ILLINOIS NATURAL HISTORY SURVEY

Genetic Analyses of Fish Species in the Upper Midwest

EXECUTIVE SUMMARY

Presented to:
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Project Background

Over the last few decades, advances in culture techniques have enabled the widespread use of fish hatcheries to supplement and/or establish fish populations in efforts to counteract the effects of overharvest, habitat loss, and/or low recruitment (Stroud 1986). This high production capability of hatcheries presents a potential for genetic manipulation that has no parallel in natural vertebrate systems (Ryman et al. 1994). It is this potential for large scale translocation of cultivated fish stocks that poses such a serious threat to the genetic diversity of native fish stocks by providing the possibility of introgression with introduced non-native stocks (Hindar et al. 1991; Nehlsen et al. 1991; Philipp 1991; Fleming 1994; Hindar and Jonsson 1994).

This situation exists because resource management efforts historically have used the "species" as the operational unit of concern, and so are often in direct conflict with conservation genetic theory. This conflict arises because such a species-based approach fails to recognize the spatially complex nature of the distribution of genetic resources within each species. As a result, management activities sometimes promote the loss of these important genetic resources rather than their conservation. For fisheries management programs to be truly effective on a long term basis, therefore, the operational unit of concern must be the stock, not the species as a whole (Philipp 1991).

Stocks are defined as randomly breeding groups of individuals (or populations) that have diverged from other such groups due to temporal, spatial, or behavioral isolating mechanisms (Kapuscinski and Philipp 1988). Distinction of the underlying genetic differences among stocks, and, therefore, identification of the spatial boundaries that define individual stocks, however, often require molecular genetic analyses. A general lack of appropriate population genetic data on even a regional scale has been the greatest hindrance to implementing management by this Stock Concept. That lack of data has been a clear hurdle for defining the boundaries for sportfish stocks.

Project Objectives

The objectives of this project were as follows: 1) to assess the usefulness of three molecular techniques for detecting genetic variation among selected fish species in the Upper Midwest; 2) to identify a set of variable genetic characters that have the potential to delineate population structure on this regional scale; 3) to conduct such a population genetic survey for selected fish species across three major drainage basins in Minnesota and Wisconsin (Mississippi River, Great Lakes, and Hudson Bay) and 4) to use these genetic data cooperatively with Minnesota DNR and Wisconsin DNR biologists to develop a regional management strategy based on conservation genetic principles.

Project Organization

Species and populations were chosen by DNR personnel in consultation with project personnel. We tried to choose three populations from each major river system within each of the three major drainage basins (Hudson Bay, Great Lakes, Mississippi River) within Minnesota. Sampling efforts for most populations were conducted by DNR field biologists. Project personnel made some of the outgroup collections. The project was divided into three phases, as described below.

Phase I consisted of an assessment of the ability of three molecular techniques (protein electrophoresis, RFLP analysis of mitochondrial DNA, and RAPD analysis of genomic DNA) to detect genetic variation in eight target species of fish (muskellunge, northern pike, bluegill, smallmouth bass, largemouth bass, johnny darter, yellow perch, and walleye). For each species, four populations were sampled, one from each of three drainage basins in the upper Midwest (Mississippi River, Great Lakes, and Hudson Bay), and an additional geographically distant location to serve as an outgroup. This design was employed in an attempt to capture as large a component of the available variation as

possible using a minimal number of collections. The data collected allowed us to assess the relative ability of each technique to detect genetic variability in each of the eight target species.

Phase II consisted of a survey of the distribution of genetic variation among populations of each of the eight target species. For each of these species (with the exception of northern pike and yellow perch, for which insufficient variability was detected to warrant continuation at this time), two molecular techniques (protein electrophoresis and RFLP analysis of mtDNA) were used to obtain independent datasets. For each species, we then used the results from the two independent datasets to construct a map defining the boundaries for genetic groupings of that species within Minnesota. Finally, the six species-specific maps were then combined to form the basis for recommending a set of nine conservation management units.

Phase III consisted of answering specific questions associated with eight additional species that were not widely distributed across Minnesota. This effort involved an assessment of the usefulness of the various molecular techniques employed (protein electrophoresis, RFLP analysis of mitochondrial DNA, and RAPD analysis of genomic DNA) for detecting genetic variation. It also involved conducting the appropriate genetic analyses needed to address the specific questions associated with the eight additional species.

Summary of Results

Phase I

For each of the eight target species, the results of the analyses using each of the three molecular techniques tested are summarized in Table 1. All of the techniques tested indicated that three of the species (bluegill, largemouth bass, and johnny darter) had relatively high levels of detectable genetic variation, whereas three others (smallmouth bass, walleye, and muskellunge) had only moderate levels and two others (northern pike and yellow perch) had quite low levels. In fact, the number of variable characters detected in northern pike and yellow perch were so low that Phase II analyses on the collected populations have been postponed until another technique is identified that will provide those needed variable characters. For the three highly variable species (bluegill, largemouth bass, and johnny darter) the absolute number of variable characters detected using either RAPD or mtDNA analyses was greater than that detected using protein electrophoresis. The reverse was true for most of the other species. This result may simply reflect a difference in the level of genetic relatedness of the outgroup used for the various species in Phase I. Because all of the techniques detected a high degree of among population variation (F_{ST} or G_{ST}, depending upon the technique) for all species except northern pike and yellow perch, the feasibility of successfully resolving genetic structure during a Phase II population survey for the remaining six target species was good.

Table 1. Summary of the genetic variation detected using three molecular techniques.

Technique	Species[1]	Number of systems analyzed	# of characters detected with each system	# characters polymorphic	% polymorphic	F_{ST}/G_{ST}
protein electrophoresis	BLG	35 enzymes	63 loci	9 loci	14.29%	0.647
	JYD	31 enzymes	53 loci	14 loci	26.42%	0.677
	LMB	33 enzymes	60 loci	8 loci	13.33%	0.512
	MUK	34 enzymes	61 loci	7 loci	11.48%	0.453
	NOP	34 enzymes	61 loci	4 loci	6.56%	0.032
	SMB	33 enzymes	60 loci	7 loci	11.67%	0.268
	WAE	31 enzymes	70 loci	9 loci	12.86%	0.149
	YEP	35 enzymes	66 loci	3 loci	4.55%	0.025
RAPD's	BLG	13 primers	84 fragments	66 fragments	78.57%	0.567
	JYD	13 primers	70 fragments	27 fragments	38.57%	0.902
	LMB	12 primers	71 fragments	34 fragments	47.89%	0.892
	MUK	14 primers	93 fragments	4 fragments	4.30%	0.379
	NOP	14 primers	111 fragments	2 fragments	1.80%	0.239
	SMB	14 primers	81 fragments	7 fragments	8.64%	0.309
	WAE	11 primers	71 fragments	8 fragments	11.27%	0.327
	YEP	15 primers	86 fragments	3 fragments	3.49%	0.461
RFLP analysis of mtDNA	BLG	9 RE's	48 restriction sites	23 restriction sites	47.92%	0.635
	JYD	9 RE's	43 restriction sites	16 restriction sites	37.21%	0.949
	LMB	9 RE's	39 restriction sites	13 restriction sites	33.33%	0.916
	MUK	15 RE's	55 restriction sites	2 restriction sites	3.64%	0.406
	NOP	13 RE's	56 restriction sites	0 restriction sites	0.00%	0.000
	SMB	14 RE's	41 restriction sites	2 restriction sites	4.88%	0.895
	WAE	15 RE's	69 restriction sites	6 restriction sites	8.70%	0.307
	YEP	9 RE's	31 restriction sites	1 restriction site	3.23%	0.000

1 : BLG = bluegill; JYD = johnny darter; LMB = largemouth bass; MUK = muskellunge; NOP = northern pike; SMB = smallmouth bass; WAE = walleye; and YEP = yellow perch.

Phase II

Based upon the characteristics of the various molecules that each of the three molecular technique addresses and the results obtained in Phase I, we chose to employ a combination of protein electrophoresis and RFLP analysis of mtDNA for all Phase II efforts. For this Phase II effort, we analyzed the variation in a total of 87 populations across the six target species within Minnesota (for sample locations, see Figure 1), plus an additional 184 populations outside of Minnesota. With the exception of the southwestern portion of Minnesota, sample coverage across the state was quite extensive. The following is the final list of polymorphic (or interspecifically diagnostic) loci used:

Muskellunge - *G3PDH-1**, *GPI-B**, *IDHP-A**, and *PGDH**.

Bluegill - s*AAT-2; FBP**, *GPI-A**, *IDHP-A**, *IDHP-B2**, *MDH-B**, and *PGM-A**.

Smallmouth Bass - *AH-1**, *LDH-C**, *MDH-B**, *SOD-1*, and *TPI-1**.

Largemouth Bass - *mAAT**, *sAAT-B**, *CK-B**, *CK-C**, *GPI-A**, *GPI-B**, *GLYDH-1**, *IDHP-B1**, *MDH-B**, and *MPI-2**.

Johnny Darter - *AH-1**, *G3PDH-1**, *GPI-A**, *GPI-B**, *IDHP-A**, *IDHP-B1**, *LDH-B**, *PGDH**, *PGM-A**, *SOD-1**, and *TPI-2**.

Walleye - *ADH-1**, *CBP-1**, *IDHP-B1**, *MDH-A**, *MDH-B**, *mMDH**, *PGM-A**, and *SOD-1**.

In addition, the following is the final list of restriction enzymes used for the genetic analyses of each of the six target species:

Muskellunge - *Pst I* and *Sca I*.

Bluegill - *Dde I*, *Dpn II*, *Hae III*, *Rsa I*, and *alpha Taq I*.

Smallmouth Bass - *Xba I*.

Largemouth Bass - *Dde I* and *Dpn II*.

Johnny Darter - *Bstu I*, *Dde I*, and *Msp I*.

Walleye - *Ava I*, *Hinc I*, *Nci, I,* and *Sca I*.

For each of the six target species, we performed the following analytical steps. First, we constructed a genetic distance matrix containing all pairwise population comparisons for that species using each of the two datasets (protein electrophoresis and RFLP analysis of mtDNA) separately. Second, using UPGMA clustering techniques with each matrix, we constructed a dendrogram illustrating the phenetic relationships among the populations for each species. Third, we assessed both resulting dendrograms for geographic patterns in the distribution of genetic variability. Fourth, we compared the geographic patterns produced from the two independent datasets (protein electrophoresis and RFLP analysis of mtDNA) and constructed a map showing the boundaries for the various genetic groupings within Minnesota (Figures 2-7). After these analyses were completed, the six species-specific maps were then combined to form the basis for the recommended set of nine conservation management units (Figure 8).

Phase III

Data were obtained for each of the remaining species (lake trout, lake sturgeon, channel catfish, flathead catfish, white crappie, black crappie, sauger), as per the specific needs of the questions pertaining to each of those species. Analyses were tailored to meet the needs of individual species' questions.

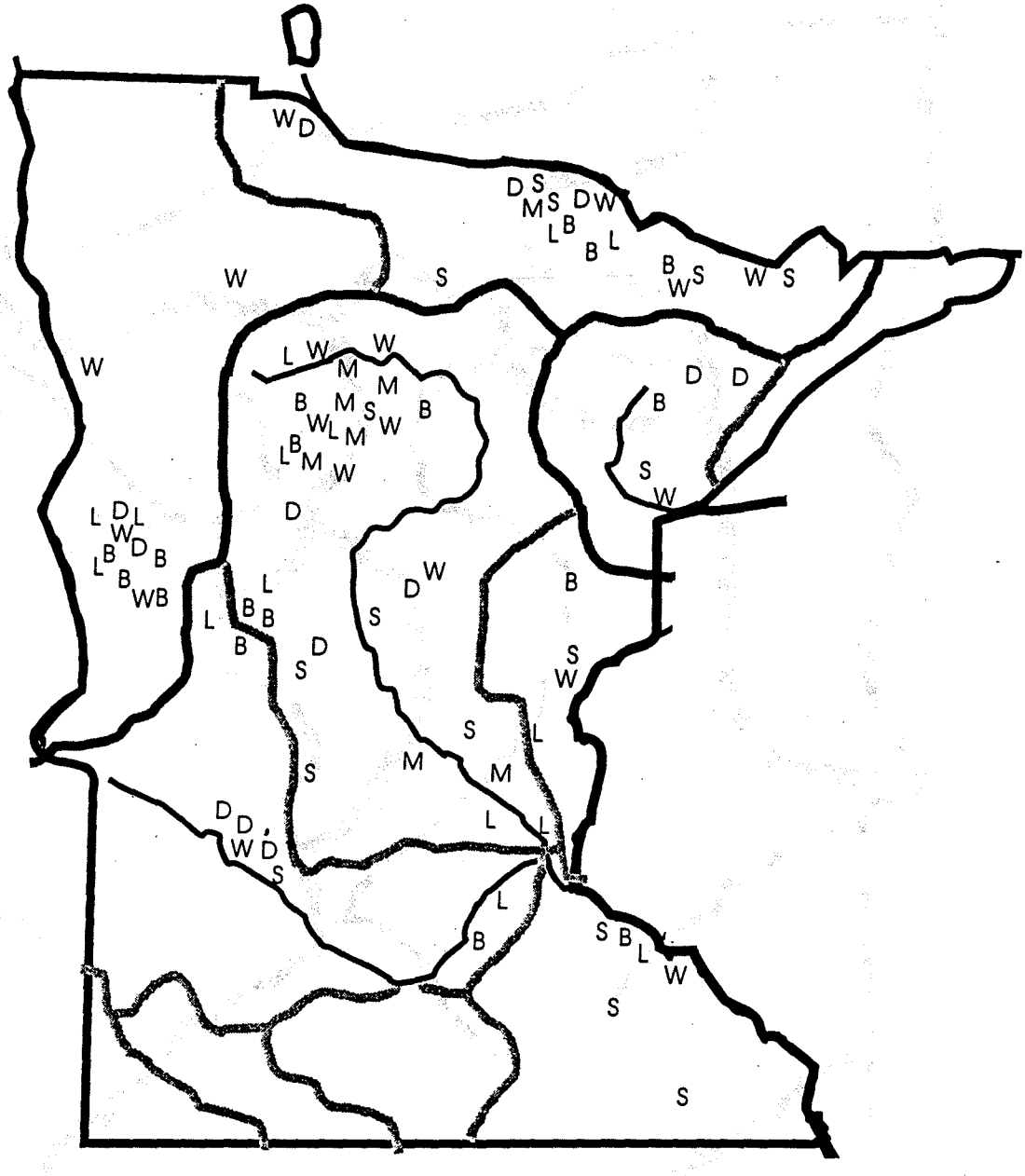

Figure 1. Map of Minnesota sampling locations for six primary species: smallmouth bass (S), largemouth bass (L), bluegill (B), walleye (W), muskellunge (M), and johnny darter (D). Heavy gray lines indicate boundaries of major drainage basins; lighter lines indicate boundaries of selected watersheds.

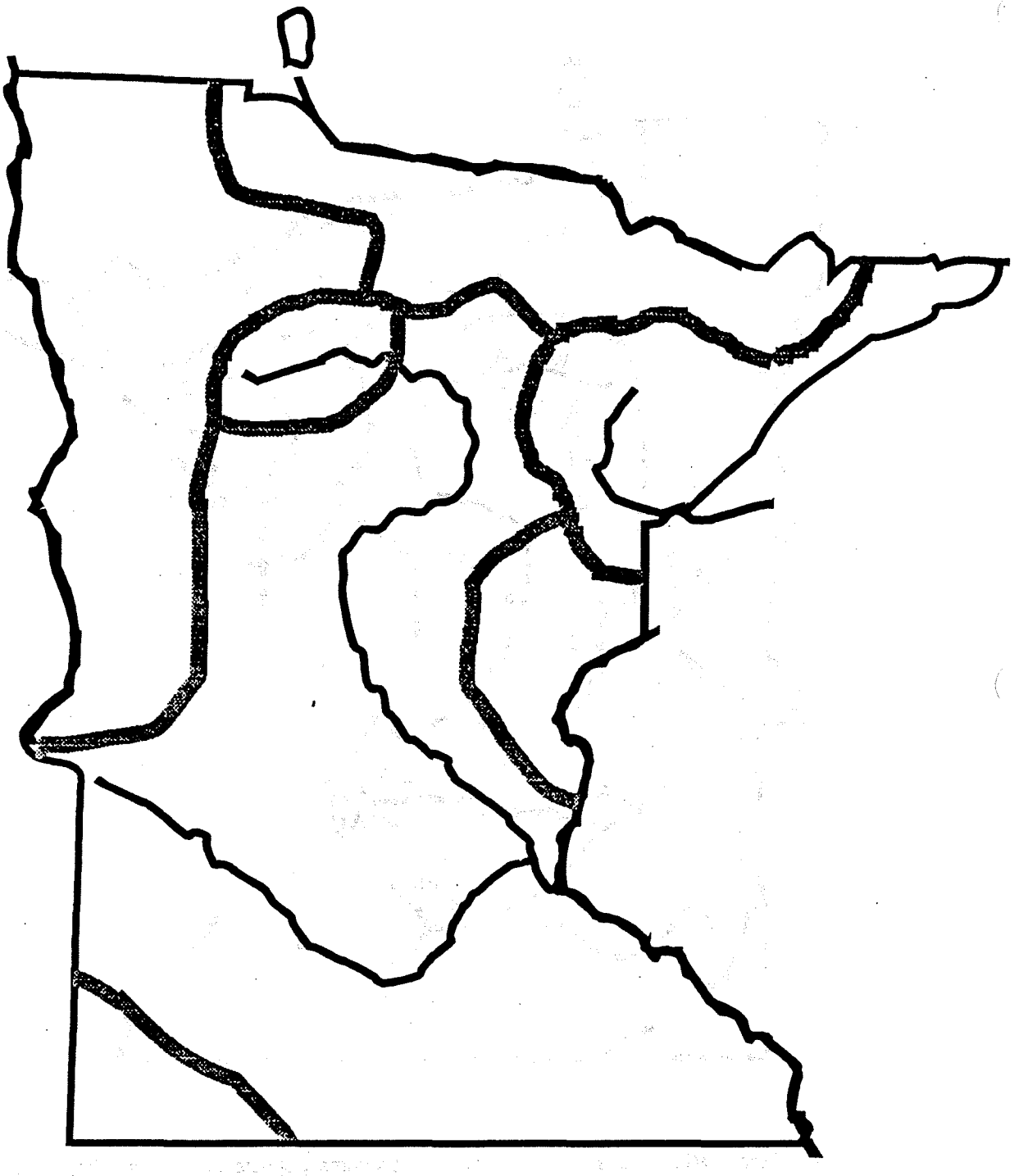

Figure 2. Map of genetic groupings of bluegill populations, based on mtDNA and allozyme analysis.

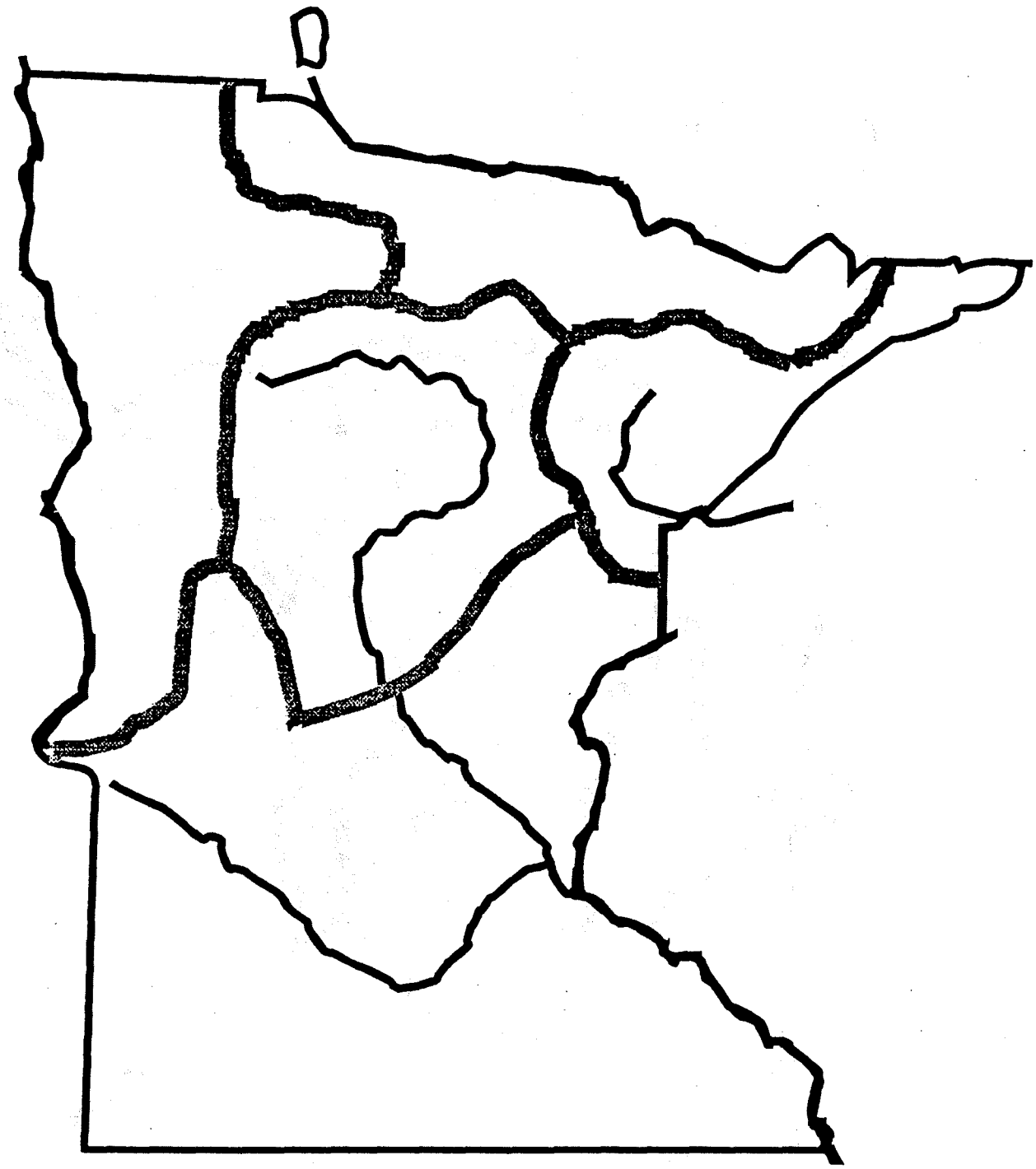

Figure 3. Genetic groupings of muskellunge populations, based on mtDNA and allozyme analysis.

Figure 4. Genetic groupings of smallmouth bass populations, based on allozyme and mtDNA analysis.

Figure 5. Genetic groupings of walleye populations, based on allozyme and mtDNA analysis.

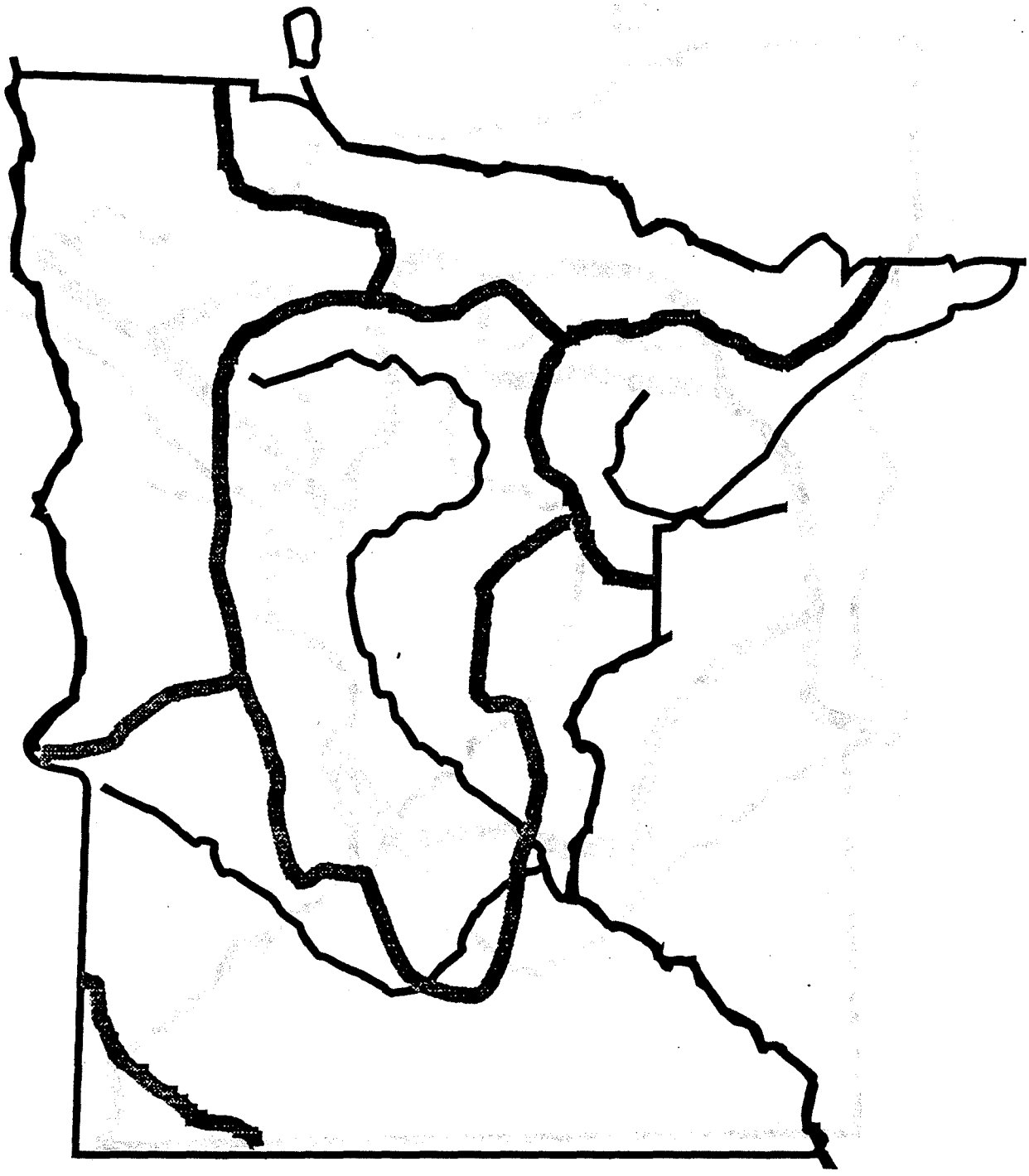

Figure 6. Genetic groupings of largemouth bass populations, based on mtDNA and allozyme analysis.

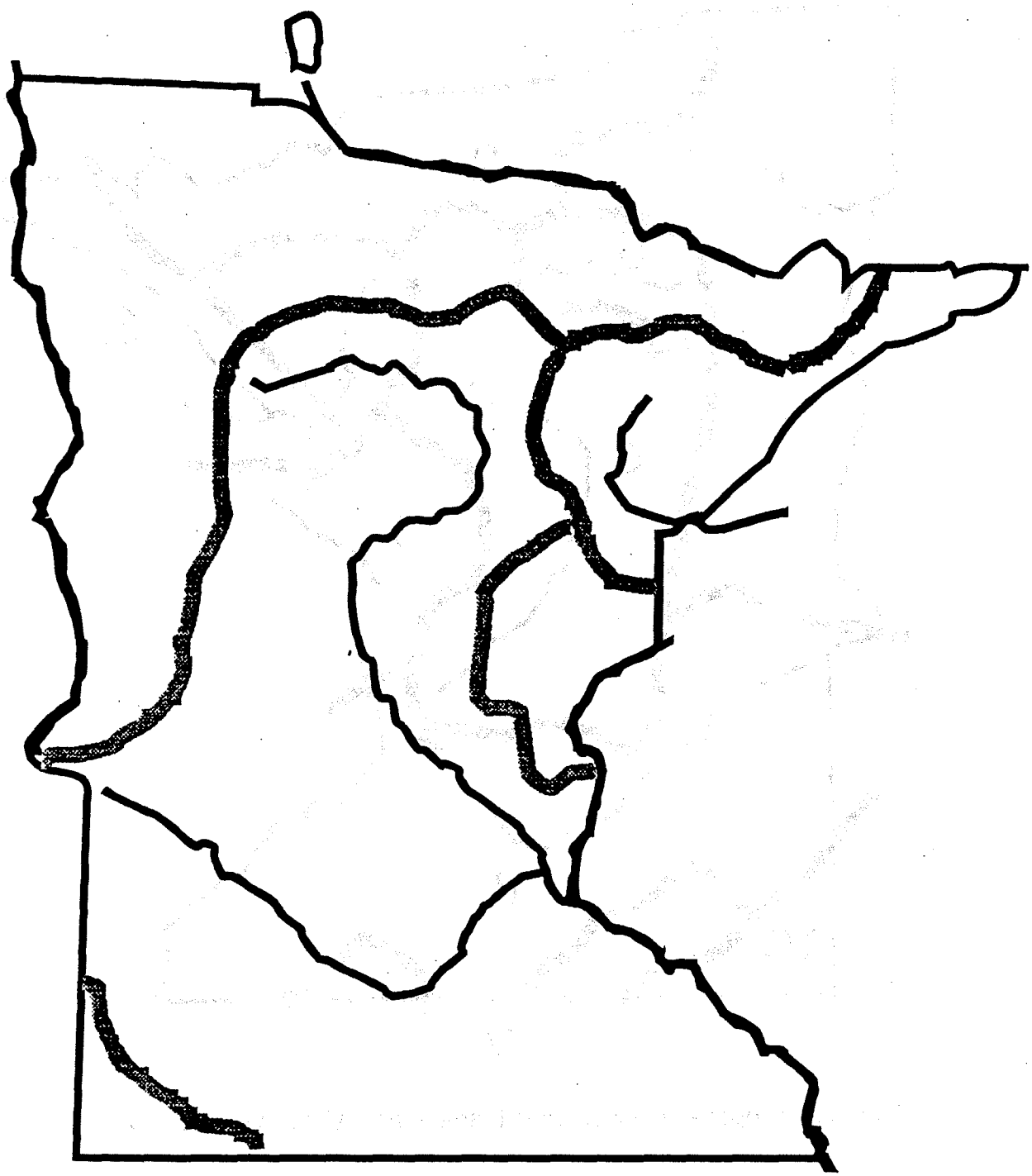

Figure 7. Genetic groupings of johnny darter, based on mtDNA and allozyme analysis.

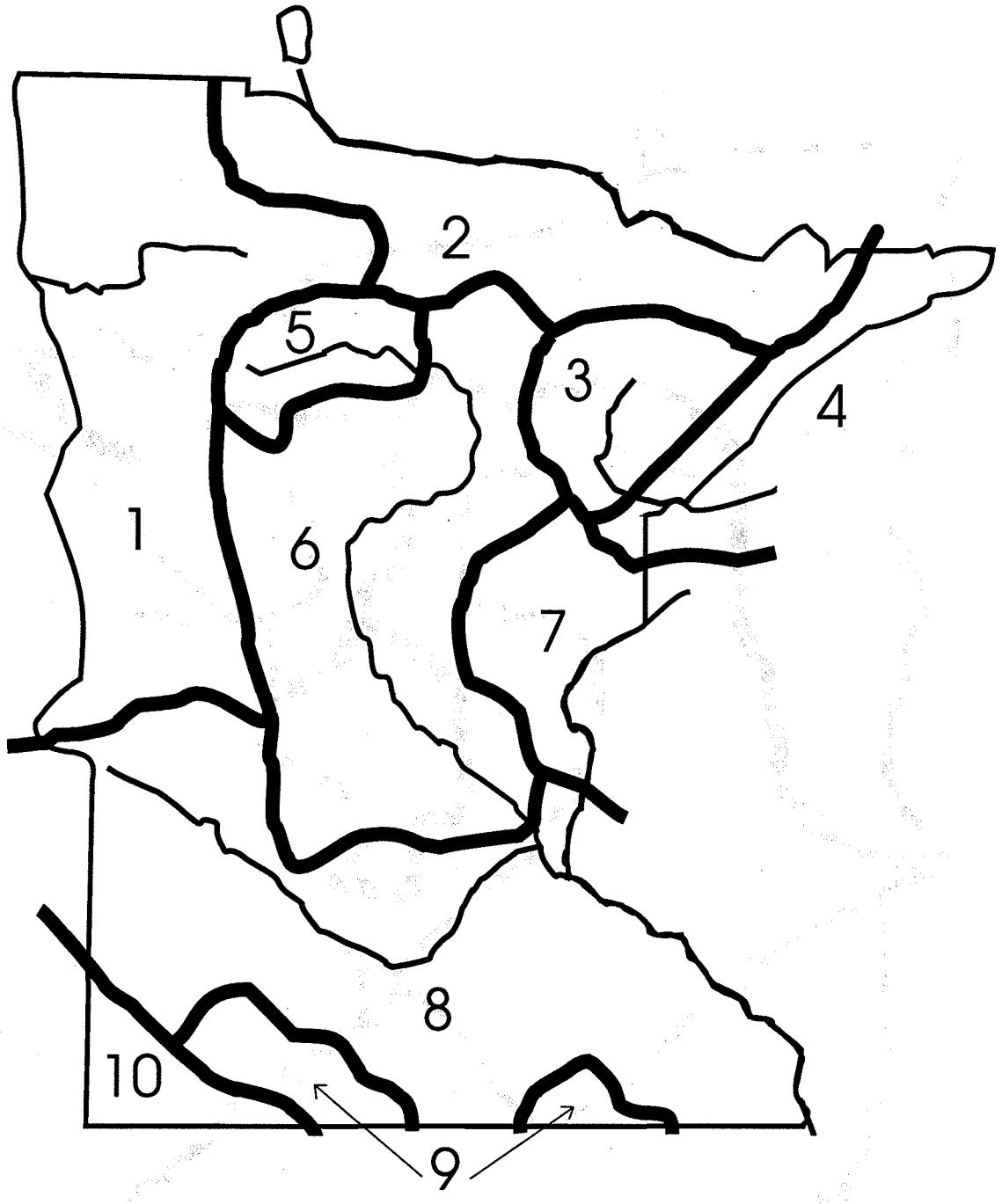

Figure 8. Proposed Conservation Management Units in Minnesota.

Management Recommendations

General Philosophy

Natural barriers to gene flow create an opportunity for differentiation among populations to occur. Two quite different isolating mechanisms can contribute to such a nonrandom distribution of genetic variation. The first mechanism is based upon the isolation among populations that results from the region's historical biogeography. To colonize the upper Midwest, freshwater fish species most likely used open water channels connecting the newly created aquatic habitats of peripheral proglacial waters (Bailey and Smith 1980). Historical watershed boundaries to gene flow may have become connected for periods of time, only to once again become separated. Because the sources for reinvasion of different parts of this region varied during this process, genetic composition of newly colonized populations varied as well. The second mechanism is based upon the spatial isolation of populations caused by the linear connectivity properties of the extant aquatic environment. Short and (rarely) long range dispersal events are limited by distance (Thompson 1931); because of this property, Wright (1943) postulated that immigrants to a population are more likely to come from immediately adjacent localities than random samples of the entire species.

By whatever mechanism, as gene flow among populations becomes and remains limited, the opportunity for differentiation among those populations to occur through the processes of selection and/or drift increases (Mayr 1942, 1982). Genetic differentiation among populations then provides the opportunity for local adaptation, which is thought to result in the formation of different gene combinations or arrangements (coadapted gene complexes) among populations (Dobzhansky 1948). Altering these favorable coadapted gene complexes through introgression (perhaps as a consequence of introductions) reduces the relative fitness of the recipient population (Hindar et al. 1991; Philipp 1991). In addition, because natural selection acts on the heritable phenotypic variation among the individuals found within a population, loss of genetic variation among the

populations within a species through such introgression would clearly restrict that species' ability to respond successfully to changing environmental conditions (Arnold 1987).

Resource management activities that involve the translocation of individuals across broad geographic landscapes often fail to recognize the fact that the genetic variation found within species is not uniformly distributed. Current stocking practices that involve long distance translocations of fish likely have negative effects on recipient populations, for example, disruption of locally adapted gene complexes that have evolved positive adaptive value in the local environment. As such, to develop truly effective management programs for a given region or jurisdiction, it is important to identify the boundaries that delineate the location of each of the component stocks (genetically distinct groups) for each species under consideration. Our project represents joint efforts by the states of Illinois, Minnesota, and Wisconsin to gather the appropriate data needed to identify stocks of a variety of fish species in the upper Midwest, propose a series of Conservation Management Units for that region, and outline a set of recommended management activities to promote the long term conservation of their fisheries resources.

Delineation of Conservation Management Units

Based on the results of the genetic analyses, we have identified distinct stocks for each of the six target species analyzed (see Figures 2-7). Because of the great deal of similarity among the different species in the location of these proposed stock boundaries, and because of the relatively short time available for differentiation to have occurred (since the last glaciation), we believe that the stock structure for many fish species within Minnesota results from a common biogeographic history. As such, we have combined the information from all six target species to propose a set of nine conservation management units within Minnesota (Figure 8), defined as follows:

1. Red River CMU

   The entire Red River drainage plus the headwaters of the Pomme de Terre and Chippewa Rivers from the Minnesota River drainage.

2. Rainy River CMU

   The entire Rainy River drainage, including the Big Fork and Little Fork River drainages.

3. St. Louis River CMU

   The entire St. Louis River drainage, above the Thomsom Reservoir Dam.

4. Lake Superior CMU

   Lake Superior, including all of the North Shore river systems, the lower portion of the St. Louis River (below Thomsom Reservoir Dam) and tributaries to the Allouez, Superior and St. Louis Bays, and including the Nemadji River drainage.

5. Mantrap Plateau CMU

   All waterbodies on the Mantrap Plateau plus the headwaters of the Mississippi River (all waters above confluence with Leech Lake River).

6. Upper Mississippi River CMU

   That portion of the Mississippi River drainage above St. Anthony's Falls up to the confluence with the Leech Lake River, including the Leech Lake, Crow Wing, Crow, and Rum River drainages.

7. Upper St. Croix River CMU

   That portion of the St. Croix River drainage above St. Croix Falls, including the Sunrise, Snake, Kettle, and Tamarack River drainages.

8. Mainstem Mississippi River CMU

   That portion of the Mississippi River drainage below St. Anthony's Falls, including the Minnesota, Blue Earth, Vermilion, Zumbro, Root, and lower St. Croix (below St. Croix Falls), River drainages.

9. Iowa CMU

   Headwaters of the Des Moines and Cedar Rivers.

10. Missouri River CMU

    All river drainages in southwest Minnesota draining into the Missouri River.

Recommended Management Activities

The following is our recommended approach toward the movement of fish (through new or supplemental introduction programs) to meet fisheries management goals. First, each body of water must be classified as to whether or not a given species of interest (1) was native and still exists, (2) was native, but is not now present, (3) was not native, but is now present and reproducing, (4) was not native, but is now present only through continued introduction programs, (5) was not native and is still not present. Second, management decisions pertaining to proposed introductions should be evaluated using the following guidelines (treating each species individually):

For fish populations in Class (1):

1. If there is substantive natural reproduction (adequate to support a self-sustaining population), or if substantive natural reproduction could be accomplished through actions other than stocking, fish should not be introduced from any source.
2. If there is not substantive natural reproduction, and supplemental stocking of additional fish is deemed essential for supporting the population at desired levels, only adult fish from the waterbody of interest should be used as broodstock, unless taking those fish is impossible or that action would harm the resident population. Under the latter scenario, broodstock should then be selected from the (geographically and ecologically) most similar waterbody within the same CMU as the proposed recipient waterbody.

For fish populations in Class (2):

1. If reintroduction is justified and populations of that species still exist within other waterbodies within the CMU, broodstock should be selected from the (geographically and ecologically) most similar waterbody within the same CMU as the proposed recipient waterbody.
2. If reintroduction is justified, but populations of that species no longer exist in any other waterbodies within the CMU, broodstock should be selected from waterbodies within the (geographically, ecologically, and if known, genetically) next most similar CMU. This action may necessitate obtaining broodstock (or production fish) from outside of Minnesota.

For fish populations in Class (3):

1. If the fish from these waterbodies originated from an unknown source or a known source outside of the CMU (a non-native stock or even non-native species):
   a. they should not be used as a source for broodstock for any proposed culture/introduction program;
   b. for a waterbody where there is not substantive natural reproduction, and supplemental stocking of additional fish is deemed essential for supporting the population at desired levels, replacement of this non-native resident stock with a native stock should be encouraged.
2. If the fish from these waterbodies originated from a source within the CMU:
   a. they could be used as a source for broodstock for stocking efforts within that same CMU;
   b. for a waterbody where there is not substantive natural reproduction, and supplemental stocking of additional fish is deemed essential for supporting the population at desired levels, adult fish from the waterbody of interest should be used as broodstock, unless taking those fish is

impossible or that action would harm the resident population; under the latter scenario, broodstock should be selected from the (geographically and ecologically) most similar waterbody within the same CMU as the proposed recipient waterbody.

For fish populations in Class (4):

1. For put-grow-and-take stocking programs, all care should be taken to minimze any risk of unintentional spread of the non-native organism.
2. Whenever possible, fish used for stocking these waterbodies should originate from a source within the same CMU as the waterbodies stocked.
3. In those cases where there is no source within the CMU, yet there is continued high demand and little risk of escapement, if at all possible, broodstock should be selected from the (geographically and ecologically) most similar CMU as the proposed recipient waterbody.

For fish populations in Class (5):

1. New put-grow-and-take stocking programs should not be initiated if they could possibly serve as a risk for unintentional spread of a non-native organism or any associated diseases.
2. In those cases where an extraordinarily high demand justifies the establishment of a new put-grow-and-take fishery, and:

   a. that species is native to the CMU, broodstock should be selected from the (geographically and ecologically) most similar waterbody within the same CMU;

   b. that species is not native to the CMU and there is no risk of unintentional spread of that non-native organism or any associated diseases, broodstock should be selected from the (geographically and ecologically) most similar waterbody from another CMU.

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