Journal of the Minnesota Academy of Science

Volume 68 | Number 1 Article 2

2004

Minnesota Banded Darters (Etheostoma zonale) Exhibit a High Degree of Genetic Similarity in Mitochondrial DNA Sequences Occurrence

Jean Porterfield
St. Olaf College

Molly Fee
St. Olaf College

Jessica Theorin
St. Olaf College

RESEARCH PAPER

MINNESOTA BANDED DARTERS (*ETHEOSTOMA ZONALE*) EXHIBIT A HIGH DEGREE OF GENETIC SIMILARITY IN MITOCHONDRIAL DNA SEQUENCES OCCURRENCE

Jean Porterfield, Molly Fee, and Jessica Theorin
Dept. of Biology, St. Olaf College, Northfield, MN 55057

ABSTRACT

hylogeography, or the historical interpretation of population genetic data, is a useful tool for addressing historical processes like the colonization history of organisms. North American freshwater fishes have experienced glacial ebb and flow in their relatively recent past (as little as 10,000 years before present in Minnesota), and studies of variation in intraspecific DNA sequences of these fishes can shed light on their radiations into previously glaciated areas. This study assessed intraspecific variation in two mitochondrial genes of the banded darter (*Etheostoma zonale*) from seven Minnesota localities. A high degree of similarity was found among all individuals in the study. This finding prevented phylogeographic analysis, but it is informative about other historical factors, such as the significance of population bottlenecks during colonization.

INTRODUCTION

The banded darter (*Etheostoma zonale*) is a small nongame fish named for the brilliant green bands that encircle the bodies of breeding males. It is a member of the family Percidae, a group that contains the popular sport fishes yellow perch and walleye as well as nearly 200 species of the smaller darters. While the family has a Holarctic distribution, all darters are limited in range to North America with the majority of species endemic to very small ranges in the Central Highland regions east and west of the Mississippi River. The banded darter is one of the relatively few darters with a widespread distribution; it ranges from the highlands of Arkansas and Tennessee northeast through the Ohio River valley, and reaches the northwest portion of its range in the southern half of Minnesota. This large distribution makes the banded darter a good species for studying population genetics questions, such as amount and patterns of migration, both past and present.

The Pleistocene was an epoch of glacial ebb and flow, with glaciers reaching their maximum southern extent in North America during the Illinoian advance between 65,000 and 122,000 years before present (YBP), and with the most recent (Wisconsinan) glaciers retreating from the northern United States about 10,000–11,000 YBP (Dawson, 1992). The banded darter, along with other aquatic life, was confined to glacial refugia during these onsets of ice, and had to colonize the northern parts of its range postglaciation. Accordingly, populations of banded darters currently inhabiting Minnesota are relatively recent in origin, and should be most closely related to populations of banded darters from one or more refugium areas.

The current distribution of the banded darter provides insight about the timing of its postglacial colonization. Because the banded darter is present only in the Minnesota River and lower Mississippi River drainages in Minnesota, it is thought to be a relatively recent migrant into the state, becoming established only after the southern outlet to glacial Lake Agassiz had closed (Underhill, 1957). The banded darter's Minnesota distribution also suggests its colonization from the lower Mississippi River refugium (Underhill, 1989), but whether colonizing individuals came from the Central Highlands regions east or west of the Mississippi River (or both) is not known.

The field of phylogeography interprets population genetic data in a historical and geographic context (Avise, 1994); it allows the analysis of intraspecific variation in DNA sequences to provide insight into processes like gene flow or colonization of new areas. For example, Strange and Burr (1997) compared intraspecific phylogenies of five Central Highlands fish species to distinguish between vicariance and dispersal as processes explaining current population distributions. Similarly, Near et al. (2001) examined DNA sequences of a widespread darter (*Percina evides*) to hypothesize how these processes have affected not only Central Highlands distribution but also the species' presence in glaciated areas of the Midwest.

Mitochondrial DNA has provided the data sets for the majority of recent phylogeographic studies. In general, the mitochondrial genome accumulates nucleotide substitutions more rapidly than its nuclear counterpart (Brown, 1987), and its uniparental inheritance and lack of recombination result in a lower effective population size, allowing for the possibility of faster fixation or extinction of

haplotypes via drift (Moore, 1995). The cytochrome *b* protein spans the inner membrane of the mitochondrion (Degli Esposti et al., 1993), and its gene has been used extensively in phylogenetic studies, including those of fishes (Lydeard and Roe, 1997). Although the phylogenetic utility of cytochrome *b* has been the subject of some debate (e.g., Graybeal, 1993; Meyer, 1994), it is still widely used in a variety of studies ranging from intergeneric (e.g., Dunn et al., 2003) to intraspecific (e.g., Van Houdt et al., 2003).

Mitochondrial NADH dehydrogenase genes, including NADH dehydrogenase subunit 2 (ND2), have also been widely used in phylogenetic analyses. Results from studies that have obtained sequences for both cytochrome *b* and ND2 are mixed; the two genes exhibit comparable variability in dabbling ducks (Johnson and Sorenson, 1998), but ND2 is more variable than cytochrome *b* in *Myotis* bats (Cooper et al., 2001) and fishes such as suckers (McPhail and Taylor, 1999) and logperches (George, 2003).

The purpose of this study was to investigate the phylogeography of Minnesota banded darters in order to elucidate the pattern of this species' radiation in Minnesota over the past 10,000 years. Banded darters were sampled from seven sites in the state, and mitochondrial DNA sequences from the cytochrome *b* and ND2 genes were analyzed. These genes were chosen because of their past success in inferring intraspecific phylogeny, and because darter sequences for these genes are readily available for comparison.

MATERIALS AND METHODS

Collection of specimens

Banded darters were collected throughout southern Minnesota during 2001-2002, and the seven sites from which sequence data were obtained are mapped in Figure 1. Locality information and number of individuals sequenced (with GenBank accession numbers) are provided in Table 1. Fish were collected using a ten-foot seine under State of Minnesota Department of Natural Resources Division of Fisheries Special Permit No. 10962, and frozen in liquid nitrogen or preserved in 95% ethanol onsite. Specimens are stored at St. Olaf College.

DNA Sequence Data

Total DNA extractions were performed on muscle tissue using a protocol modified from the PUREGENE DNA Purification Kit (Gentra Systems) by T. Near (personal communication). The polymerase chain reaction (PCR) was used to amplify a 550-nucleotide region of the cytochrome *b* gene of banded darters using primers THR (Song et al., 1998) and ZON635F (5'-ACT CCG ACG CCG ATA AAG TGT C-3'). PCR was also used to amplify the entire ND2 gene using primers ND2Met and ND2Trp (Kocher et al., 1995). Primers were annealed at 50 C in all reactions. The primers THR (for the cytochrome *b* gene) and ND2Met (for the ND2 gene) were used in sequencing reactions of clean PCR product (QIAquick PCR Purification Kit, Qiagen, Inc.), which were done at the DNA Synthesis and Sequencing Laboratory of the University of Medicine and Dentistry of New Jersey's Robert Wood Johnson Medical School (cytochrome *b* sequences) or the Auburn University Genomics and Sequencing Laboratory (ND2 sequences).

Sequence Analysis

Chromatograms were viewed and sequences were edited using EditView (version 1.0.1, Applied Biosystems), and ClustalW (Thompson et al., 1994) was used to align sequences. A cytochrome *b* DNA sequence from an Illinois banded darter (Porterfield, 1998) was aligned along with the sequences from Minnesota individuals. Uncorrected pairwise distances among individuals were calculated with PAUP* version 4.0b10 (Swofford, 2000), and MacClade version 3.03 (Maddison and Maddison 1992) was used to assess variable nucleotide and amino acid positions.

RESULTS AND DISCUSSION

Cytochrome *b* sequence data were obtained for 29 Minnesota banded darters, which resulted in a data set of 30 individuals (including the individual from Illinois). While the amount of sequence obtained per individual varied, the data set was reduced to the length of the shortest high-quality sequence (426 nucleotides). These nucleotides correspond to nucleotide positions 637 to 1062 of this 1140-nucleotide gene (amino acid positions 213 to 354 out of 380). ND2 sequences of 712 nucleotides in length were obtained for eight of the 29 banded darters (representing seven Minnesota localities; see Table 1); these nucleotides represent the first 712 positions (first 237 amino acids) of this 1047-nucleotide gene. Only single-stranded sequence was obtained in this study; although double-stranded sequence is more desirable, the lack of variation found when examining the single-strand data set suggested that obtaining double-stranded sequence would not be cost-effective (since sequencing the other strand is more likely to contradict putative substitutions than to identify more substitutions).

Of the 426 nucleotides of cytochrome *b* for all 30 individuals, only two were variable (not

including the 14 positions where an undetermined nucleotide [N] was present in one individual). This resulted in an average uncorrected genetic distance of only 0.0007 in all pairwise comparisons among the 30 sequences. One of the variable nucleotides (position 768) was a third-position silent substitution unique to the individual from the Zumbro River. The other (position 1061) was a second-position substitution found in four individuals (two from Cobb River and two from Yellow Medicine River); it resulted in an amino acid change from alanine to glycine. These amino acids are very similar in structure and biochemical properties, with alanine exhibiting slightly more hydrophobicity than glycine. Of the 712 nucleotides of ND2 for nine banded darters, the only variable position was nucleotide 48 where an undetermined nucleotide (N) was present in one individual. Contamination is not likely based on other PCR and sequencing reactions that were performed at the same time.

Based on the cytochrome *b* sequence data, three haplotypes were found among Minnesota banded darters, and the most common haplotype is shared with the banded darter from Illinois. This high degree of similarity not only within Minnesota fish but also among Minnesota and Illinois fish is consistent with the glacial history of the Midwest. All 30 individuals were collected from regions that experienced the last (Wisconsinan) glacial retreat just 10,000–11,000 YBP, so they represent relatively recently established populations. Near et al. (2001) also found high genetic similarity among central Minnesota and northern Wisconsin gilt darters (*Percina evides*), with the three individuals from this region exhibiting pairwise sequence distances of only 0.1–0.2% (based on all 1141 nucleotides of the cytochrome *b* gene). Both the banded darter and the gilt darter are hypothesized to be relatively recent migrants (Underhill, 1957). In contrast, Kassler et al. (1997) found some among-population mtDNA variation in their study of 36 Minnesota and Wisconsin populations of johnny darters (*Etheostoma nigrum*). The johnny darter is hypothesized to be one of the earliest postglacial arrivals to Minnesota (Underhill, 1957).

While the data from this study are too homogeneous for phylogeographic analysis, the lack of variation among Minnesota banded darter mitochondrial DNA sequences is informative about other aspects of this species' colonization history. Patterns of low nucleotide and haplotype diversity are consistent with historical bottlenecks during colonization (e.g., Gamache et al., 2003; Wang et al., 2000). Considerably more variation is found within and among Central Highlands populations of the banded darter (Porterfield, unpublished data), but if there were relatively small numbers of colonizing banded darters, it is probable that genetic diversity among colonizers was low. Estimates of the rate of cytochrome *b* evolution range from 0.70%/Myr for ectothermic vertebrates (Johns and Avise, 1998) to 0.76%/Myr for European cyprinid fishes (Zardoya and Doadrio, 1999). Based on these estimates, banded darters would be expected to exhibit little cytochrome *b* variation after 10,000 years in Minnesota, presuming genetic uniformity among colonizing fishes.

A different molecular marker is necessary to address phylogeographic patterns among Minnesota banded darters. Microsatellite loci have proven valuable for uncovering genetic variation within and among populations (Koskinen et al., 2002; Pope et al., 2000), and may be an appropriate direction for the study of Minnesota freshwater fishes including the banded darter. For now, the high degree of genetic similarity among Minnesota banded darters, even in the fast-evolving mitochondrial genome, provides some insight into the postglaciation colonization of these fish into the state. This information is also important in the context of the future evolution and conservation of Minnesota populations, as these populations represent the northern edge of the banded darter's range.

ACKNOWLEDGMENTS

Funding for this research was provided by a St. Olaf College Faculty Development Grant to Jean Porterfield and by a Howard Hughes Medical Institute Undergraduate Science Education grant to St. Olaf College. Matt Berkseth and Kathryn Huber helped with sequencing of the ND2 gene. Figure 1 was compiled by Charles Umbanhowar Jr., and Diane Angell, Patrick Ceas, Jay Hatch, Gary Phillips, and Charles Umbanhowar Jr. provided helpful comments on the manuscript.

Literature Cited

1. Dawson, A. 1992. *Ice Age Earth*. London: Routledge.
2. Underhill. J. 1957. The distribution of Minnesota minnows and darters in relation to Pleistocene glaciation. *Occas. Papers Minn. Mus. Nat. Hist.* 7:1-45.
3. Underhill, J. 1989. The distribution of Minnesota fishes and late Pleistocene glaciation. *J. Minn. Acad. Science* 55:32-37.
4. Avise, J. 1994. *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall.
5. Strange, R., and Burr, B. 1997. Intraspecific phylogeography of North American highland fishes: a test of the Pleistocene vicariance hypothesis. *Evol.* 51: 885-897.
6. Near, T., Page, L., and Mayden, R. 2001. Intraspecific phylogeography of *Percina evides* (Percidae: Etheostomatinae): an additional test of the Central Highlands pre-Pleistocene vicariance hypothesis. *Mol. Ecol.* 10:2235-2240.
7. Brown, W. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* 18:269-292.
8. Moore, W. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evol.* 49:718-726.
9. Degli Esposti, M., De Vries, S., Crimi, M., Ghelli, A., Patarnello, T., and Meyer, A. 1993. Mitochondrial cytochrome *b*: evolution and structure of the protein. *Biochim. Biophys. Acta* 1143:243-271.
10. Lydeard, C., and Roe, K. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among actinopterygian fishes. In. T. Kocher and C. Stepien (eds.), *Molecular Systematics of Fishes*. San Diego: Academic Press.
11. Graybeal, A. 1993. The phylogenetic utility of cytochrome *b*: lessons from bufonid frogs. *Mol. Phylogenet. Evol.* 2:256-269.
12. Meyer, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends Ecol. Evol.* 9:278-280.
13. Dunn, K., McEachran, J., and Honeycutt, R. 2003. Molecular phylogenetics of myliobatiform fishes (Chondrichthyes: Myliobatiformes), with comments on the effects of missing data on parsimony and likelihood. *Mol. Phylogenet. Evol.* 27:259-270.
14. Van Houdt, J., Hellemens, B., and Volckaert, F. 2003. Phylogenetic relationships among Palearctic and Nearctic burbot (*Lota lota*): Pleistocene extinctions and recolonization. *Mol. Phylogenet. Evol.*29:599-612.
15. Johnson, K., and Sorenson, M. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome *b* and ND2) in the dabbling ducks (Tribe: Anatini). *Mol. Phylogenet. Evol.* 10:82-94.
16. Cooper, S., Day, P., Reardon, T., and Schulz, M. 2001. Assessment of species boundaries in Australian *Myotis* (Chiroptera: Vespertilionidae) using mitochondrial DNA. *J. Mamm.* 82:328-338.
17. McPhail, J., and Taylor, E. 1999. Morphological and genetic variation in northwestern longnose suckers, *Catostomus catostomus*: the Salish sucker problem. *Copeia* 1999:884-893.
18. George, A. 2003. Conservation genetics of the imperiled logperches *Percina burtoni*, *Percina jenkinsi*, and *Percina rex* (Teleostei: Percidae). 17th Annual Meeting of the Society for Conservation Biology.
19. Song, C-B., Near, T., and Page, L. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. *Mol. Phylogenet. Evol.* 10:343-353.
20. Kocher, T., Conroy, J., McKaye, K., Stauffer, J., and Lockwood, S. 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. *Mol. Phylogenet. Evol.* 4:420-432.
21. Thompson, J., Higgins, D., and Gibson, T. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673-4680.
22. Porterfield, J. 1998. Phylogenetic systematics of snubnose darters (Percidae, *Etheostoma*), with discussion of reproductive behavior, sexual selection, and the evolution of male breeding color. Unpublished Ph.D. Dissertation, University of Illinois Urbana-Champaign.
23. Swofford, D. 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sunderland: Sinauer Associates.
24. Maddison, W., and Maddison, D. 1992. MacClade version 3.03. Sunderland: Sinauer Associates.
25. Kassler, T., Fields, R., and Philipp, D. 1997. Determining stock boundaries of johnny darter, *Etheostoma nigrum*, in the upper Midwest. Chapter 5 *in* R. Fields, M. Desjardins, J. Hudson, T. Kassler, J. Ludden, J. Tranquilli, C. Toline, and D. Philipp. 1997. Genetic Analyses of Fish Species in the Upper Midwest. *Illinois Natural History Survey,*

Center for Aquatic Ecology Technical Report 97/5, volume 3, Champaign.
26. Gamache, I., Jaramillo-Correa, J., Payette, S., and Bousquet, J. 2003. Diverging patterns of mitochondrial and nuclear DNA diversity in subarctic black spruce: imprint of a founder effect associate with postglacial colonization. *Mol. Ecol.* 12:891-901.
27. Wang, P., Hsu, C., and Chiang, Y. 2000. Mitochondrial DNA phylogeography of *Acrossocheilus paradoxus* (Cyprinidae) in Taiwan. *Mol. Ecol.* 9:1483-1494.
28. Johns, G., and Avise, J. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. *Mol. Biol. Evol.* 15:1481-1490.
29. Zardoya, R., and Doadrio, I. 1999. Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. *J. Mol. Evol.* 49:227-237.
30. Koskinen, M., Nilsson, J., Veselov, A., Potutkin, A., Ranta, E., and Primmer, C. 2002. Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. *Heredity* 88:391-401.
31. Pope, C., Estoup, A., and Moritz, C. 2000. Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. *Mol. Ecol.* 9:2041-2053.

Table 1. Banded darters (*Etheostoma zonale*) collected and sequenced for this study. Localities (with number of individuals collected), drainages, and number of individuals sequenced for both the cytochrome *b* gene and the ND2 gene are provided. GenBank accession numbers are AY354674–AY354702 for cytochrome *b* sequences and AY354703–AY354710 for ND2 sequences. A map of localities is provided in Figure 1.

Locality (number collected)	**Drainage**	**Cytochrome *b* Sequences**	**ND2 Sequences**
Hawk Creek at County Road 52, Renville County, MN, 18 June 2002 (10)	Minnesota River	4	2
Yellow Medicine River about 2 river miles upstream of Highway 67 bridge, Yellow Medicine County, MN, 18 June 2002 (8)	Minnesota River	4	1
Big Cobb River at County Road 16, Blue Earth County, MN, 15 July 2002 (14)	Minnesota River	5	1
North Branch Root River at Highway 63, Olmsted County, MN, 9 July 2002 (15)	Lower Mississippi River	5	1
Zumbro River at County Road 125, Olmsted County, MN, 9 July 2002 (12)	Lower Mississippi River	1	1
tributary of North Fork Zumbro River and County Road 71, Wabasha County, MN, 1 July 2002 (16)	Lower Mississippi River	5	1
Middle Fork Zumbro River at Highway 5, Olmsted County, MN, 9 July 2002 (14)	Lower Mississippi River	5	1

Figure 1

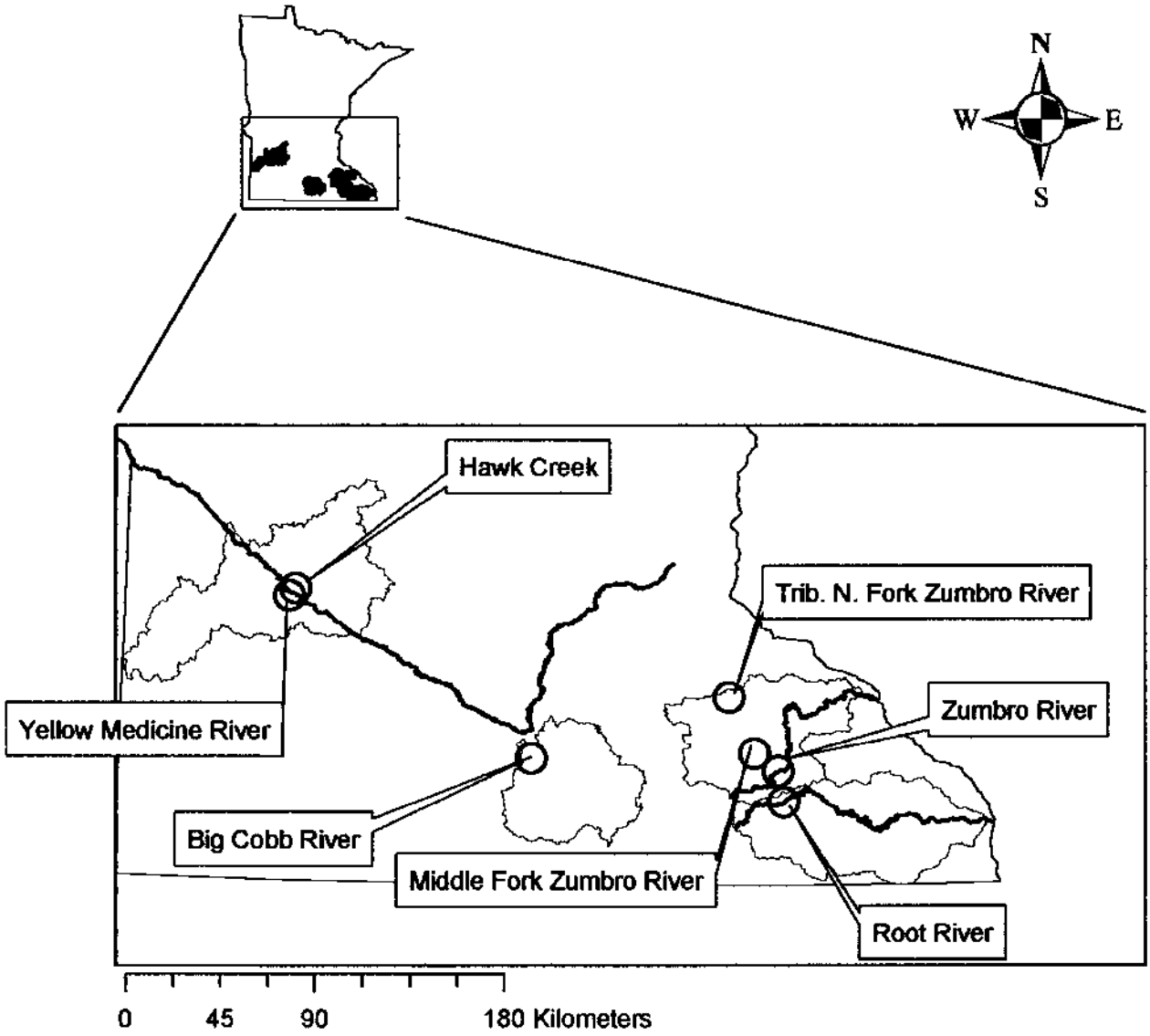

Figure 1. Map of southern Minnesota river systems with circles indicating the localities of banded darters (*Etheostoma zonale*) collected and sequenced for this study. Map was compiled with data from the Minnesota Land Management Information Center (www.lmic.state.mn.us) and the Minnesota Department of Natural Resources GIS Data Deli (deli.dnr.state.mn.us).