** | **0.021** | **0.013** | **0.010** | **0.030** | **0.018** | **0.012** | 0.012 | 0.013 | **0.015** | **0.029** | 0.004 | −0.005 | – |

Bold values indicate differentiation that was statistically significant, adjusted $\alpha = 0.00938$; letter and number codes for localities are cross-referenced with Table 1 and Fig. 1

($F_{ST}$) between populations was mapped onto stream sections that connect them and analyzed using the statistical methods in STREAMTREE (Kalinowski et al. 2008). The fit of the STREAMTREE model was assessed using a coefficient of determination ($R^2$). This method is useful for freshwater organisms that can only disperse through stream corridors that connect pairs of populations (Kalinowski et al. 2008). Finally, a bias in the direction of gene flow within drainages was investigated. If gene flow was biased in a downstream direction, it is expected that genetic diversity would decrease in an upstream direction (Alp et al. 2012). To test for this a linear regression using SYSTAT 13 (Systat Software, San Jose, CA, USA) was performed between the mean allelic richness for all loci within each population and the distance of each population in the drainage to the confluence with the Mississippi River.

To investigate the spatial structure of genetic diversity a nested analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed using ARLEQUIN version 3.5.1.3. Individuals were grouped by locality and drainage. Pairwise $F_{ST}$ values were used with significance of each estimator based on 10,000 permutations. The B-Y correction was applied.

To further assess population structure, a Bayesian assignment test was implemented using STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). The method was used to infer the number of genetic clusters ($K$) that fit the dataset and assign individuals to clusters based on their genotypes without the need a priori to define units. Twenty repetitions each of $K = 1$ through $K = 15$ were analyzed. The number of possible $K$ values selected was one more than the number of sampling localities based on recommendations in Pritchard et al. (2000). A burn-in of $2.5 \times 10^5$ and $1 \times 10^6$ MCMC iterations were used. The admixture model of ancestry and independent allele frequencies were assumed (François and Durand 2010).

Sampling localities were used as a prior for the LOCPRIOR model (Hubisz et al. 2009). The LOCPRIOR model can be helpful for discerning structure when the genetic signal is weak, while not causing overestimation of fit in cases when a priori population assumptions are not informative (Hubisz et al. 2009). The proposed standard deviation of alpha (ALPHAPROPSD) was set to 0.1 to promote mixing and convergence of alpha (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE HARVESTER WEB version 0.6.93 (Earl and von Holdt 2012) was used to determine the most appropriate $K$ value using the $\Delta K$ method (Evanno et al. 2005) and best log likelihood method (Pritchard et al. 2000). The replicates for the selected $K$ were aligned in CLUMPP using the *Greedy* algorithm (Jakobsson and Rosenberg 2007). Plotting of results was accomplished in DISTRUCT version 1.1 (Rosenberg 2004).

### Historical and contemporary demographic processes

The program BOTTLENECK version 1.2.02 (Piry et al. 1999) was used to identify evidence of past changes in effective population size. BOTTLENECK employs the heterozygosity excess test, which is based on the observation that severe demographic bottlenecks decrease allelic diversity more rapidly than heterozygosity (Cornuet and Luikart 1996). The distribution of expected heterozygosities was calculated under the two-phased model (TPM), which is most appropriate for empirical microsatellite data (Di Rienzo et al. 1994). The variance was set to 10 and the proportion of stepwise mutations set to 90 (Garza and Williamson 2001). Localities were pooled by drainage and as a single population. Significance was tested using a one-tailed Wilcoxon signed-rank test and the B-Y correction was applied.

Contemporary, short-term migration rates were estimated using BAYESASS version 3.0 (Wilson and Rannala 2003) for localities within drainages and between drainages. This method estimates asymmetric migration over the last two to three generations using Bayesian inference. Delta values, the maximum amount parameters are allowed to change between iterations, were adjusted to ensure that the proposed changes between chains were between 40 and 60 % of the total number of iterations (Wilson and Rannala 2003). The values were selected based on pilot runs. Delta parameters for the analyses between drainages were set to 0.40 for allele frequency (a), 0.02 for migration rate (m) and 0.50 for inbreeding (f). Delta parameters for the analyses within drainages were set to $a = 0.70$, $m = 0.50$ and $f = 1.60$ for the Upper Iowa River; $a = 1.2$, $m = 0.70$ and $f = 2.70$ for the Turkey/Volga River; $a = 0.70$, $m = 0.50$ and $f = 1.20$ for the Maquoketa River and $a = 0.80$, $m = 0.50$ and $f = 1.70$ for the Cedar River. Ten independent runs each with a different initial seed value were performed for $3 \times 10^7$ iterations with a burn-in of $3 \times 10^6$ and sampling every 2,000 generations for each analysis. The run length was sufficient for the posterior probability to achieve convergence. Migration rates were averaged for the 10 independent runs.

Historical, long-term migration rates between drainages were estimated using the coalescent approach and Markov chain Monte Carlo techniques implemented in MIGRATE-N version 3.4.4 (Beerli and Felsenstein 1999, 2001). Estimates of migration rates within drainages were unable to be determined due to the small sample size in some localities. Parameter distributions were estimated using the Bayesian implementation of MIGRATE (Beerli 2006). The continuous Brownian mutation and full migration matrix models were used. An initial analysis using $F_{ST}$ was run to find start parameters for all subsequent analyses. Exponential priors (minimum, mean, maximum) were placed on both $\Theta$ (0, 1, 100) and $M$ (0, 100, 1000), and a static heating scheme using four Markov chains set to 1, 1.5, 3.0 and 10,000,000.0 was employed. Ten independent runs were performed with a different random number seed, $5 \times 10^6$ generations and burn-in of 10,000 generations. The results were generally stable, suggesting the Markov chains had converged on astationary distribution. Estimates of $M$ were averaged across all runs.

Values of long-term, historical estimates of gene flow ($M$) estimated in MIGRATE were converted to proportion of migrants ($m$) so that the values were more comparable to short-term, contemporary estimates of $m$ estimated in BAYESASS. The conversion was calculated using the formula: $m = M\mu$ (Howes et al. 2009; Apodaca et al. 2012) where $\mu = 5.56 \times 10^{-4}$ (Yue et al. 2007). A Wilcoxon matched-pairs test implemented in SYSTAT 13 was used to determine whether the two methods differ significantly in estimates of gene flow (Apodaca et al. 2012).

## Results

Null alleles were present for the loci Eca6 and Eca22 when all individuals were pooled as a single population. However, the detection of null alleles varied with a finer analysis based on drainages. Locus Eca6 had null alleles present for the Cedar River drainage, while Eca22 had null alleles for the Maquoketa and Turkey river drainages. Based on these results, these loci were not included in any further analyses. There was no evidence of scoring errors or allelic dropout in the eight remaining loci and they were used in all subsequent analyses.

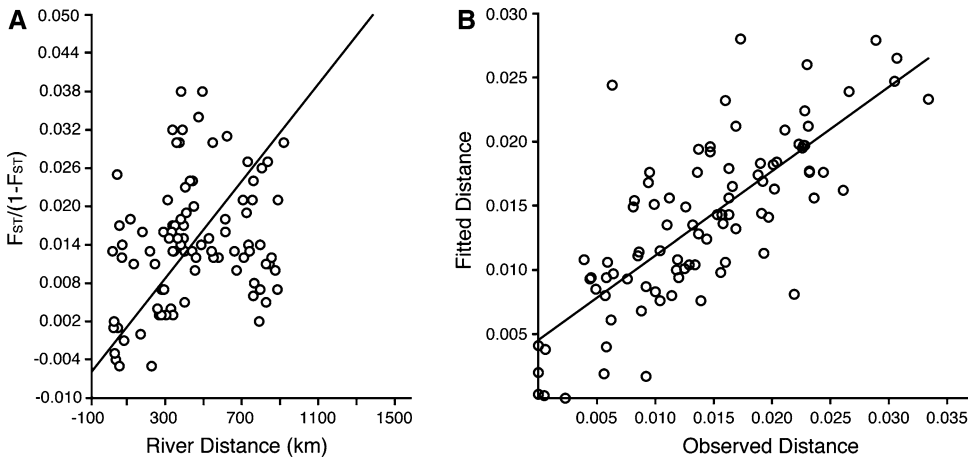


**Fig. 2** A. Scatter plot of Mantel test for isolation by distance using river distance across all localities (r = 0.2799, $P = 0.045$). B. Scatter plot of the results of the STREAMTREE analysis ($R^2 = 0.578$)

Patterns of genetic diversity and population structure

All tests of deviation from HWE and linkage disequilibrium were corrected for multiple tests (adjusted $\alpha = 0.01840$). With all individuals pooled together as a single population significant deviation from HWE was found in two loci, Eca10 ($P = 0.0035$) and Eca14 ($P = 0.0013$). However, with all individuals grouped by drainage there was no evidence of significant deviation from HWE. With all individuals pooled together as a single population there was no evidence of linkage disequilibrium between pairs of loci. However, with all individuals grouped by drainage there was evidence of linkage disequilibrium between loci Eca14 and Eca46 ($P = 0.0045$) in the Cedar River. Due to the inconsistencies between pooled datasets all loci were included in subsequent analyses.

In order to assess genetic diversity and population differentiation, individuals were grouped by locality, drainage, and as a single population. All sampled localities had high genetic diversity (Table 1). The mean expected heterozygosity ranged from 0.799 (SE 0.021) to 0.865 (SE 0.018) and the average number of alleles per locus ranged from 8.125 (SE 0.693) to 13.250 (SE 1.146). The results were very similar with individuals pooled by drainage (Table 1); mean expected heterozygosity ranged from 0.864 (SE 0.014) to 0.869 (SE 0.0178) and the average number of alleles per locus ranged from 14.250 (SE 1.031) to 16.375 (SE 1.625). The genetic diversity was consistent and uniform across localities and drainages. The number of private alleles was low, ranging between 3 and 5 across drainages. Over all individuals there was evidence of heterozygote excess (F = −0.016 SE 0.012; Table 1). Population structure was not apparent across sampling localities; less than half of all pairwise comparisons were significant (Table 2). All significant genetic differentiation was low with $F_{ST}$ values ranging from 0.010 to 0.037. In addition, there was no clear geographic pattern to this differentiation. Comparisons between localities grouped by drainage revealed similar levels of genetic differentiation with $F_{ST}$ values ranging from 0.006 to 0.021; all values were significant.

Genetic differentiation among localities was positive, but weakly correlated with river distance ($r = 0.2799$, $P = 0.045$; Fig. 2a). There was no evidence of IBD using straight-line distance ($r = 0.1727$, $P = 0.116$) or a correlation with number of dams between localities ($r = 0.1770$, $P = 0.1716$); data not shown. Genetic differentiation between populations was weakly explained by contemporary patterns of stream connectivity. The STREAMTREE model resulted in a $R^2 = 0.578$ (Fig. 2b).There was no evidence of a downstream bias in gene flow. The mean allelic richness showed no correlation with the distance to the confluence of the Mississippi River ($R^2 = 0.184$, $P = 0.126$); data not shown.

Across the four river drainages, the AMOVA revealed that most of the variation was explained by differences within localities (98.45 %; Table 3). Very little of the variation was explained by differences among drainages (1.05 %) and even less among populations within drainages (0.50 %). However, the fixation indices were low (Table 3) indicating very little overall differentiation.

The analysis of the Bayesian assignment test results revealed a pattern where all individuals clustered together indicating a single population. The $\Delta K$ method identified 5 genetic clusters. However, because needing a prior $K$ value is mathematically inherent to the $\Delta K$ method, this strategy cannot identify the best $K$ when only one cluster is present in the data (Evanno et al. 2005). Upon evaluation of the best mean log likelihood values as outlined by Pritchard et al. (2000), the dataset contained only one genetically homogeneous group. The graphical display of STRUCTURE results based on the $K = 5$ value were consistent with one cluster as well (Fig. 3), with only minimal membership coefficients for additional groups.

**Table 3** Results of the analysis of molecular variance (AMOVA)

| Source of variation | df | Sum of squares | Variance component | % Variation | Fixation indices |
|---|---|---|---|---|---|
| Among drainages | 3 | 23.641 | $V_a = 0.03201$ | 1.05 | $F_{CT} = 0.01050$[a] |
| Among localities within drainages | 10 | 35.690 | $V_b = 0.01535$ | 0.50 | $F_{SC} = 0.00508$[a] |
| Within localities | 526 | 1579.943 | $Vc = 3.00369$ | 98.45 | $F_{ST} = 0.01553$[a] |

[a] Statistical significance; adjusted $\alpha = 0.00938$

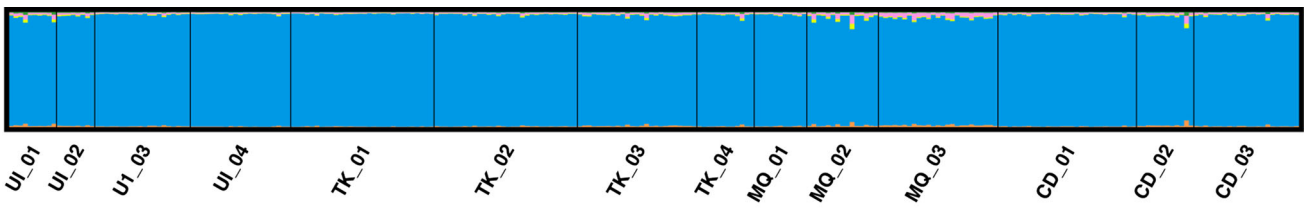


**Fig. 3** Estimated membership coefficients in *Etheostoma caeruleum* based on admixture analyses for $K = 5$ genetically defined populations for each individual sampled. *Vertical axis* indicates membership coefficient and the *horizontal axis* indicates the locality of each individual. Sampling localities codes are cross-referenced with Table 1

### Contemporary and historical demographic processes

Results of the tests for population bottlenecks for both localities pooled by drainage and as one population did not detect any significant deviations in heterozygosity (adjusted $\alpha = 0.0184$). Short-term estimates of gene flow between drainages ranged from 0.0087 to 0.2603 with an average of 0.0771 (Table 4). Asymmetric gene flow between drainages, indicated by no overlap in 95 % confidence intervals between estimates, was observed in four of the six comparisons. However, there was no consistent pattern in the direction and magnitude of the asymmetry. Asymmetric gene flow was not observed between the Upper Iowa and Maquoketa, and Cedar and Maquoketa river drainages, which was indicated by overlap in 95 % confidence intervals between estimates. The migration rates between these systems were also the lowest observed (Table 4). The highest estimate of contemporary gene flow was from the Upper Iowa to the Turkey/Volga River drainage.

Short-term estimates of gene flow within each drainage ranged from 0.0056 to 0.2796 with an average of 0.1288 (Table 5). The estimates of downstream migration encompassed both the highest and lowest values observed within drainages ranging from 0.0056 to 0.2796 with an average of 0.1015. Upstream migration ranged from 0.0369 to 0.2500 with an average of 0.1440. There was only a single case of asymmetrical gene flow observed in the Turkey/Volga River drainage. Almost all 95 % confidence intervals encompassed zero.

The long term, historical estimates of gene flow among drainages from MIGRATE were low and varied less than the short-term estimates (Table 4). The pattern of asymmetric gene flow among drainages was consistent with the short-term estimates. The only difference was no asymmetric gene flow was observed between the Upper Iowa and Cedar river drainages. Interestingly, the direction of gene flow with the greater magnitude was opposite from the short-term estimates. The Wilcoxon test indicated that the long-term and short-term rates were not statistically different ($P = 0.374$).

## Discussion

The analyses of eight microsatellite loci from 14 localities of *E. caeruleum* detected a single genetic population in tributaries of the upper Mississippi River in northeast Iowa. The amount of genetic diversity observed within localities and drainages was high (Table 1); however, the distribution of this diversity was almost uniform across the study area. Few private alleles were found across localities and drainages. There was no evidence of significant population subdivision at any spatial level (Tables 2 and 3; Fig. 3). There was evidence of a weak correlation of genetic differentiation with river distance (Fig. 2a) and with contemporary patterns of stream connectivity (Fig. 2b). However, there was no correlation with genetic differentiation and straight-line distances. This indicated that localities in close geographic proximity to one another but in different river drainages are not any more genetically similar than localities from the same drainage. Finally, there was no correlation of genetic differentiation with the number of dams (barriers) between localities.

**Table 4** Rates of historical (MIGRATE) and contemporary (BAYESASS) migration rates (*m*) between the four drainages

| Drainages | MIGRATE | 95 % confidence interval | BAYESASS | 95 % confidence interval |
|---|---|---|---|---|
| UI → TK | *0.0125* | 0.0021–0.0228 | *0.2603* | 0.1760–0.3446 |
| U ← TK | *0.0500* | 0.0355–0.0648 | *0.0491* | 0.0000–0.1207 |
| UI ← MQ | 0.0201 | 0.0080–0.0330 | 0.0087 | 0.0000–0.0250 |
| UI ← MQ | 0.0170 | 0.0056–0.0288 | 0.0371 | 0.0000–0.1003 |
| UI → CD | 0.0202 | 0.0075–0.0312 | *0.0115* | 0.0000–0.0335 |
| UI ← CD | 0.0248 | 0.0121–0.0373 | *0.1306* | 0.0655–0.1957 |
| TK → MQ | *0.0501* | 0.0340–0.0616 | *0.0119* | 0.0000–0.0365 |
| TK ← MQ | *0.0109* | 0.0010–0.0207 | *0.1754* | 0.0590–0.2918 |
| TK → CD | *0.0743* | 0.0609–0.0874 | *0.0442* | 0.0000–0.0923 |
| TK ← CD | *0.0189* | 0.0079–0.0295 | *0.1706* | 0.0996–0.2416 |
| MQ → CD | 0.0162 | 0.0043–0.0275 | 0.0162 | 0.0000–0.0472 |
| MQ ← CD | 0.0181 | 0.0066–0.0289 | 0.0090 | 0.0000–0.0259 |

Letter codes for drainages are cross-referenced with Table 1 and Fig. 1; italicized values indicate no overlap in 95 % confidence intervals between pairs of drainage comparisons

These results were interesting given the patchy, disjunct distribution of *E. caeruleum* in tributaries of the upper Mississippi River in Iowa and the large river distances separating areas of concentration within the region (Fig. 1). There are two potential explanations for the observation of a single genetic population in northeast Iowa. First, the Rainbow Darter could be highly migratory across the region resulting in a uniform population structure. This suggests that recent alterations of the landscape and the patchy distribution are not significantly impacting the genetic signature. The second explanation for the absence of population subdivision is that the genetic signature is predominantly a product of historical geologic and climatic processes.

Based on what is known about the life history of *E. caeruleum* and darters in general, it is unlikely that the spatial distribution of genetic diversity observed represents contemporary demographic patterns in northeast Iowa. Most darters exhibit strict habitat requirements, which limit their distribution and dispersal abilities. Darters are not typically known to undergo long-distance migrations and are generally limited to a few kilometers over their life span (Page 2000). Rainbow Darters are commonly found in gravel and rubble riffles. There is evidence that adult individuals migrate seasonally from riffles to nearby pools and runs depending on fluctuations in current velocity and water depth (Schlosser and Toth 1984; Harding et al. 1998). It is estimated that Rainbow Darters have low to moderate gene flow even though they are widely distributed and locally abundant (Turner and Trexler 1998; Page 2000). However, a comprehensive knowledge of darter movement in contemporary landscapes is lacking and future studies are needed to improve the understanding of darter vagility.

The best explanation of the pattern of genetic diversity observed in the Rainbow Darter in the upper Mississippi River drainage is that historical processes had a stronger influence than contemporary processes. The genetic signature revealed in this study is indicative of population expansion into the region following the retreat of glacial advances and the establishment of suitable habitat. This is evidenced by the homogeneous genetic pattern and relatively uniform migration rates observed across the large geographic area (Hewitt 1996). The genetic signature is only weakly associated with contemporary river structure (Fig. 2), and there is no evidence of a downstream bias in gene flow or a pattern of asymmetrical gene flow between drainages (Tables 4 and 5). The lack of observed population substructure resulting from contemporary factors is also a function of effective population size ($N_e$) and time. Even though the Rainbow Darter is generally localized, it tends to be very abundant and often the most common fish in a stream (Page 2000). The Rainbow Darter likely has a large $N_e$ and therefore it may take tens of thousands of generations to reach equilibrium between genetic drift and gene flow following habitat fragmentation (Varvio et al. 1986; Zellmer and Knowles 2009). This time lag makes it difficult to detect the effects of contemporary landscape changes on the genetic pattern (Zellmer and Knowles 2009).

Throughout the Quaternary period, populations of Rainbow Darter were unlikely able to survive in the upper Mississippi River basin during periodic glacial cycles. It is more plausible that following glacial retreat populations expanded their ranges northward from southern refugia and recolonized the region when suitable habitat became available (Bernatchez and Wilson 1998; Hewitt 2000; Berendzen et al. 2008; Berendzen et al. 2010). After colonization of the region, the Rainbow Darter presumably

**Table 5** Rates of contemporary (BAYESASS) migration rates (*m*) within the four drainages

| Drainage | Locality | BAYEASS | 95% confidence interval |
|---|---|---|---|
| Upper Iowa | | | |
| Downstream | UI2 ➔UI3+4 | 0.1036 | ± 0.1404 |
| | UI3+4 ➔UI1 | 0.0450 | ± 0.1244 |
| | UI2 ➔UI1 | 0.0603 | ± 0.0981 |
| Upstream | UI1 ➔UI3+4 | 0.1177 | ± 0.1591 |
| | UI3+4 ➔UI2 | 0.2500 | ± 0.1691 |
| | UI1 ➔UI2 | 0.1644 | ± 0.1707 |
| Turkey / Volga | | | |
| Downstream | TK1+2 ➔TK3 | 0.2018 | ± 0.2193 |
| | TK3 ➔TK4 | 0.0130 | ± 0.0242 |
| | TK1+2 ➔TK4 | 0.0056 | ± 0.0108 |
| Upstream | TK4 ➔TK3 | 0.0878 | ± 0.1390 |
| | TK3 ➔TK1+2 | 0.2303 | ± 0.1537 |
| | TK4 ➔TK1+2 | 0.2208 | ± 0.1414 |
| Maquoketa | | | |
| Downstream | MQ1 ➔MQ2 | 0.0647 | ± 0.1218 |
| | MQ3 ➔MQ2 | 0.1037 | ± 0.2083 |
| Upstream | MQ2 ➔MQ1 | 0.1483 | ± 0.1759 |
| | MQ2 ➔MQ3 | 0.1108 | ± 0.1457 |
| | MQ1 ➔MQ3 | 0.1182 | ± 0.1662 |
| | MQ3 ➔MQ1 | 0.1908 | ± 0.2258 |
| Cedar | | | |
| Downstream | CD1 ➔CD2 | 0.0264 | ± 0.0841 |
| | CD2 ➔CD3 | 0.2127 | ± 0.1357 |
| | CD1 ➔CD3 | 0.2796 | ± 0.1032 |
| Upstream | CD3 ➔CD2 | 0.0369 | ± 0.1070 |
| | CD2 ➔CD1 | 0.0882 | ± 0.1250 |
| | CD3 ➔CD1 | 0.1074 | ± 0.1884 |

Downstream migration rates are followed by upstream migration rates. Letter and number codes for localities are cross-referenced with Table 1 and Fig. 1

had a large and widespread distribution in the upper Mississippi River basin. As the climate continued to warm and habitats changed, populations were locally extirpated. The modern distribution of the Rainbow Darter in the region represents a few established populations that were able to survive in areas that retained suitable habitat. The observed genetic signature within the region supports this conclusion owing to the fact that a pattern of extinction and recolonization can act as a form of gene flow that limits genetic differentiation (McCauley 1991).

This conclusion is consistent with other genetic studies of the Rainbow Darter and observations in other native fishes with a similar distribution. In a phylogeographic study of the entire range of the Rainbow Darter based on mtDNA, Ray et al. (2006) identified four major allopatric clades consistent with geographic distributions. Populations from the upper Mississippi River basin, including drainages of Iowa, were included in a clade with samples from drainages of the northern Ozarks. They noted individuals from previously glaciated regions in the north had reduced nucleotide diversity compared to unglaciated regions in the south. These results suggested recent colonization of the glaciated northern portion of the range of the Rainbow Darter from southern refugia (Ray et al. 2006).

The pattern of a discontinuous distribution in the upper Mississippi River basin and northern Ozarks has been repeatedly observed in other fishes as well; e.g. *Campostoma oligolepis*, Large Scale Stoneroller (Blum et al. 2008), *Hypentelium nigricans*, Northern Hogsucker (Berendzen et al. 2003), *Notropis percobromus*, Carmine Shiner (Berendzen et al. 2008), *Notropis nubilus*, Ozark Minnow (Berendzen et al. 2010), *Noturus exilis*, Slender Madtom (Hardy et al. 2002), and *Percina evides*, Gilt Darter (Near et al. 2001). A phylogeographic study of the Ozark Minnow based on mtDNA revealed that the upper Mississippi region, including drainages in northeast Iowa, was colonized by populations expanding out of northern Ozark drainages during the late Pleistoscene (Berendzen et al. 2010). Subsequently, populations in southern Iowa and northern Missouri were extirpated due to loss of suitable habitat. Although Ray et al. (2006) and Berendzen et al. (2010) employed mtDNA sequence data, the patterns are directly comparable to the observation in the Rainbow Darter in tributaries of the upper Mississippi River based on genotypic data.

A landscape genetic study of the Rainbow Darter in the previously glaciated Great Lakes region revealed a similar pattern of expansion following glacial retreat in the eastern portion of the range (Haponski et al. 2009). This study utilized both mtDNA sequence data and microsatellite genotype data to investigate the spatial distribution of genetic diversity of the Rainbow Darter in drainages of the lower Great Lakes. Results of the analyses revealed a genetic signature shaped by Pleistocene glacial cycles. Populations in the Lake Erie and Ohio River catchments were genetically distinct, having diverged during the early Pleistoscene. They hypothesized that the independent lineages recolonized the lower Great Lakes from two separate refugia; the Lake Erie group from a Mississipian refugium and the Ohio River from an Atlantic Slope refugium (Haponski et al. 2009).

The ability of the Rainbow Darter to rapidly colonize a region is further supported by observations in contemporary landscapes (Smith 1985; Cessna et al. 2014). In the Potomac River on the Atlantic Slope of eastern North

America, the Rainbow Darter is a non-native fish that was likely transferred to the system through bait bucket introductions. Within 25 years of the initial introduction, the Rainbow Darter expanded to over 400 river kilometers downstream of the initial site (Cessna et al. 2014).

In general, aquatic organisms in southern regions of North America display more population substructure when compared to populations distributed in previously glaciated regions in the North (Hewitt 1996; Avise 2000). Unfortunately, to date, there are no landscape genetic studies of the Rainbow Darter in unglaciated regions of its distribution available for comparison. However, there are several studies on other species of darters distributed south of the maximum glacial advance in eastern North America; e.g. Bluemask Darter, *Etheostoma akatulo* (Robinson et al. 2013); Okaloosa Darter, *Etheostoma okaloosae* (Austin et al. 2011); Yazoo Darter, *Etheostoma raneyi* (Sterling et al. 2012). As expected, the genetic signature in these species contrasts with the Rainbow Darter in the upper Mississippi River drainage.The observed genetic signature included significant population subdivision, genetic divergence strongly correlated with geographic distance, and constant population sizes reflecting populations that were not directly impacted by periodic glacial cycles (Austin et al. 2011; Sterling et al. 2012; Robinson et al. 2013).

## Conclusion

This study highlights the importance of understanding the temporal and spatial dynamics that shape the observed genetic diversity, in addition to the importance of considering life history characteristics when interpreting the data. If taken at face value, the results from the analyses of the genetic diversity of the Rainbow Darter in tributaries of the upper Mississippi River in northeast Iowa suggests high levels of contemporary gene flow and connectivity over a relatively large geographic area. This would lead to the potentially false conclusion that contemporary habitat alterations in the region have not impacted populations. This may be particularly true for species that are specialists and have low dispersal abilities. However, when taking life history, distribution, and historical processes into account, the best explanation of the spatial distribution of genetic diversity within the Rainbow Darter in the upper Mississippi River drainage is a signature of historical geologic and climatic events rather than alterations of the landscape resulting from recent human activity.

The conclusion that historical processes have a stronger influence on the genetic signature emphasizes the fact that species-specific life histories are another important component in considering temporal and spatial scale of genetic variation (Brown and Knowles 2012). It is possible that, given the life and demographic histories of the Rainbow Darter in northeast Iowa, a larger sample size, greater number of variable microsatellite loci, or new techniques using high-throughput sequencing may give enough power to detect the subtle signature of population subdivision (Emerson et al. 2010; Lowe and Allendorf 2010). Although the results of genetic studies are valuable, the reliance on these methods alone for addressing connectivity can misdirect applications to management (Lowe and Allendorf 2010). Direct in-stream measurements of movement, habitat associations, and population densities of *E. caeruleum* in the upper Mississippi River drainage are necessary to compliment the genetic data in order to fully understand the effects of contemporary habitat fragmentation on the demographic connectivity.

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