

Research Article

Genetic change versus stasis over the time course of invasions: trajectories of two concurrent, allopatric introductions of the Eurasian ruffe

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Abstract

Few investigations have examined whether population genetic changes occur over the course of nonindigenous species invasions, which would facilitate understanding their trajectories and ecological successes. The Eurasian ruffe fish *Gymnocephalus cernua* unintentionally was introduced from ballast water released into western Lake Superior of the North American Laurentian Great Lakes in ~ 1986, likely originating from the Elbe River region where ruffe is indigenous. The ruffe spread quickly to several other areas in the upper Great Lakes, but despite early predictions, has not invaded the lower Great Lakes. In ~ 1991, the ruffe was introduced to Bassenthwaite Lake in northern England through bait bucket releases from southern England, where it is indigenous (and genetically distinct from the Great Lakes invasion). The genetic trajectories of these two independent invasions offer intriguing evolutionary and ecological comparisons. This study tested two alternative hypotheses for the genetic compositions of the two invasions, with reference to two native northern European populations (Elbe River and Vistula Lagoon): whether they have (1) experienced temporal consistency or (2) changed over their respective 30-year time courses. At each invasion stage (early, middle, or later), population genetic diversity may (A) remain similar, (B) decrease due to drift, or (C) increase over time due to addition and establishment of new propagules. Analyses of variation at 10 nuclear DNA microsatellite loci and entire mitochondrial DNA control region sequencing revealed that both invasions overall have lower effective population sizes, allelic richness, and observed heterozygosity compared to the native populations, indicating founder effects. The genetic compositions of both invasions significantly changed over 30 years, supporting hypothesis 2. Diversity has remained relatively consistent overall (A), with decreased allelic richness at the middle invasion stage (B), followed by recovery (C), suggesting arrival of new propagules. Moreover, population differentiation occurred with spread in the upper Great Lakes, with no overall differences in genetic diversity levels (scenario 2A). Ruffe numbers in the Great Lakes have been declining over time, and its continued absence from the lower Great Lakes may reflect limited genetic diversity stemming from a single source population area and few new supplements. The genetic diversity reservoirs of both ruffe invasions, along with continued measures to prevent new inoculants, may constrain their populations and limit long-term ecological success.

Key words: Baltic Sea, Bassenthwaite Lake, Eurasian ruffe, North Sea, Great Lakes, invasion genetics, invasive species

Introduction

The inherent genetic composition and population diversity of nonindigenous species invasions are believed to significantly influence their relative ecological successes across temporal and spatial trajectories (Williamson 1999; Stepien et al. 2005; Allendorf et al. 2013). Invasion theory predicts that new introductions undergo founder effects, reducing their genetic diversity and related adaptive potential (Baker and Stebbins 1965; Williamson 1999; Bock et al. 2015). However, some invasions possess genetic diversity that is equivalent or even higher than those of their native source populations, resulting from large numbers of introduced propagules, multiple founding sources, and/or repeated introductions (Roman and Darling 2007; Lockwood et al. 2013; Rius et al. 2015). For example, invasions of zebra mussel *Dreissena polymorpha* (Pallas, 1771), quagga mussel *D. rostriformis* (Deshayes, 1838) (Brown and Stepien 2010; Stepien et al. 2013), and round goby *Neogobius melanostomus* (Pallas, 1814) (Stepien and Tumeo 2006; Brown and Stepien 2008, 2009) in the North American Laurentian Great Lakes (via Eurasian ballast water) all show relatively high genetic diversity and significant population differentiation across their introduced ranges. This is attributed to large numbers of introduced propagules from multiple introduction events across several Eurasian sources. Such cases of high genetic variability are believed to enhance the adaptive potential of invasions (see Bock et al. 2015).

Relatively few investigations have examined temporal population genetic trajectories of invasions, which may undergo a variety of pathways and alternatives (Adrian-Kalchauer et al. 2016; Snyder and Stepien 2017). This stems from lack of detection at the early stages of invasions when numbers of individuals are few, along with limited long-term collection and storage of samples, which may span decades. Moreover, molecular genetic techniques often change over time, rendering comparisons with earlier results problematic. Invasions for which long-term data are available (mostly plants) generally show declining genetic variation over several decades, followed by a rebound, especially in the case of larger populations and multiple ongoing introductions (Dlugosch and Parker 2008).

After establishment, an invasive species' genetic composition may remain relatively consistent over time (hypothesis 1), or may change with drift, gene flow, and/or the arrival of new genotypes (hypothesis 2). Corollaries under these hypotheses are that overall genetic diversity levels across the temporal course of an invasion might remain statistically

similar (A), decrease (B), or increase (C) over time. Scenario (1A) would be expected to occur when a newly established invasion experiences high reproductive success and all or most of its allelic variation is retained over time. Furthermore, the reproductive output of early-established individuals may outpace or outcompete any later arrivals, limiting their relative representation in the population, or eliminating them altogether (Waters et al. 2013). This stasis may occur even in the absence of selection, due to relative preponderance of the genotypes of those individuals that established early on and their comparative reproductive output. Alternative scenario (2B) would result from changes in allelic frequencies and diversity due to small population size and drift. Scenario (2C) would result if new propagules with additional genetic variation arrive, become established, and augment the population. In the case of change in genetic composition (2), turnover of alleles having approximately the same overall genetic diversity levels would show scenario (2A), which might occur stochastically (in the absence of selection), or when later arrivals are superior competitors.

For example, round goby populations in the Great Lakes were founded from various Eurasian sources (Dillon and Stepien 2001; Stepien and Tumeo 2006; Brown and Stepien 2008, 2009) and have remained relatively genetically consistent over 25 years, with each area retaining its respective regional genetic distinctiveness and consistent diversity levels (scenario 1A; Snyder and Stepien 2017). Conversely, early-established zebra mussel genotypes in the Hudson River ca. 1991 were replaced by later arrivals ca. 2003 (Strayer et al. 2011), which were genetically different (according to neutral microsatellite markers) and likely originated from other European sources, yet had similar genetic diversity levels (scenario 2A) (Brown and Stepien 2010; Stepien et al. 2013). These scenarios also may change over the time course of an invasion. For example, the spiny waterflea *Bythotrephes longimanus* (Leydig, 1860) initially displayed a founder effect after invading the Great Lakes (2B), which then dissipated over time (2C) due to multiple new introductions from the native range (Berg et al. 2002). Likewise, a round goby expansion population in Lake Superior underwent initial founder effect (2B), then received supplementation from the invasion's core area (2C), and since has remained genetically consistent over time (1A) (Snyder and Stepien 2017).

The present case study of two concurrent, independent invasions

In 1986, the Eurasian ruffe *Gymnocephalus cernua* (Linnaeus, 1758) first appeared in the Great Lakes at

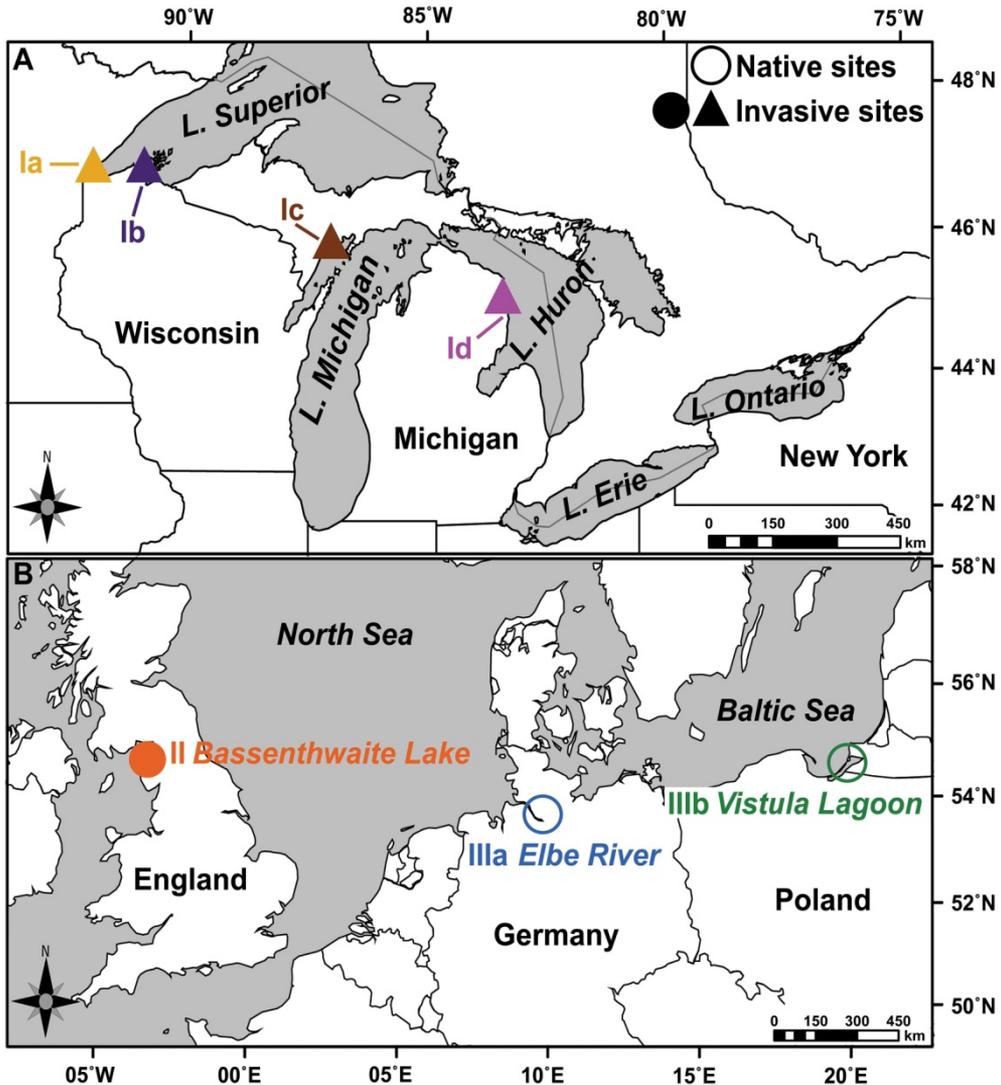


Figure 1. Ruffe populations sampled in (A) North America (triangles) and (B) northern Europe (circles, filled circle = invasive population, open circle = native population). Great Lakes sites: Ia. St. Louis Harbor, Ib. Sand River, Ic. Little Bay de Noc, and Id. Thunder Bay. Full information in Table 1.

St. Louis Harbor (Duluth, Minnesota) in southwestern Lake Superior (site Ia, Figure 1A), attributed to accidental release via ballast water discharge from transoceanic shipping (Simon and Vondruska 1991; Pratt et al. 1992). Its likely European genetic source (based on nuclear (n) and mitochondrial (mt) DNA sequences) traced to the northern Elbe River region, which drains into the eastern North Sea (site IIIa, Figure 1B). Its introduction coincided with increased trade between the Great Lakes and a newly reunified Germany (Stepien et al. 2004, 2005). In 1991, invasive ruffe was transported via shipping 300 km northeast to Thunder Bay Harbor in northern Lake

Superior, Ontario, Canada (Pratt et al. 1992). It also spread eastward from St. Louis Harbor via natural dispersal and shipping (USGS 2018), reaching Thunder Bay*, Michigan in Lake Huron in 1995 (site Id, Figure 1A; *note this is a separate Thunder Bay from the Canadian location). The ruffe's invasive range expanded along northern Lake Michigan from 2002–7 (site Ic, Figure 1A).

The ruffe's invasive European range includes Bassenthwaite Lake, Cumbria in northern England (location II, Figure 1B), where it was transported in bait buckets from its native southern England distribution in ~1991 (Winfield 1991; Winfield et al. 1996).

Other introduced ruffe populations (Supplementary material Figure S1) are located in Scotland and Wales (Maitland et al. 1983; Adams and Maitland 1998), western Norway (Kålås 1994), and along the Mediterranean Sea in France and northern Italy (Matthey 1966; Chiara 1986).

Ecology, distribution, and background genetics of the ruffe

The ruffe's relatively small size and sharp spines render it of low fishery value, both in North America and Europe (Kováč 1998; Stepien and Haponski 2015; Gutsch and Hoffman 2016). Its habitat and prey overlap with North American yellow perch *Perca flavescens* (Mitchill, 1814) (Souter et al. 1992; Ogle et al. 1995) and European perch *P. fluviatilis* Linnaeus, 1758 (Bergman 1991; Schleuter and Eckmann 2006; Lorenzoni et al. 2007), which also are in family Percidae. Yellow and European perch both support valuable sport and commercial fisheries (Stepien and Haponski 2015). U.S. and Canadian fishery agencies have been concerned that further spread of the ruffe would seriously impact yellow perch in the lower Great Lakes (Busiahn 1997; Brenton 1998), and maintain active monitoring (Great Lakes Fisheries Commission, <http://www.glf.org/>).

The ruffe's native distribution is widespread (Figure S1), including southern England, northeastern France, rivers entering the North, Baltic, and White seas, and most of central Europe and Siberia (Stepien and Haponski 2015). It lives in diverse eutrophic and oligotrophic habitats (Ogle 1998; Gunderson et al. 1998; Hölker and Thiel 1998), displaying wide tolerances to temperature, salinity, depth, flow rate, and substrate type (Pratt et al. 1992; Gunderson et al. 1998; Gutsch and Hoffman 2016). The geographical and ecological breadth of its native range may predicate invasive success through generalist strategies and phenotypic plasticity (see Bock et al. 2015). The ruffe matures early (males at 1 yr and females 1–2 yrs) and at small sizes (≤ 10 cm), exhibiting short generation time and high reproductive potential (Winfield et al. 2011; Volta et al. 2013; Gutsch and Hoffman 2016). Females can produce more than one clutch per season (Fedorova and Vetkasov 1974; Ogle 1998; Gutsch and Hoffman 2016); such high fecundity may enhance an invasive species' success and spread (Keller et al. 2007; Blackburn et al. 2015).

Previous genetic studies of the ruffe's native and invasive distributions revealed five mtDNA control region sequence haplotypes (1024 base pair (bp); GenBank #AF025355–9; lettered A–E on Figures 2 and S1), which showed pronounced population divergence and partitioning across Eurasia (Stepien et al.

1998, 2004, 2005). Populations sampled from the native eastern North Sea, western Baltic Sea, and Black Sea regions and the Great Lakes possessed haplotype A alone. Invasive populations in Bassenthwaite Lake and Loch Lomond, Scotland both had haplotype B. Native eastern European populations contained haplotypes C, D, or E, denoting geographic distinctiveness (Figure S1). The haplotypes are divided into two primary clades (A and B, versus C–E; Figure 2), which are widely genetically separated (Stepien et al. 1998, 2004). Sequencing the nDNA lactose dehydrogenase (Ldh) A6 intron further delineated a close genetic relationship between ruffe from the Great Lakes and Elbe River populations, diverging from Black Sea samples (Stepien et al. 2004, 2005).

Population genetic questions and hypotheses

The present investigation's aim was to evaluate the genetic compositions and diversity of ruffe populations across the temporal courses (~ 30 years) of two concurrent invasions in separate continents (the Great Lakes in North America and Bassenthwaite Lake in England), in reference to two native European populations (Elbe River and Vistula Lagoon). We tested the alternatives of (1) genetic similarity (i.e., the null hypothesis) versus (2) difference in allelic composition, and the scenarios of genetic diversity levels (A) remaining similar, (B) declining (with drift), or (C) increasing over time. We compared their estimated effective population sizes, differentiation in genetic composition (using pairwise and hierarchical comparisons, and Bayesian assignment tests), and genetic diversities (observed heterozygosity, allelic richness, and private alleles) at 10 nDNA microsatellite (μ sat) loci, and determined haplotypic variation of mtDNA control region sequences. We additionally assessed population variation across the four primary regions of the ruffe's geographic distributional and temporal spread in the upper Great Lakes, which had not been previously investigated.

Materials and methods

Sampling design and collections

Samples were collected by agency scientists and collaborators (see Acknowledgements) following their protocols and the University of Toledo IACUC (to C.A.S.). Figure 1 shows the seven sampling locations (lettered), from which 462 individual ruffe were analyzed (Table 1) including: the initial establishment site in the Great Lakes (Ia) at St. Louis Harbor, Duluth, MN in southwestern Lake Superior, (Ib) Sand River, Bayfield, WI in southern Lake Superior,

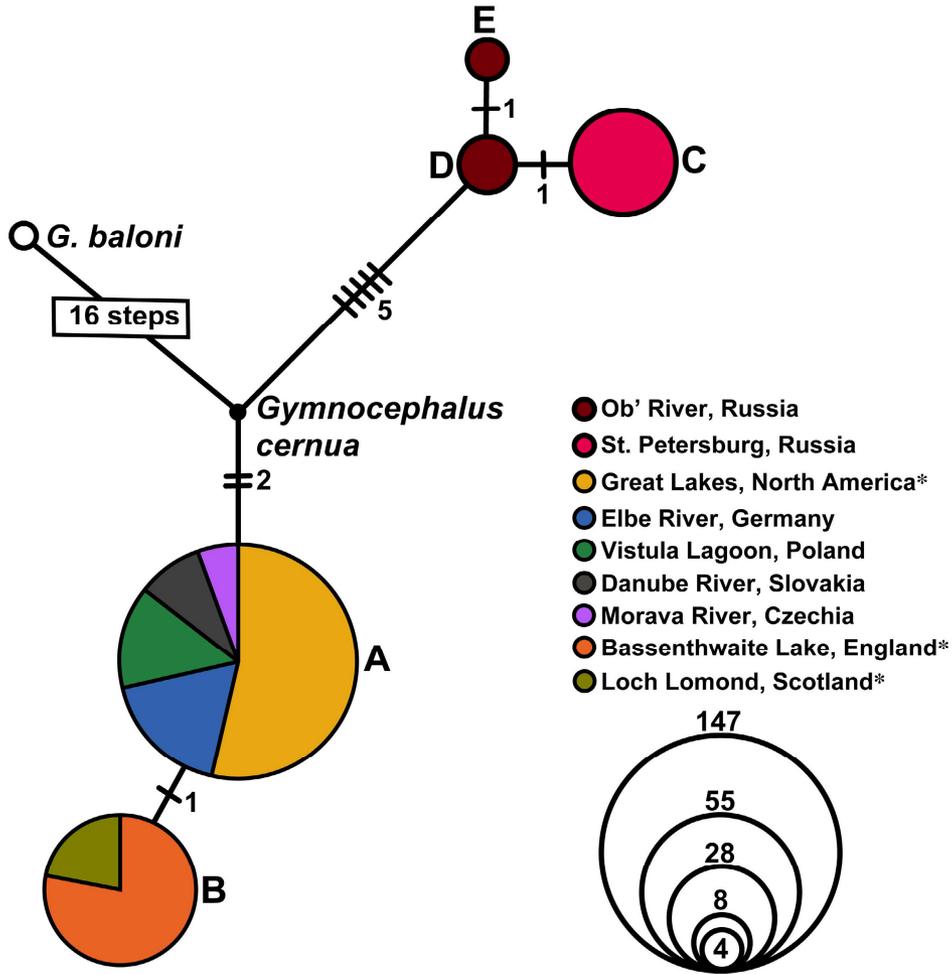


Figure 2. Parsimony network depicting evolutionary genetic relationships of mtDNA control region sequence haplotypes (lettered A–E) for ruffe (*Gymnocephalus cernua*), in relation to its sister species (Balon’s ruffe *G. baloni*). Circles denote haplotypes, with sizes proportional to number of individuals with that haplotype (here and from Stepien et al. 1998, 2004, 2005). Colors denote locations where haplotypes were found, mapped on Figure S1. Hatch marks (and numbers) denote number of nucleotide substitutions between haplotypes. * = invasive population.

(Ic) Little Bay de Noc at Escanaba, MI in northern Lake Michigan, (Id) Thunder Bay at Alpena, MI in Lake Huron, (II) Bassenthwaite Lake, Cumbria, England, (IIIa) Elbe River at Hamburg, Germany, and (IIIb) Vistula Lagoon at Tolkmicko, Poland. The latter two are native populations in the North and Baltic seas region of Europe. Temporal genetic patterns were evaluated at the three invasion stages (Table 1), including: early (close to the time of initial invasion and establishment), middle, and later (2014–15) for samples from (Ia) St. Louis Harbor and (II) Bassenthwaite Lake. Results were compared to the native populations (IIIa and b) from the early and later time periods (Table 1), which served as controls. Samples were fixed in 95% ethanol in the field, labeled, and archived in fresh ethanol at room temperature in our laboratory.

DNA extractions and amplifications

Genomic DNA was extracted using DNeasy® Blood and Tissue kits (Qiagen Inc., Valencia, CA, USA), evaluated with a Nanodrop™ 2000 spectrophotometer (Thermo Fisher Scientific™ Inc., Waltham, MA, USA), and visualized on 1% agarose mini-gels with ethidium bromide. We analyzed genetic variation at 10 nDNA microsatellite loci (Table S1) and from sequences of the entire mtDNA control region.

For microsatellites, polymerase chain reactions (PCR) contained 10 µL of 0.035 units AmpliTaq® DNA polymerase (ABI; Applied Biosystems™, Foster City, CA, USA), 1X GeneAmp® PCR Buffer I (ABI), 106 mM trehalose, 100 µM dNTPs, 100 µM spermidine, 0.5 µM each of forward and reverse

Table 1. Sampling locations of ruffe populations, with coordinates (in decimal degrees), year of first discovery at that location, invasion stage (Early, Middle, Later), collection year, and number (*N*) of individuals (total in parentheses). * = time periods grouped together for analysis, due to low and/or disparate sample sizes.

Population Site	Latitude, Longitude	Discovery Year	Invasion Stage	Collection Year	<i>N</i> (Total)
I. Great Lakes (invasive)					
a. St. Louis Harbor, Lake Superior Duluth, MN	46.743956°N -92.10236°W	1986	Early	1995	50
			Middle	2006	20
			Later	2014	50
					(120)
b. Sand River, Lake Superior, Bayfield, WI	46.837050°N -90.99517°W	1988	Early*	1995	9
			Later*	2014	2
					(11)
c. Little Bay de Noc, Lake Michigan Escanaba, MI	45.733028°N -87.02650°W	2002	Early*	2002	2
			Middle*	2014	5
			Later*	2015	9
					(16)
d. Thunder Bay, Lake Huron Alpena MI	45.039183°N -83.41278°W	1995	Early*	1996	10
			Middle*	2003	49
					(59)
II. Bassenthwaite Lake (invasive)					
Cumbria, England	54.666287°N -3.228960°W	1991	Early	1996	49
			Middle	2003	30
			Later	2014	50
					(129)
III. Native North and Baltic Sea Region					
a. Elbe River Hamburg, Germany	53.570136°N 9.6615180°E	-	Early	1996	16
			Later	2015	50
					(66)
b. Vistula Lagoon Tolknicko, Poland	54.607988°N 20.001843°E	-	Early	2008	50
			Later	2014	11
					(61)

primers, and 2 μL of ≥ 30 ng/ μL DNA on a C1000TM Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Amplifications were 3 min initial 95 °C denaturation, followed by 34 cycles of 30 sec at 95 °C, 30 sec annealing (temperatures in Table S1), and 1 min 72 °C extension, capped by 5 min at 72 °C, conducted separately per locus. Products were diluted 1:50 with ddH₂O, 2 μL was added to 13 μL of formamide and ABI GeneScanTM-500 LIZ[®] size standard solution, and loaded onto 96-well plates. Samples were denatured for 2 min at 95 °C and analyzed on an ABI 3130xl Genetic Analyzer with GeneMapper[®] 4.0 software (ABI). Allelic size variants were confirmed on output profiles.

Ten individuals per sample site and temporal replicate (Table 1), which had not been previously sequenced, were randomly sequenced for the entire 1,024 bp mtDNA control region, and added to a larger published reference database for ruffe across Eurasia and North America (Stepien et al. 1998, 2004, 2005). Single 25 μL PCRs contained 1.5 units AmpliTaq[®] DNA polymerase, 1.2X GeneAmp[®] PCR Buffer I (ABI), 85 μM dNTPs, 0.5 μM each of forward primer (Gcern1F: CTCAAAGAAAGGAG ATTCTA) and reverse primer (Gcern4R: GGAGC

TTTCTAGGGCTCAT), 2 μL of ≥ 30 ng/ μL DNA template, and ddH₂O. Settings were 3 min 95 °C denaturation, then 38 cycles of 45 sec at 95 °C, 50 sec at 54 °C, and 2 min at 72 °C, capped by 5 min at 72 °C. Results were visualized on 1% agarose mini-gels with ethidium bromide, purified with QIAquick[®] PCR Purification Kits (Qiagen), and quantified via NanodropTM. Sequences from ABI 3730 DNA analyzers (Cornell University Biotechnology Resource Center; <http://www.biotech.cornell.edu/biotechnology-resource-center-brc>) were identified and aligned with Bioedit v7.2.5 (Hall 1999).

Genetic data analyses

MtDNA sequences were aligned with our previously published data, and parsimony networks (Templeton et al. 1992) constructed in Popart v1.7 (Leigh and Bryant 2015). Since there was no mtDNA sequence variation within our populations (see Results), no further analyses were conducted with those data.

For the microsatellite data, population samples were tested at each locus for conformance to Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium expectations, using 10,000 Markov Chain

Monte Carlo (MCMC) chains, 1,000 batches, and 10,000 iterations in Genepop v4.3 (Rousset 2008). Significance levels were adjusted with standard Bonferroni correction (Rice 1989). Loci were evaluated for heterozygote deficiency or excess, null alleles, scoring errors, and/or large allele dropout using Micro-Checker v2.2.3 (Van Oosterhout et al. 2004). Number of alleles (N_A) and genetic divergences (F_{ST}) were calculated with Fstat v2.9.3.2 (Goudet 1995).

Genetic diversity metrics for microsatellite data included observed/expected heterozygosity (H_O/H_E) in Genepop and allelic richness (A_R) in Fstat. Friedman's (1937, 1939) rank-sum tests, post-hoc analyses on mean H_O and A_R , and paired two-tailed t -tests were run in R v3.2.1 (R Development Core Team 2015) to test for significant differences, with sequential Bonferroni correction to probabilities (p) (Rice 1989). Standard errors (SE) were calculated in Microsoft® Office Excel 2016 (Redmond, Washington USA). Numbers of private alleles (N_{PA}), i.e., those found exclusively at single sampling sites or in a single temporal sample, were determined in Convert v1.31 (Glaubitz 2004). Relative proportions of private alleles (P_{PA}) were calculated by dividing the number of private alleles by the total number of alleles among all loci for that sample. Mean frequency of private alleles ($F_{PA} \pm SE$) was calculated using Fstat. Siblings were identified with Colony v2.0.6.1 (Jones and Wang 2010), and NeEstimator v2 (Do et al. 2014) estimated effective population sizes for samples having > 30 individuals using default settings without a temporal method.

Pairwise genetic divergences included unbiased θ_{ST} estimates (Weir and Cockerham 1984) in Fstat, and pairwise exact tests of differentiation (χ^2) in Genepop (Raymond and Rousset 1995), using 1,000 batches and 10,000 iterations; both with p -values adjusted via sequential Bonferroni correction. Analysis of molecular variance (AMOVA) in Arlequin v3.5 (Excoffier and Lischer 2010) assessed distribution of genetic variation among population groupings and their component population and temporal samples.

Relationships among population groups were visualized using three-dimensional factorial correspondence analysis (3dFCA; Benzécri 1973) in Genetix v4.05 (Belkhir et al. 1996). Bayesian assignment tests in Structure v2.3.4 (Pritchard et al. 2000) analyzed the likelihood of individuals belonging to K (1–15) hypothetical population groups, with burn-ins of 100,000, 500,000 replicates, and 10 iterations each using a hierarchical approach. K values were evaluated with ΔK (highest ΔK value) and $Ln P(D)$ (optimal K when the mean reached maximum or plateaued) (Evanno et al. 2005) in Structure Harvester

v0.6.94 (Earl and vonHoldt 2012). GeneClass2 (Piry et al. 2004) assigned individuals to populations (the four Great Lakes sites and the Elbe River), with “enable probability computation”, default parameters, and 100,000 simulated individuals.

Results

Population genetic diversity and composition

All ruffe from the Great Lakes and native locations (Elbe River and Vistula Lagoon) were monotypic for mtDNA control region sequence haplotype A (GenBank AF025355) (Figures 2 and S1). All from Bassenthwaite Lake had haplotype B (AF025356). The two haplotypes diverged by a single nucleotide substitution, and both differed from other Eurasian ruffe haplotypes (C–E; Figures 2 and S1). Since all populations were respectively monotypic, they were not further analyzed.

A total of 151 alleles occurred among 10 microsatellite loci (Table S1), averaging 15.1 alleles per locus (range = 3–27). All loci and population samples conformed to HWE expectations and were unlinked. Slight homozygote excess occurred in one sample (Bassenthwaite Lake 1996), at a single locus (Sv114). We included all 10 loci, since there was no evidence of null alleles, scoring errors, and/or stuttering, and just < 0.005 of the data were unscorable. Colony identified full siblings in the Bassenthwaite Lake population alone (12 pairs), whose inclusion did not alter the results ($\theta_{ST} = 0.0015 \pm 0.0001$).

Native populations had higher genetic diversity values than invasive ones (Table 2A), and native effective population sizes were estimated as infinite (Table 3). The Elbe River's observed heterozygosity was $H_O = 0.52 \pm 0.06$, with a mean of 10.2 alleles/locus, and allelic richness $A_R = 4.91 \pm 0.33$. Diversity values for the native Vistula Lagoon population were statistically similar ($H_O = 0.55 \pm 0.06$, 9.50 alleles/locus, $A_R = 5.08 \pm 0.33$) to the Elbe River (Table 2A). H_O and A_R of the Elbe River population were significantly greater than for invasive populations from Great Lakes ($p = 0.016, 0.013$) and Bassenthwaite Lake ($p = 0.004, 0.004$). Effective population sizes were markedly smaller for the five invasive population samples evaluated (three from the Great Lakes and two from Bassenthwaite Lake), ranging from 28.3–360.6 (Table 3). Genetic diversity values for the overall Great Lakes were $H_O = 0.37 \pm 0.03$, 7.00 alleles/locus, and $A_R = 3.09 \pm 0.01$ (Table 2A). Bassenthwaite Lake had $H_O = 0.36 \pm 0.03$, 5.90 alleles/locus, and $A_R = 2.85 \pm 0.11$. Paired t -tests showed that H_O and A_R were statistically similar between the two invasions, with slightly more alleles in the Great Lakes.

Table 2. Genetic variation of ruffe population samples (labels from Figure 1) based on 10 nDNA microsatellite loci, including number of individuals (N), observed heterozygosity (H_O) \pm SE, total number of alleles (N_A), allelic richness (A_R) \pm SE, number of private alleles (N_{PA} ; i.e., in that sample alone), proportion of private alleles (P_{PA}), and mean frequency of private alleles (F_{PA}) \pm SE.

A. Population Comparisons								
Population area	Sampling site	N	H_O	N_A	A_R	N_{PA}	P_{PA}	F_{PA}
I. Great Lakes (invasive)	a. St. Louis Harbor	120	0.41 \pm 0.04	54	3.32 \pm 0.11	3	0.06	0.005 \pm 0.001
	b. Sand River	11	0.28 \pm 0.09	28	2.99 \pm 0.48	0	0	0
	c. Little Bay de Noc	16	0.41 \pm 0.10	36	3.18 \pm 0.41	0	0	0
	d. Thunder Bay	59	0.39 \pm 0.05	38	2.86 \pm 0.15	2	0.05	0.017 \pm 0.000
	Total Great Lakes	206	0.37 \pm 0.03	72	3.09 \pm 0.01	5	0.07	0.010 \pm 0.003
II. Bassenthwaite Lake (invasive)	II. Bassenthwaite Lake	129	0.36 \pm 0.03	54	2.85 \pm 0.11	6	0.11	0.071 \pm 0.065
III. North and Baltic seas region (native)	a. Elbe River	66	0.52 \pm 0.06	96	4.91 \pm 0.33	22	0.23	0.012 \pm 0.002
	b. Vistula Lagoon	61	0.55 \pm 0.07	89	5.08 \pm 0.33	17	0.19	0.023 \pm 0.002
B. Temporal Comparisons								
Ia. St. Louis Harbor (invasive)	Early	50	0.44 \pm 0.07	39	3.17 \pm 0.42	1	0.03	0.010 \pm 0.000
	Middle	20	0.44 \pm 0.08	29	2.92 \pm 0.45	0	0	0
	Later	50	0.46 \pm 0.07	44	3.58 \pm 0.50	4	0.09	0.023 \pm 0.006
II. Bassenthwaite Lake (invasive)	Early	49	0.44 \pm 0.07	46	3.25 \pm 0.41	3	0.07	0.013 \pm 0.003
	Middle	30	0.34 \pm 0.08	25	2.49 \pm 0.49	1	0.04	0.017 \pm 0.000
	Later	50	0.38 \pm 0.08	37	2.82 \pm 0.48	2	0.05	0.010 \pm 0.000
III. North and Baltic seas region (native)	a. Elbe River – Early	16	0.58 \pm 0.10	60	5.39 \pm 1.10	7	0.12	0.040 \pm 0.006
	a. Elbe River – Later	50	0.58 \pm 0.08	83	5.30 \pm 1.03	15	0.18	0.017 \pm 0.003
	b. Vistula Lagoon – Early	50	0.62 \pm 0.06	85	5.43 \pm 0.97	12	0.14	0.018 \pm 0.006
	b. Vistula Lagoon – Later	11	0.61 \pm 0.08	48	5.10 \pm 0.82	2	0.04	0.205 \pm 0.160

Genetic divergences between the native and invasive populations

Ruffe populations from the Great Lakes overall (analyzed together as a single group), Bassenthwaite Lake, and the native Elbe River and Vistula Lagoon all significantly differed in genetic compositions ($\theta_{ST} = 0.091\text{--}0.244$, $p < \alpha$ for all; Table 4A); results from pairwise exact tests were equivalent. Bassenthwaite Lake was the most divergent population in our study (Table 4A). The 3dFCA (Figure 3) and Structure analyses (Figure 4) similarly scored Bassenthwaite Lake as very genetically different from the Great Lakes' invasion and from the native populations analyzed.

Genetic divergence analyses also showed that the two native populations differed significantly ($\theta_{ST} = 0.044$, $p < 0.0001$), with the Elbe River genetically closer to the Great Lakes' population (Table 4A); as shown in the 3dFCA (Figure 3). AMOVA partitioning of genetic variation (Table 5A) revealed 7.11% of the genetic variation between the Great Lakes versus native European populations, and 2.81% among their respective population samples, with both being significant.

Bayesian Structure analyses (Figure 4) supported $K = 2$ (ΔK) or $K = 5$ ($\ln P(D)$) respective population groups for ruffe (Figure S2). At $K = 2$ (Figure S2), which was the best-supported, the Great Lakes, Elbe River, and Vistula Lagoon populations comprised a single group (colored aqua) and the Bassenthwaite Lake samples another (orange). For $K = 5$, individuals from

Table 3. Effective population size (N_e) for population samples having $N > 30$, based on microsatellite data using NeEstimator v2 (Do et al. 2014).

Population sample	N_e
Ia. St. Louis Harbor – Early (invasive)	283.8
Ia. St. Louis Harbor – Later (invasive)	28.3
Id. Thunder Bay (invasive)	74.8
II. Bassenthwaite Lake – Early (invasive)	360.6
II. Bassenthwaite Lake – Later (invasive)	166.4
IIIa. Elbe River (native)	Infinite
IIIb. Vistula Lagoon (native)	Infinite

the Great Lakes primarily cross-assigned to two groups (maroon and gold), while those from Bassenthwaite Lake (orange), Elbe River (blue), and Vistula Lagoon (green) mostly separately assigned (Figure 4B). Hierarchical analyses for component single populations and population regions did not resolve additional structure (defaulting to $K = 2$, showing approximately equal cross-assignment to two groups for all individuals).

GeneClass2 assignment tests (Table 6) depicted appreciable mis-assignments (18–39%) of the Great Lakes' populations to the hypothesized source region (Elbe River). Individuals from St. Louis Harbor, which was the site of initial establishment for the ruffe in the Great Lakes, had 39% misassignment to the Elbe River and 56% self-assignment. In contrast, all individuals from Elbe River correctly self-assigned (Table 6).

Table 4. Pairwise genetic divergences (θ_{ST} ; below diagonal), and comparisons of allelic richness and observed heterozygosity (commas between respective probabilities from paired two-tailed *t*-tests; above diagonal), from 10 microsatellite loci. (A) primary populations analyzed, combining all Great Lakes locations and temporal samples, (B) individual populations (temporal samples combined), (C) temporal samples from St. Louis Harbor invasive population, Great Lakes, and (D) temporal samples from Bassenthwaite Lake, England invasion. * = $p < 0.05$, ** = Significant both before and after sequential Bonferroni correction, NS = $p > 0.05$ (or without * below diagonal).

A. Population	I	II	IIIa	IIIb
I. Great Lakes (invasive)	–	NS, NS	*, *	*, **
II. Bassenthwaite Lake (invasive)	0.175**	–	** , **	** , **
IIIa. Elbe River (native)	0.091**	0.170**	–	NS, NS
IIIb. Vistula Lagoon (native)	0.112**	0.244**	0.044**	–
B. Sampling Location in Great Lakes	Ia	Ib	Ic	Id
Ia. St. Louis Harbor (invasive)	–	NS, NS	NS, NS	*, NS
Ib. Sand River (invasive)	0.035**	–	NS, NS	NS, NS
Ic. Little Bay de Noc (invasive)	0.023**	0.006	–	NS, NS
Id. Thunder Bay (invasive)	0.012**	0.039**	0.031**	–
C. Temporal – St. Louis Harbor (Ia)	E	M	L	
Early (E)	–	NS, NS	NS, NS	
Middle (M)	0.014*	–	** , NS	
Later (L)	0.011**	0.007	–	
D. Temporal – Bassenthwaite Lake (II)	E	M	L	
Early (E)	–	** , *	*, NS	
Middle (M)	0.015**	–	NS, NS	
Later (L)	0.014**	0.017**	–	

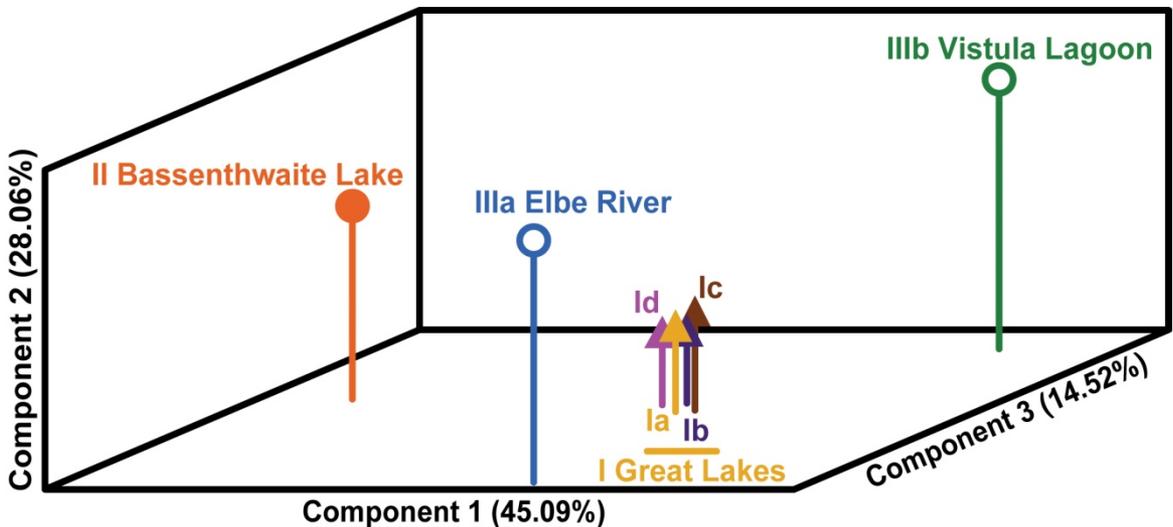


Figure 3. Three-dimensional factorial correspondence analysis (3dFCA) illustrating relationships among ruffe populations (colored; sampling years combined), using 10 microsatellite loci.

Divergences among locations in the Great Lakes’ invasion

Pairwise genetic divergence analyses discerned that all populations within the Great Lakes, except for the Sand River and St. Louis Harbor comparison, significantly differed after Bonferroni correction (Table 4B, below diagonal). Allelic richness and heterozygosity values did not significantly differ among the Great Lakes’ population samples (Table 4B, above diagonal),

except for slightly lower (but not significant) allelic richness in Thunder Bay.

All population samples in the Great Lakes tightly clustered in 3dFCA (Figure 3). Structure (Figure 4) depicted little differentiation among the Great Lakes’ populations. GeneClass revealed high self-assignments (Table 6) among Great Lakes’ population samples, except for the Thunder Bay expansion site, with 41% of its individuals mis-assigning to the St. Louis Harbor (the invasion’s original location).

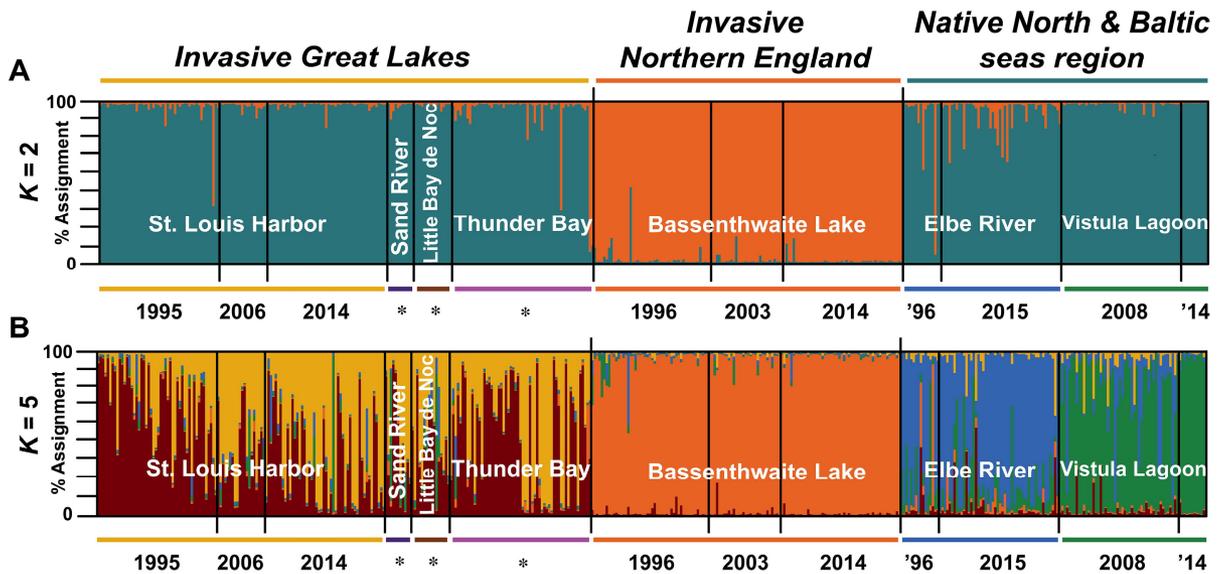


Figure 4. Bayesian Structure analyses depicting alternatives of (A) two population groups ($K = 2$; colored aqua or orange), or five population groups ($K = 5$; maroon, gold, orange, blue, or green), with each individual fish as a thin vertical line whose color(s) indicates percent likelihood of assignment to each population group. * = population sample for which multiple years were pooled.

Table 5. Analysis of Molecular Variance (AMOVA) of scenarios (A and B) for partitioning genetic variation from microsatellite data. Source of variation, percentage (%) of variation, degrees of freedom (d.f.), fixation index, and p value. * = $p < 0.05$.

Level	Comparison	% Variation	d.f.	Fixation index	p value
A. Groups	Between the Great Lakes (I) and native Europe (III)	7.11	1	0.071	< 0.001*
Populations	Among the Great Lakes (Ia–Id) and native Europe (IIIa, b)	2.81	4	0.030	< 0.001*
B. Groups	Between St. Louis Harbor (Ia) and Bassenthwaite Lake (II)	17.43	1	0.174	< 0.001*
Samples	Among their temporal samples (Early, Middle, Later)	1.07	4	0.012	< 0.001*

Table 6. GeneClass2 assignment tests, using microsatellite data. Number of individuals (and relative proportion; in parentheses) assigning to and from the four Great Lakes sites and the invasion's hypothesized native population source area. Self-assignments in italics, along diagonal.

Site	Assigned to				
	Ia	Ib	Ic	Id	IIIa
Ia. St. Louis Harbor (invasive)	67 (0.56)	1 (0.01)	3 (0.03)	2 (0.02)	47 (0.39)
Ib. Sand River (invasive)	1 (0.09)	7 (0.64)	1 (0.09)	-	2 (0.18)
Ic. Little Bay de Noc (invasive)	4 (0.25)	2 (0.13)	7 (0.44)	-	3 (0.19)
Id. Thunder Bay (invasive)	24 (0.41)	2 (0.03)	1 (0.02)	15 (0.25)	17 (0.29)
IIIa. Elbe River (native)	-	-	-	-	66 (1.00)

Temporal comparisons

In Structure, all populations appeared relatively temporally consistent in self-assignments (Figure 4). There were no significant differences over time in pairwise genetic compositions, allelic richness, or heterozygosity values for either of the native populations (Table 2). In contrast, AMOVA (Table 5B) for the two invasions showed relatively small, yet significant partitioning of genetic variation (1.07%)

across their temporal courses (early, middle, and later stages). Significant difference in pairwise genetic divergence occurred for the St. Louis Harbor population between its early and later periods (Table 4C, below diagonal), and over all time periods for Bassenthwaite Lake (Table 4D, below diagonal); these denote changes in genetic compositions. The middle time period for the St. Louis Harbor varied from the early one prior to, but not after, Bonferroni correction, and the middle and later periods did not differ (Table 4C, below diagonal).

Both invasions declined in effective population size over time, from early through later stages (Table 3). For genetic diversity, the invasive populations showed no significant temporal differences overall (from early to later stages of the invasion) in either allelic richness or heterozygosity values (Table 4C and D, above diagonal). Significant difference in allelic richness occurred between the middle and later time periods for St. Louis Harbor (Table 4C, above diagonal), with the middle having the lowest value and the later period with the highest (Table 2B). Its U-shaped pattern depicted slight decline in allelic richness between the early and middle stages (not significant), followed by significant increase. The later stage also contained four low-frequency private alleles (Table 2B). No differences occurred in its observed heterozygosity values (Table 2B), which rose slightly from 0.44 in the early and middle stages to 0.46 in the later period.

For Bassenthwaite Lake, significant genetic divergences ($\theta_{ST}=0.014\text{--}0.017$) occurred across the invasion (Table 4D, below diagonal). Allelic richness (Table 4D, above diagonal) significantly declined from the early to the middle stages, and overall varied slightly from the early through the later period (the latter was not significant post-Bonferroni correction). The early stage possessed the highest allelic richness value, the middle the lowest, and the final stage was intermediate (Table 2B), with a U-shaped pattern. Heterozygosity values also slightly dipped between the early versus the middle time periods (not significant after Bonferroni correction), in a U-shaped pattern. All temporal samples contained a few low-frequency private alleles (Table 2B).

Discussion

Genetic divergences between the two invasions and their respective origins

The mtDNA and nDNA data sets both revealed pronounced genetic divergences between the two ruffe invasions, indicating that the Great Lakes and Bassenthwaite Lake populations each originated from separate genetic sources. The Great Lakes and both native European populations shared mtDNA control region haplotype A, and were monotypic. The Bassenthwaite population was monotypic for haplotype B. MtDNA haplotypes from other Eurasian regions have been absent from the Great Lakes and Bassenthwaite Lake populations (this study and Stepien et al. 1998, 2005). The microsatellite data further differentiated between the two native north European population samples—Elbe River and Vistula Lagoon—with the Great Lakes' invasion appearing genetically closer

to the Elbe River, suggesting this, or nearby, as the founding source. This was corroborated by nuclear Ldh-A6 intron sequences (Stepien et al. 2004, 2005).

Genetic diversity and founder effect in the respective invasions

Genetic diversity levels of the ruffe, as well as effective population sizes, were greater in the native European populations than in the invasive populations. Results indicate that both ruffe invasions experienced significant founder effects, following scenario (2B), especially in the initial invasion stage. Effective population sizes have decreased temporally over the courses of both invasions. Low effective population sizes that are retained over time after nonindigenous species introductions, accompanied by genetic drift, likely limit their overall success through inbreeding depression and reduced adaptive potential (Allendorf et al. 2013; Blackburn et al. 2015; Vera et al. 2016). Our Colony analyses discerned 12 pairs of full siblings in the Bassenthwaite Lake samples, indicating potential inbreeding depression. For neutral loci, such as microsatellites, allelic richness generally decreases more than does heterozygosity, since rare alleles are more readily affected by drift than are common ones (Nei et al. 1975; Bai and Zhang 2014); this pattern was observed for ruffe. In our genetic diversity comparisons, allelic richness values thus differed more than did heterozygosity across the temporal course of the invasions.

Roman and Darling (2007) showed that just 37% of introduced populations significantly lost genetic diversity (scenario B), as indicated for the ruffe invasions. In contrast, introductions of dreissenid mussels and round goby in the Great Lakes were large and very genetically diverse, involving several likely source populations and introduction events, showing no founder effects (hypothesis 1; Stepien et al. 2005, 2013; Brown and Stepien 2008, 2009, 2010; Snyder and Stepien 2017). Founder effect and genetic drift in the Great Lakes' ruffe invasion likely account for its genetic divergence from the putative founding source population (at or near the native Elbe River region), shown in our θ_{ST} and Structure results, along with their effective population size differential.

The Great Lakes' ruffe, zebra mussel, quagga mussel, and round goby populations all were introduced and established prior to U.S. Coast Guard (2004, 2018) control regulations, which require a ballast water management plan by ships entering the Great Lakes. Canadian and U.S. legislation now require ships to flush their tanks with open-ocean seawater (Canada Shipping Act 2006; Ballast Water Working Group 2009, 2011). Research indicates that regulations

have reduced invasive species entering the Great Lakes (Bailey et al. 2011; GLANSIS 2018), and may have prevented significant genetic supplementation of the ruffe invasion (scenario 2C). It is essential that international management continue regulations to circumvent new propagules from reaching the Great Lakes, which might “refuel” the ruffe’s genetic diversity and/or directly introduce individuals into the lower Great Lakes. It also is important to avoid transport of ruffe via intra-lake shipping, to alleviate gene flow and further spread.

Temporal genetic changes across the ruffe invasions

This investigation found that genotypic compositions have varied over the three-decades of the Great Lakes and Bassenthwaite Lake ruffe invasions, in contrast to the native populations. Our results thus support the alternative hypothesis of genetic change (2), across the respective temporal courses of the two concurrent invasions in separate continents. Both invasions have declined in effective population size over time (scenario 2B).

In Bassenthwaite Lake, genetic divergence occurred over the course of the ruffe’s invasion (hypothesis 2), with allelic richness significantly declining and slight heterozygosity difference from the early to the middle stages (scenario 2B). This was followed by slight gain in allelic richness and consistency in heterozygosity between the middle to later periods (scenario 2A). The later Bassenthwaite Lake sample, although showing some recovery, remained lower in allelic richness and heterozygosity (not significant) than the early sample. Over the span of years following an invasion, this pattern of a “U”-shaped dip and slight recovery in allelic richness is common in invasions that have been studied, primarily plants (Dlugosch and Parker 2008). This genetic diversity pattern may have resulted with or without propagule supplement. Population diversity recovery is postulated to stem from higher reproductive success and gene flow from neighboring colonization areas (Blackburn et al. 2015). Bassenthwaite Lake may have had some supplements, since two new private (but rare) alleles appeared in the later sample; however, this may reflect sampling variation, since sample sizes were limited.

In the St. Louis Harbor time series, genetic divergence of introduced ruffe significantly differed between the early and later periods (hypothesis 2), accompanied by decline in effective population size (scenario 2B). Heterozygosity values were relatively consistent, with a slight (non-significant) increase in the later sample, accompanied by a gain in allelic richness between the middle and later stages (scenario 2C).

The later sample contained four private alleles, and thus might have been supplemented by new propagules. Since these private alleles were very rare, their prior absence might be a stochastic sampling effect.

Two North American mosquitofish species (the eastern mosquitofish *Gambusia holbrooki* Girard, 1859 and the western mosquitofish *G. affinis* (Baird and Girard, 1853)) have been widely introduced for insect control around the world and together are listed in the world’s top 100 worst invasive species (Lowe et al. 2000). Native eastern mosquitofish populations exhibited high spatial heterogeneity and temporal stasis (scenario 1A) (McClenaghan et al. 1985), like native populations of round goby (Brown and Stepien 2008) and ruffe (this study). Despite low numbers founding their European introductions and low mtDNA variability (scenario 2B), eastern mosquitofish introductions since have retained much of their nDNA diversity and population structure (1A) due to high reproduction and large population sizes (Sanz et al. 2013; Diez-del-Molino et al. 2013). Invasive round goby populations in the Great Lakes underwent no appreciable post-establishment genetic changes in composition or diversity over 25 years (Snyder and Stepien 2017), supporting the hypothesis of high diversity and relative temporal stasis (scenario 1A). The temporal invasion scenarios for eastern mosquitofish (2B, followed by 1A) and the round goby (consistently 1A) each appear to differ from the ruffe invasions, with the latter being more stochastic over time in Bassenthwaite Lake (2B, then 2A) and the Great Lakes (2B, then 2C). These may be attributed to low effective population sizes of the ruffe invasions.

Genetic divergence across the Great Lakes’ ruffe expansion and recent population trends

The ruffe displayed some genetic heterogeneity across its Great Lakes range, as found for invasive round goby (Brown and Stepien 2009; Snyder and Stepien 2017) and zebra and quagga mussel populations (Stepien et al. 2005, 2013; Brown and Stepien 2009). In contrast to the ruffe, those other invasions possessed diversity levels similar to native populations and indicated that multiple Eurasian founding sources were involved. Similar to the ruffe, the Asian paddle crab’s *Charybdis japonica* (Milne-Edwards, 1861) New Zealand invasion had lower genetic diversity than in its native range (hypothesis 2), but, unlike the ruffe, appeared genetically homogenous across the invasive range (Wong et al. 2016).

In the present study, the Great Lakes’ ruffe invasion displayed significant genetic subdivision between most locations (except between the Sand

River and Little Bay de Noc). This genetic divergence pattern may have stemmed from strong genetic drift during the expansion process, as occurred during eastern mosquitofish invasions (Vera et al. 2016). Population samples of ruffe mostly self-assigned, except for greater mis-assignments between the St. Louis Harbor (invasion's origin) and the Thunder Bay expansion site in Lake Huron. Interestingly, since ~2003 the ruffe has become very rare in Thunder Bay and in Lake Huron overall, despite targeted sampling by the U.S. Fish and Wildlife Service (Bowen and Keppener 2015), including electrofishing, trawls, and trapping (Bowen and Keppener, *pers. comm.*). During 2008, 2011, and 2012, just four individuals were captured from two locations (30 and 60 km upstream of Thunder Bay; Bowen and Keppener 2015). The ruffe's decline in Lake Huron may be attributable to its considerable geographic distance from the invasion core area in western Lake Superior, and lack of recent supplements.

Original predictions were that ruffe would spread quickly throughout all the Great Lakes and threaten the yellow perch population and fishery in Lake Erie (Busiahn 1997; Brenton 1998). In reality, the ruffe's spread has been considerably slower and much less extensive (restricted to the upper Great Lakes). An environmental (e)DNA assay targeting ruffe suggested a potentially larger expansion front in the Great Lakes, possibly extending into southern Lake Michigan (Tucker et al. 2016). However, eDNA often does not equate to the living organism, as positives can result from the droppings of predators (birds, larger fishes, etc.) and/or long-distance spread by currents (see Trebitz et al. 2017). Environmental RNA may be more effective at pinpointing the organism's actual nearby presence (Pochon et al. 2017). Results of our investigation, and others, suggest a continued need and interest to effectively monitor occurrence and persistence of ruffe in the Great Lakes in case further introductions occur and/or it invades the lower Great Lakes.

Since the Great Lakes' ruffe invasion appears to genetically trace to a single source region (the Elbe River or nearby) and experienced founder effect, it is possible that temperature tolerance, reproductive behavior, or other factors may have slowed its potential spread into the lower Great Lakes. The genetic variability of invasive populations frequently depends on whether they were founded from single or multiple sources, which may significantly influence phenotypic plasticity and adaptive potential (Bock et al. 2015). Moreover, low and declining effective population sizes, as indicated for both introductions in our results, may limit their adaptive capacity (see Blackburn et al. 2015; Vera et al. 2016), barring successful genetic

supplementation from other population sources. Although our study investigated selectively neutral genetic variation, Vera et al. (2016) discerned that mosquitofish diversity losses at genomic scales during its European invasion equally affected coding and noncoding regions. Since many fitness-related traits may be polygenic, they might retain adaptive variation despite the influence of genetic drift (Dlugosch and Parker 2008). Thus, examination of genomic loci that are under selection will be important to elucidate relative adaptive trends in ruffe invasions.

The ruffe has not extended its range beyond the upper Great Lakes and its population densities today have been undergoing sharp declines in most locations, including the initial establishment site at St. Louis Harbor in Lake Superior (Gutsch and Hoffman 2016; G. Cyzpinski and J. Hoffman, *pers. comm.*). This trend has not been observed in some European ruffe invasions, whose population densities have remained consistent (Lorenzoni et al. 2007; Winfield et al. 2011; Volta et al. 2013). However, our finding of sustained (and declining) low effective population size for ruffe in Bassenthwaite Lake and the presence of full siblings, may indicate that this population also is in decline. Results of the present study suggest that the ruffe's limited population genetic diversity and small effective population sizes may curb potential for further success, persistence, and expansion in the Great Lakes and Bassenthwaite Lake invasions, unless significant genetic supplementation occurs. Further genetic investigation and future sampling are merited.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Summary of 10 microsatellite loci analyzed for ruffe.

Figure S1. Native range and invasive range of the ruffe in Eurasia and North America.

Figure S2. Delta *K* curve and plot of mean *Ln p* from microsatellite data.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2018/Supplements/AI_2018_Stepien_et_al_SupplementaryMaterial.pdf