

RISK ANALYSIS AND GENETIC IDENTITY OF THE EURASIAN SOURCE POPULATION FOR THE RUFFE (*Gymnocephalus cernuus*) INVASION IN THE GREAT LAKES

Carol A. Stepien, Alexander M. Ford, Alison K. Dillon-Klika, Great Lakes Environmental Genetics Laboratory, Center for Environmental Science, Technology and Policy, Cleveland State University, MC 219, Euclid Ave., Cleveland, OH 44115, USA. c.stepien@csuohio.edu, and Mark A. Tumeo, Vice Provost for Research, Cleveland State University.

Introduction. Risk analysis methodology, coupled with the genetic characterization of invasive and potential source populations, may allow scientists and managers to predict the spread patterns and successes of nonindigenous species invasions. In this study, we compare nuclear and mitochondrial DNA sequence data from potential source populations in relation to the original colonization and spread sites for the Eurasian ruffe *Gymnocephalus cernuus*. The ruffe colonized the western Lake Superior region of the North American Great Lakes (Fig. 1) during the mid-1980s, after its accidental introduction via shipping ballast water discharge from one or more unknown Eurasian source populations. The ruffe is now an abundant nearshore benthic fish in western Lake Superior. During the early 1990s, it spread rapidly eastward along Lake Superior's southern shore and since then slowed in range progression, contrary to predictions by some fishery scientists. Shipping traffic also was the likely vector for the ruffe's 1992 introduction 300 km ne to Thunder Bay Harbor, Canada as well as its 1995 establishment in Lake Huron at Thunder Bay River, near Alpena, MI (site 11, Fig. 1). In 2002, it appeared in Little Bay de Noc of Lake Michigan near Escanaba, MI (site 12, Fig. 1).

The potential North American range of the ruffe originally was predicted to extend from the Great Plains to the eastern seaboard and north into Canada (Busiahn 1993). Its widespread Eurasian range led scientists to predict that the ruffe would quickly colonize the lower Great Lakes and potentially outcompete the yellow perch *Perca flavescens*. International efforts have focused on preventing new ballast water introductions and the Ruffe Control Program has sought to stem its spread. Here we analyze the ruffe's genetic characters in native and invasive populations in Eurasia and North America, based on nuclear DNA sequences of the LdhA6 intron and the mitochondrial DNA control region, in relation to risk analysis predictions and its spread patterns.

Methods. Ruffe were sampled from seven Eurasian population sites (Fig. 2), including: the Danube River at Gabčíkovo, Slovakia (site 1, N=13), the Morava River, Czech Republic (site 2, N=8), the Elbe River near Magdeburg, Germany (site 3, N=11), Bassenthwaite Lake, United Kingdom (an invasive location; site 4, N=12), Loch Lomond, Scotland (another invasive location; site 5, N=12), St.

Petersburg, Russia (site 6, N=15), and the Ob' River at Novosibirsk, Siberia, Russia (site 7, N=12).

Collections from North America included three sites off Lake Superior, representing the initial North American introduction (Fig. 1): the St. Louis River, MN (site 8, N=4), Superior Harbor, WI (site 9, N=12), and the Amnicon River, WI (site 10, N=10). Individuals also were examined from the Thunder Bay River, a tributary of Lake Huron at Alpena, MI (site 11, N=6), as well as from Little Bay de Noc in Lake Michigan at Green Bay, WI (Site 12, N=3).

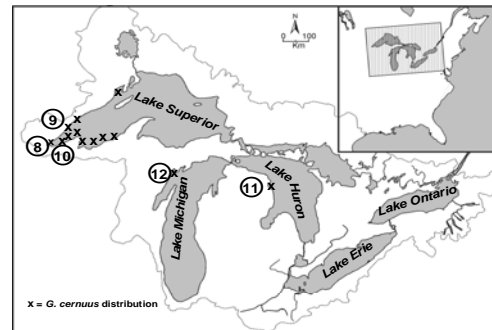


Figure 1. North American range of the ruffe (X), with numbered collection sites in circles.

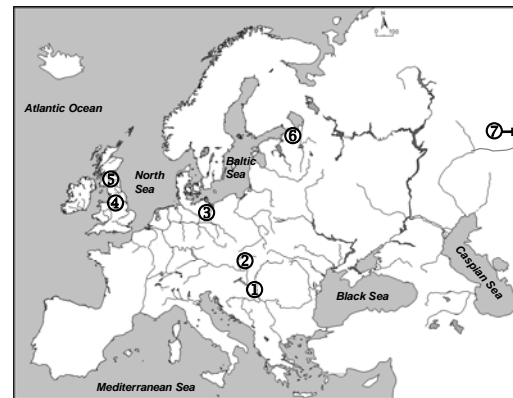


Figure 2. Eurasian collection sites for ruffe.

Methodology for DNA extraction, PCR amplification, and DNA sequencing of the mtDNA control region followed Stepien et al. (1998). Procedure for the sixth intron of the nuclear muscle-type lactate dehydrogenase A (LdhA6) locus followed Quattro and Jones (1999). Sequences were

run either manually (Stepien et al. 1998) or on a Beckman-Coulter CEQ 8000 capillary autosequencer separately in both directions. Data analysis of relationships among haplotypes included neighbor-joining trees in the programs Mega 2.1 using Kimura 2-parameter genetic distances and maximum parsimony using the branch-and-bound algorithm in PAUP*, each with 1000 bootstrap replications. Distributions of haplotypes among sites and genetic divergences were analyzed with the programs AMOVA in Arlequin 2.1 and Genepop.

Results. The mtDNA control region of ruffe and its congeners comprised 1,024 bp. Sequences were deposited in GenBank as Accession Numbers AF025355-62. Ruffe had 5 different haplotypes (A-E), and all population sites contained only a single type - except for the Ob' River, which had two (D and E) (Fig. 3). All samples from the Great Lakes were haplotype A, matching ruffe from the Danube, Morava, and Elbe Rivers. Haplotypes from western and central Europe (A and B) were widely diverged from those in northern Eurasia (C, D, and E).

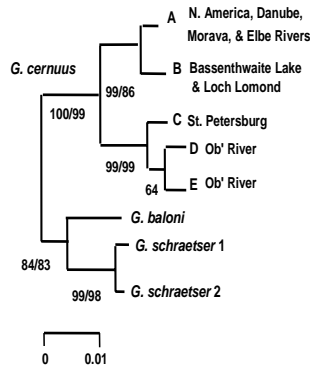


Figure 3. Neighbor-Joining and maximum parsimony tree of relationships among mtDNA haplotypes, with 1000 bootstrap replications.

The nuclear DNA LdhA6 intron for ruffe and its congeners comprised 198 bp and were deposited as Accession numbers AY034781-3 in GenBank. This nuclear intron was significantly more variable than mtDNA, having 16 different haplotypes. Each sampling location housed 3-7 LdhA6 haplotypes. Three haplotypes occurred in the North American Great Lakes, and did not significantly differ in their representation among those sites. The Elbe River also contained only 3 haplotypes identical to those in the Great Lakes, in statistically similar frequencies. One haplotype was only found in North America (24% of samples) and the Elbe River (27%). Samples from other Eurasian sites significantly differed from those in the Great Lakes and the Elbe River.

Discussion. The genetic composition of the nonindigenous introduction of ruffe in the Great Lakes matches that from the Elbe River drainage region in nw Europe (which empties into the Baltic and North Seas) for both nuclear and mitochondrial DNA sequences. All later colonization areas in the Great Lakes appear to stem from the original introduction in the St. Louis River region of Lake Superior. There thus appears to have been a single colonization source for the introduction, from the vicinity of the Elbe River. Ports on the Elbe River increasingly opened to shipping traffic with North America preceding the 1990 reunification of Germany, coinciding with the time of the anthropogenic introduction of the ruffe. This population may be better pre-adapted to the temperatures and other environmental conditions in the upper Great Lakes, and less well suited to habitats in the lower Great Lakes - which should be further analyzed. As in our genetic studies of the dreissenid mussel and round and tubenose goby invasions in the Great Lakes, the new ruffe population has a similar level of genetic variation to the founding population, indicating that a large number of individuals were introduced and there was no apparent "founder effect". Risk analyses suggest that founding success may increase with introduction of large numbers of individuals representing the "normal" amount of genetic variation in native populations. Relatively slow spread may be due to the introduction of a single source population and ballast control efforts.

Acknowledgements. This study was supported by grants to C. Stepien from the NOAA National Sea Grant Program #RF-707294, the Ohio Sea Grant Program, the Minnesota Sea Grant Program, the National Fish and Wildlife Fund, the US Fish and Wildlife Service, and the Lake Erie Protection Fund. This project is part of the Environmental Risk Analysis Program of Cleveland State University's Center for Environmental Science, Technology and Policy. We thank V. Kovac, I. Winfield, C. Adams, J. Gunderson, D. Jensen, A. Hintz, J. Clay Bruner, T. Busiahn, G. Czepinski, V. Panov, G. Spangler, D.L. Stein, M. Burnham-Curtis, J. Selgeby, and the Biological Resources Division of the US Geological Survey for specimens. We also thank E. Whippo-Cline for logistic support.

References. Busiahn, T.R. 1993. Can the ruffe be contained before it becomes your problem? *Fisheries* 18: 223.

Stepien, C.A., A.K. Dillon, and M.D. Chandler. 1998. Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* in the North American Great Lakes. *Journal of Great Lakes Research* 24: 361-378.

Quattro, J.M. and W.J. Jones. 1999. Amplification primers that target locus-specific introns in Actinopterygian fishes. *Copeia* 1999, 191-195.