Minnesota State Wildlife Grants Program

12 March 2008

Final Report

1. **Project Title:** Distribution, abundance and genetic diversity of the longear sunfish (*Lepomis megalotis*) in Minnesota, with determination of important populations

2. **Principal Investigators:**

Jean Porterfield
St. Olaf College
Northfield, MN 55057
507.786.3850
porterfi@stolaf.edu

Patrick Ceas
St. Olaf College
Northfield, MN 55057
507.786.3560
ceas@stolaf.edu

3. **DNR Liaison:**

Konrad Schmidt
Division of Ecological Resources
Minnesota Department of Natural Resources
500 Lafayette Rd Box 25
Saint Paul, MN 55155
651.297.2565
konrad.schmidt@dnr.state.mn.us

4. **Type of Project:** Research, Wildlife Survey

SUMMARY

The northern longear sunfish (*Lepomis megalotis peltastes*) is recognized by the Minnesota Department of Natural Resources (MN DNR) as a Species in Greatest Conservation Need (SGCN) due to its extremely spotty distribution in Minnesota (known only from 26 lakes) and its threatened status in Wisconsin. This study, funded primarily by the Minnesota State Wildlife Grant (SWG) program and by a smaller amount from the Chippewa National Forest (CNF), serves as an initial effort to begin to understand the distribution, habitat requirements, relative abundance, and genetic variation of the longear sunfish in Minnesota. A total of 119 lakes and one river were visited during June–August 2006, and June, August, and October 2007. Seventeen of the 26 historical lakes were sampled. Longear sunfishes were found in 23 bodies of water (22 lakes or major bays and one river). Twelve of these waters represent new distributional records for Minnesota, and 11 serve as confirmation of historic records.

Longear sunfishes were quite restricted in their habitat use, which included high-quality waters with shorelines that have relatively undisturbed stretches of emergent aquatic plants, extensive shallows (< 3' depths), and a firm substrate that was usually rich with organic detritus and submerged plants. Individuals live in these shoreline shallows at least during the warmer months of the year (May-October). During the summer spawning season the colonies of saucer-shaped nests can be found within or nearby the emergent plant beds, and often right next to the shoreline. The species is rarely found in deeper waters, even if these waters support large populations of other sunfish species. Lakes that did not produce longears did not possess the combination of habitat traits that longear sunfishes seem to require. Although we characterized some populations as being "abundant" in terms of the numbers of individuals within a lake, in no lakes did the density of longears ever approach that found for bluegills or pumpkinseeds, which were always more abundant and widespread.

The number of lakes/streams from which the longear sunfish has been collected now stands at 37, and we recognize eleven geographic clusters of populations within Minnesota. This number, although likely to rise when additional collecting occurs, still represents a tiny fraction of the waters within Minnesota. Until such time that the longear sunfish is found to be widespread and common throughout its range, or that the species can be shown not to be under any danger of population decimation/extirpation, this species must remain listed as a Species in Greatest Conservation Need in Minnesota. We further suggest that, until more is known about the species, all 37 populations should be considered "important" in terms of management decisions. We offer some additional subdividing/ranking of these populations based on the current and perceived threats to the lakes/habitats, and divide the populations into the following four categories: "Most Secure," "Uncertain," "Concern," and "Most at Risk."

Given the number of lakes that contain potentially "good" habitat, we expect that continued sampling will result in the discovery of additional new populations of longear sunfishes, and will allow for an even greater understanding/baseline of the distribution and relative abundance of the species. Furthermore, since this is a species whose decline can be used as an indicator of a water body's health, once the baseline data has been gathered then long-term monitoring will identify any downward trends in populations within a given water body. These trends can then be used to inform managers that a particular lake/stream may be experiencing a decrease in water/habitat quality. Decreases in populations have already been observed in lakes where the shorelines have been be modified extensively.

We addressed the amount and distribution of genetic variation in Minnesota's *L. megalotis* populations using 645 base pairs of mitochondrial cytochrome *b* sequence (108 individuals) and four nuclear microsatellite loci (301 individuals). Analysis of both data sets showed lower levels of variation in the upper Midwest compared with the unglaciated south, and that Minnesota's sampled genetic variation likely consists of widespread ancestral variation with some potential locally evolved differentiation especially in the Mississippi Headwaters, Crow Wing River, and Otter Tail River Watersheds. Average heterozygosities calculated from the microsatellite data were fairly uniform across the 18 Minnesota collection sites, with the exception of lower heterozygosities in the two Voyageurs National Park sites. With respect to sources for potentially stocking or supplementing managed populations in the future, we recommend maintaining watershed fidelity.

INTRODUCTION

NEED AND JUSTIFICATION FOR THIS STUDY

The northern longear sunfish (*Lepomis megalotis peltastes*) is recognized by the Minnesota Department of Natural Resources (DNR) as a Species in Greatest Conservation Need (SGCN) due to its extremely spotty distribution in Minnesota and its threatened status in Wisconsin. Excluding two uncertain records (Cedar River, Dodge/Mower counties, 1932; and Keller Lake, Ramsey County, 1978), populations of longear sunfish have been found in only three of the eight major MN basins as identified by the DNR (http://www.dnr.state.mn.us/watersheds/map.html). Records within the three basins are limited to only nine watersheds, and within these nine watersheds the species has been collected from just 26 lakes (Map 1, Appendix A; Table 1, Appendix B). These lakes are found in a roughly triangular area from some northern border lakes in/near Voyageurs National Park (VNP) & Superior National Forest, southwest to the Whitefish Chain area, and west-northwest to the Becker/Mahnomen/Clearwater County junction, which includes the entire Chippewa National Forest (CNF). Each of these 26 lakes is identified on one of four maps (Maps 2, 4, 6, and 8, Appendix A).

All but four of these lake populations have been discovered within the past 20 years (Table 1), and in most cases we know little more than the fact that the species was present in the lake. There has been no effort to identify the species' habitat requirements within Minnesota. Given the potential to overlook longear sunfish during game fish surveys (the species does resemble the better-known pumpkinseed sunfish, *Lepomis gibbosus*), the distribution and abundance of longear sunfish in Minnesota is likely underrepresented in the historical database (K. Schmidt, pers. comm.). We suspected that additional sampling would uncover more populations.

The DNR's publication titled *Tomorrow's Habitat for the Wild and Rare: An Action Plan for Minnesota Wildlife*, *Comprehensive Wildlife Conservation Strategy* (CWCS; http://www.dnr.state.mn.us/cwcs/strategy.html) lists seven categories of Priority Conservation Actions (CWCS, page 36). Included are general strategies for habitat management, species management, surveys, and research. In the draft version of the CWCS (under which this project was funded), Priority Conservation Goal I, Problem 2, Action A calls for the management of "important" SGCN populations. The CWCS indicates that important populations could be those that are found in high-density clusters, contain large numbers of individuals, or are associated with high quality habitats. We would add that genetic biodiversity and/or uniqueness is another key factor in identifying important populations for management purposes.

There does not exist enough information to designate important populations of *L. m. peltastes*, thus management goals cannot yet be defined or achieved. To help identify important populations, CWCS Priority Conservation Goal II ("Improve knowledge about SGCN") Strategy II A calls for the survey and inventory of SGCN populations and habitats. Furthermore, Strategy II B calls for research studies to gather information on life history and habitat requirements. Thus, we proposed that there is a need for: 1) extensive and thorough surveys with an eye towards developing an understanding of the species' habitat needs and basic life history traits, and 2) an assessment of genetic biodiversity and uniqueness within and among populations. Below we elaborate on these two needs.

GOAL AND OBJECTIVES OF THIS STUDY

This study was undertaken to serve as an initial effort to gather baseline data so that we can begin to understand the distribution, habitat requirements, relative abundance, and genetic variation of the longear sunfish in Minnesota waters.

Given the survey and research needs as detailed in the previous section, our project had the following five objectives:

- ***Is the Longear sunfish a Native Species?*** Given its very limited distribution within Minnesota, and that nearly all of the records have been found within the past 20, there was some concern within the DNR that the longear sunfish could be an introduced species rather than a native member of the fish fauna, and not qualify as a SGCN. Therefore, our first priority was to determine if the species is native to Minnesota, or alternatively might have been introduced from other regions of the country.
- ***Fish Surveys***. Conduct thorough surveys of a subset of the lakes that contain historical records of longear sunfish, and survey candidate lakes to an attempt discover previously unknown populations. Qualitatively assess population sizes and overall condition of populations in all lakes sampled; quantifying population sizes was beyond the scope of this project. Document additional SGCN fish that are encountered.
- ***Lake Conditions***. Document qualitative observations on habitat type/condition for all SGCN fish encountered, and human development/use in all lakes sampled in relation to the presence/absence of longear sunfish.
- ***Population Genetics***. Characterize the amount and geographic distribution of genetic diversity within and among Minnesota *L. m. peltastes* populations, and advise about appropriate practices for transferring longear sunfish among populations should supplementation and/or restoration become necessary.
- ***Recommend Important Populations***. Synthesize the above information on population size and distribution, habitat quality, and genetics to recommend potential important populations for management.

METHODS

(refer to Appendix A for Maps; Appendix B for Tables; and Appendix C for Figures)

SELECTION OF LAKES

Lakes were chosen using two methods: (1) the MN DNR "Lake Finder" database (http://www.dnr.state.mn.us/lakefind/index.html) was used to identify those lakes that might have a potentially good combination of shallow shoreline depths, emergent aquatic plants, and water quality; and (2) field reconnoitering of lakes determined if sampling was warranted in these selected lakes. Identification of the Major Watersheds and basins follows the delineation outlined by the MN DNR (http://www.dnr.state.mn.us/watersheds/map.html). Lakes were sampled from all three of the historic basins and all nine historic Major Watersheds.

Additional Lakes Included in this Report. In 2007 a contract from the Chippewa National Forest (CNF) was awarded to Ceas & Porterfield to sample 12-15 lakes within the CNF to further our understanding of the distribution and population status of the longear sunfish in the CNF. SWG-funded sites sampled in 2006 and planned for 2007 naturally included lakes within the CNF. The funding received from the CNF in 2007 was intended to compliment the SWG-funded sampling efforts within the CNF. As often is the case with cooperative funding efforts, the combined 2007 funding from SWG and CNF allowed for a synergistic level of lake sampling, and more lakes within the CNF were surveyed than would have been possible if only one of the two projects were funded. Therefore, in the spirit of cooperation among government agencies, the results of the sampling efforts from the SWG-funded project were included in the CNF final report. In a reciprocal agreement the results of the CNF-funded efforts are included in this SWG final report.

FISH AND HABITAT SURVEYS

Fish Surveys. Initial efforts included sampling a variety of habitats and depths, but sampling focused on shoreline areas with wide shallows (e.g., depths of 3 feet or less even at distances often exceeding 150' from shore) because we quickly determined that this is where longear sunfish live. The field sampling methods relied almost exclusively on using small-mesh minnow seines, including a 5' x 30' bag seine, and a 5' x 20' minnow seine. Backpack electrofishing and boat electrofishing were used sparingly because of safety concerns brought about by weather conditions (see "Weather Conditions" below), and because seining alone proved to be a highly effective and efficient means of catching longear sunfish. We were assisted in the field at various times by the following persons: C. Cook, E. Onuma, and D. Schackman (USFS); A. Plain (MN DNR); J. Brown, C. Eggebroten, S. Ellingson, D. Gruner, J. Morrison, and J. Rolfes (St. Olaf College summer research students); and P. Jackson (St. Olaf College Dept. Environ. Studies).

Sampling was concentrated in June & July each year since those months represented the putative spawning season, and since this is the easiest time of the year to find individuals, which would at least allow us to determine presence/absence within lakes. Additional sampling took place in August and October to obtain late Summer/Fall data concerning seasonal movements, and to obtain specimens for food habits analysis (to be incorporated into a future life history study).

Shorelines around a particular lake were either sampled or reconnoitered, and survey efforts continued until the species was found or, in the professional judgment of P. Ceas, it was reasonably determined that the species was not present in appreciable numbers. In general, when the species was present in a lake it was not difficult to locate and capture individuals, regardless of the calendar date (and assuming decent weather conditions – see discussion below), since the species lives in a fairly well-defined habitat.

Habitat Assessment. No effort was made to quantify habitat characteristics since this was beyond the scope of the project. We did take notes on shoreland development, substrate type, depth, general water quality, and aquatic vegetation that were used to develop an overall picture of the preferred habitat of the longear sunfish.

Abundance. No attempt was made to quantify the numbers of individuals per unit area or catch per unit effort since such an effort was beyond the scope of this project. We did, however, assess qualitatively whether the species was "abundant," "common," or merely "present." Since sampling efforts were focused during the breeding season, the species was considered "abundant" if numerous breeding colonies were located within a lake, "common" if only a few breeding colonies were located, and "present" if only lone individuals were located.

Vouchers. Voucher photographs were taken, and preserved specimens will be deposited in the permanent holdings of the University of Minnesota Bell Museum, where they will be available to researchers for study.

Weather Conditions. The 2006-2007 field seasons were characterized by an ongoing drought in Minnesota. Low water levels in lakes prevented us from launching our boat on numerous occasions; these lakes, identified in Table 2 and in the Results should be sampled at a later date.

The Summer and Fall of 2007 further proved to be one of somewhat problematic sampling conditions in northern Minnesota. Strong winds (often accompanied by heavy localized rains) frequently blew across the lakes, making boating a risky endeavor during many days while in the field. Anecdotal comments from long-time residents and fisheries personnel indicated that 2007 was a most unusual year in terms of the frequent strong winds. These windy conditions certainly affected the daily location/movements of fishes, our ability to travel on lakes during such conditions and sampling success, which is why some lakes that were visited in 2007 (Table 2; and in the Results) need to be resampled to confidently determine the absence or (if present) the relative abundance of longear sunfish.

GENETICS

Choice of Markers. We assessed genetic variation using two types of molecular markers: mitochondrial DNA sequences and nuclear microsatellite loci. Both markers have the potential for relatively rapid evolution and thus for accumulating differences even between recently divergent populations. Mitochondrial DNA generally exhibits a higher substitution rate than nuclear DNA, especially at near-neutral base positions such as those in the third codon position of this genome's protein-coding genes. We chose to sequence the gene encoding the mitochondrial cytochrome *b* protein because of the availability of GenBanked *L. megalotis* (and other centrarchid) sequences for comparison. Nuclear microsatellite loci are regions of the nuclear genome exhibiting a repeated DNA element (e.g., the tetranucleotide repeat $AGAT_n$). The source of genetic variation in microsatellite markers is DNA fragment size differences due to differences in the number of repeats in an allele. The insertion/deletion mutation rate, largely due to replication slippage, is relatively high in these repeats, and microsatellite regions are generally thought to be selectively neutral. Together, these data sources (mitochondrial and microsatellite) provide a suite of independent estimates of genetic variation in *L. megalotis*.

Sampling & DNA Isolation. Sampling of *L. megalotis* for genetic purposes was conducted concurrently with the lake sampling described above. At each site harboring an *L. megalotis* population, up to 24 (usually 20) individuals were fin-clipped (tip of upper lobe of caudal fin) and the tissue was stored in 95% ethanol. At these sites, fin clips from *L. gibbosus*, *L. macrochirus*, and possible hybrids were taken as well. Fin clips were obtained from the Black River, Missouri and from three Wisconsin localities by Konrad Schmidt (MN DNR), Robert Hrabik (MO Dept. Conserv.), Roy Weitzel (MPCA), and John Lyons (WI DNR), and from three Illinois localities by aquarium hobbyist Uland Thomas. The DNEasy extraction kit (Qiagen) was used to extract total genomic DNA from each fin clip. DNA was isolated from individuals from all populations where longear sunfish were collected (see Table 2). Five previously published mitochondrial cytochrome *b* sequences of *L. megalotis* were downloaded from GenBank (Harris et al. 2005, GenBank accession numbers AY828973-AY828977).

Mitochondrial DNA Sequencing. The genomic DNA preps were used as template in PCR amplification of a portion of the cytochrome *b* gene. Preliminary sequence was amplified using the forward and reverse primers from Song et al. (1998). These sequences were then used along with previously published *L. megalotis* sequences (Harris et al. 2005) to design a new primer pair: LmegCytb1F {5'-ATG GCA AGC CTA CGA AAA ACC C} and LmegCytb702R {5'-GCT GCA AAG CCA AGG AGG TCT TTA}. The optimized conditions for each 50 µl PCR amplification using GoTaq DNA polymerase (Promega) were: reaction buffer at 1X, 1.5 mM $MgCl_{2,}$ 0.2 mM dNTP, 0.4 uM each primer, 2.5 units of Taq polymerase, and 100 ng of template DNA. Thermocycling conditions were: initial denature at 94ºC (3 min); 35 cycles of 94 ºC (40 sec), 52ºC (60 sec) and 72ºC (90 sec); a final extension at 72 ºC (10 min). PCR products were cleaned using the QIAQuick Purification kit (Qiagen) and then diluted to 28 ng/ul for commercial sequencing at Northwoods DNA (Solway, MN). Primer LmegCytb1F was the sequencing primer used, and a total of 108 sequences representing 20 sites were sequenced.

Mitochondrial DNA Analysis. MacClade 4.08 (Maddison and Maddison 1992) was used to characterize codon position data and amino acid sequence. Multiple alignments were conducted with ClustalW (Thompson et al. 1994) and checked by eye. ClustalW was also used to calculate the uncorrected p-distance, transition rate, and transversion rate for all pairwise comparisons. These values were calculated in two ways, first as one large group including all 108 sequences, and second divided into two geographic groups (12 sequences from Missouri and Tennessee, and 96 sequences representing all other locations sampled). A heuristic search algorithm in PAUP* (Swofford 2000) was used to hypothesize relationships among the mitochondrial haplotypes using maximum parsimony criteria, and TCS (version 1.13, Clement et al. 2000) implemented parsimony criteria to construct haplotype networks.

Optimization of Microsatellite Loci. Because it was not optimal for us to develop new species-specific microsatellite locus primer pairs for *L. megalotis*, we screened nine published primer pairs developed for *L. marginatus* (Lmar1, Lmar8, Lmar9, Lmar10, Lmar11, Lmar12, Lmar14, Lmar16, Lmar18; Schable et al. 2002), and four published primer pairs developed for *L. macrochirus* (Lma21, Lma29; Colbourne et al. 1996: Lma116, Lma120; Neff et al. 1999).

Amplification & Visualization of Microsatellite Loci. To visualize microsatellite variation we used a LiCor 4300 DNA Analyzer that detects infrared fluorescence at both 700 nm and 800 nm. In order to fluorescently label any given PCR-amplified microsatellite region with one of these two tags, we used a tailed primer approach. In this approach, a forward and reverse flanking primer are both used, but one of the two primers contains an extra sequence at the 5' end. This extra sequence is complementary to an M13 sequence rather than to any *L. megalotis* sequence, thus it forms a "tail" hanging off when the primer is bound to complementary genomic DNA. After the first cyle of PCR, any new product synthesized from elongation of this bound primer includes this "tail" sequence, so after a second PCR cycle some new product will include its complementary sequence. There is a third primer included in the reaction mix: it is composed of the same M13 sequence as the "tail" and is labeled with a fluorescent tag. In all remaining PCR cycles, a subset of the fragments are primed with this third M13 primer and thus become labeled for visualization. This approach greatly reduced primer costs as we only needed to purchase expensive labeled M13 primers that could be used with all of the inexpensive unlabeled locus-specific primer pairs. LiCor markets two different M13 primers, each available with either a 700 nm or an 800 nm tag, so we duplexed our PCRs when possible (Lmar10 with Lmar12, and Lmar11 with Lmar14). We ran 20 µl PCRs, and while conditions were similar to those used for amplification of mitochondrial DNA, each reaction differed with respect to primer concentration and annealing temperature in a touchdown program (see Table 3). Reaction products were separated using polyacrylamide gel electrophoresis on the LiCor 4300 DNA Analyzer, and Saga software (LiCor) was used to manually call alleles.

Microsatellite Analysis. Microsoft Excel was used for compiling and graphing allele frequency statistics, including the Shannon-Weaver diversity index for each locus. Excel was also used for data organization for constructing other programs' input files. Convert (Glaubitz 2004) was used to construct both Arlequin (version 2.0, Schneider et al. 2000) and Microsat (HPGL, Stanford University) input files. Arlequin was used to perform the following population genetic tests: linkage disequilibrium between loci (Slatkin and Excoffier 1996), Hardy-Weinberg genetic equilibrium (Guo and Thompson 1992), and analysis of molecular variance (AMOVA, Weir 1996). Microsat was used to identify unique population-specific alleles, to calculate pairwise chord distances (Cavalli-Sforza and Edwards, 1967), and to calculate average heterozygosities by locus and overall. PAUP* (Swofford 2000) was used to construct neighbor-joining trees based on the chord distance matrices. These calculations and analyses were performed on four data sets that differed in population groupings: the first grouped all 301 individuals by collection site, the second by Major Watershed, the third by Basin, and the fourth grouped the 289 Minnesota samples into their 18 collection sites. Not all calculations and analyses were performed on each of the four data sets.

RESULTS & DISCUSSION

(refer to Appendix A for Maps; Appendix B for Tables; and Appendix C for Figures)

THE LONGEAR SUNFISH IS NATIVE TO MINNESOTA

A primary goal of this project was to determine if the few Minnesota populations of longear sunfish are native to the state, or alternatively have been introduced from other regions of the country. After examining all specimens that were captured it is the professional judgment of P. Ceas that populations found in Minnesota are representative of the northern longear sunfish (*L. m. peltastes*), and therefore are native to Minnesota. This conclusion (P. Ceas, unpublished data) is the result of a study of the morphology (coloration, body proportions, meristics) of the specimens compared to specimens from south of Minnesota that represent individuals of the subspecies *L. m. megalotis*. Some diagnostic characteristics that are useful in identifying *L. m. peltastes* vs. *L. m. megalotis* are included in Figure 1. In their recently published *Michigan Fish Atlas*, Bailey *et al.* (2004) elevated *peltastes* to species level. However, they did not include any data to support this move. Even though it is a widely held belief that the *peltastes* form does represent a distinct species, we will continue to treat it as a subspecies until a systematic study has been published.

IDENTIFICATION: LONGEAR SUNFISH VS. OTHER MINNESOTA SUNFISHES (See Figures 2-5)

The longear sunfish can be distinguished in the field from the other Minnesota sunfishes (bluegill, pumpkinseed, green sunfish) by the following characteristics:

Morphology:

- Pectoral fin is short, and the tip is rounded
  (compare to bluegill and pumpkinseed, in which the fin is long, and the tip is moderately to sharply pointed; when bent forward and pressed flat against the body the tip of the fin will extend to or beyond the anterior rim of the eye).
- Mouth is small, upper jaw does not extend beyond anterior rim of eye
  (compare to green sunfish, which has a moderately large mouth, upper jaw does extend beyond anterior rim of eye).

Coloration:

- Cheeks & opercles with wavy blue lines in longears and pumpkinseeds, but anal fin of longears is orange/red with distal margin dusky blue
  (compare to pumpkinseed, which lacks the dusky blue margin).
- Opercle ("ear") flap greatly elongated in adults, dark; entire margin outlined in red
  (compare to pumpkinseed, in which the flap is not greatly elongated, with red spot but margin not completely outlined in red).

Size:

- Adult longear sunfish in Minnesota have a small body size when compared to other sunfish species. Adult longear sunfish readily fit in the palm of one's hand (see Figure 3), and the breeding male's coloration is much more brilliant than a comparable-sized juvenile pumpkinseed, bluegill, or green sunfish (see Figures 4-5).

Habitat Preferences of Longear Sunfish

It was beyond the scope of this project to conduct a quantitative assessment of habitat variables. However, using an assortment of gear (seines, electrofishing, and data from DNR trap nets sampling) and sampling a variety of habitats & depths we were quickly able to determine the "key" habitat type of the longear sunfish. This habitat type held throughout the entire sampling periods in 2006 & 2007.

Lake Habitat. With the exception of the one known Turtle River locality, longear sunfish in Minnesota are associated with deep lakes (i.e., depths > 15') possessing the following characteristics:

- high water quality,
- shorelines of relatively undisturbed stretches of emergent aquatic plants such as bulrush (*Scirpus sp*.) combined with extensive shallows (e.g., the water may be only 3' deep at distances of 150' from shore – see Figures 6 & 7). See Figure 8 for an example of a bathymetric map of a lake that contains extensive shallows (longears present) and shoreline bottoms that quickly become too deep for longear sunfish.
- substrates that are generally a firm mixture of sand/marl/silt and often "carpeted" with submerged plants such as bushy pondweed (*Najas flexilis*), coontail (*Ceratophyllum demersum*), flat-stem pondweed (*Potamogeton zosteriformis*), Canada waterweed (*Elodea canadensis*), and muskgrass (*Chara sp*.).

Individuals live in these shoreline shallows at least during the warmer months of the year (May-October). During the summer spawning season the colonies of saucer-shaped nests can be found in clearings within or nearby the emergent plant beds, and often right next to the shoreline (Figure 6). The species is rarely found in deeper waters, even if these waters support large populations of other sunfish species.

Some lakes may superficially appear to provide suitable habitat since these lakes have a combination of extensive shallows & bulrush beds; however, instead of having a substrate of mixed sand/silt/marl these lakes have a substrate of quite "clean" sand (low quantities of organic matter). Such "sand lakes" often have large numbers of fishes in the shallows (mainly perch, bluegill, pumpkinseed, and banded topminnow) and are clearly high-quality waters, but these lakes do not appear to support populations of longear sunfish. See Figure 9 for examples.

River Habitat. Longear sunfish were found at one locality in the Turtle River just within the western boundary of the CNF (at CR 207, known locally as Three Culverts Rd). Turtle River at CR 207 can be characterized as a moderately-flowing stream of exceptional water clarity & quality. The margins of the stream (Figure 7, bottom photo) were lined with wild rice and bulrush beds, and well-defined shallow-pool habitats were dispersed along these margins. The species was considered abundant within these pools, but its distribution within the river appears to be severely restricted to these pools; longears were not found in area with current.

Lakes/Streams Visited

A total of 119 lakes (or major bays within a lake) and one river were visited during June–August 2006, and June, August, and October 2007. The Turtle River just within the western boundary of the CNF (at CR 207, known locally as Three Culverts Rd, and at Hwy 22) was sampled. Lakes were sampled in all three of the Basins and all nine of the watersheds that include historical records of longear sunfish. Seventeen of the 26 historical lakes were sampled.

The yellow rectangles on Map 1 encompass the general regions in Minnesota where these lakes can be found. Maps 3, 5, 7, 9, and 10 provide a closer view of where the lakes are located within the state. The areas/watersheds represented on each map include the following:

Map 3: Becker Co./Park Rapids Area. Includes lakes within the Otter Tail River Watershed (Red River of the North Basin), and Crow Wing River Watershed (Upper Mississippi River Basin). Sites 1-13 (Table 1) are found on this map.

Map 5: Chippewa National Forest & Nearby Areas. Includes lakes within the Big Fork & Little Fork River Watersheds (Lake of the Woods Basin), and the following Watersheds within the Upper Mississippi River Basin: Mississippi River (Grand Rapids), Mississippi River (Headwaters), and Leech Lake River. Sites 14-23 (Table 1) are found on this map.

Map 7: Pine River System. Includes lakes within the Pine River Watershed (Upper Mississippi River Basin). Sites 24-98 (Table 1) are found on this map.

Map 9: Voyageurs National Park & Area; Boundary Waters Canoe Area Wilderness. Includes lakes within the Lake of the Woods Basin. Sites 99-116 (Table 1) are found on this map.

Map 10: Lakes along Echo Trail Corridor (Hwy 116) through BWCA. Includes lakes within the Lake of the Woods Basin. Sites 117-120 (Table 1) are found on this map.

Table 2 includes the sampling results from lakes that are found on Maps 3, 5, 7, 9, or 10, and arranges the lakes hierarchically first by map, and then by Major Watershed – Basin within each map. Lake surveys were considered completed or incomplete depending on whether we were able to reasonably determine the presence/absence of *L. m. peltastes* in the lake.

COMPLETED SURVEYS

A lake was deemed to be satisfactorily sampled if: (1) longear sunfish were found, or (2) after sufficient sampling, it was reasonably determined, in the professional judgment of P. Ceas, that the species was not present in appreciable numbers. This conclusion was reached primarily by failing to locate spawning colonies. Such completed surveys were documented for 91 of the 120 bodies of water visited.

Lakes with Longear Sunfish. Longear sunfish were found in 23 bodies of water (includes lakes, rivers, and significant bays of large border lakes). Longears were found in all three of the historic Basins, and eight of the nine Major Watersheds (Hustler Lake, Rainy River/Headwaters was not sampled). Twelve of these waters represent new distributional records for Minnesota, and 11 serve as confirmation of historic records. Photos of some representative lakes are included in Figures 6 & 7.

New Records. Longear sunfish were found in 12 bodies of water that represent new distributional records for Minnesota. New lakes include: Many Point (Site #4), Potato (12), Turtle (25), Movil (26), Ten Mile (60), Girl (67), Eagle #2 (87), Balsam (96), Junction Bay of Namakan Lake (105), and Staege Bay (111) and Feldt Channel of Sand Point Lake (112). The Turtle River @ CR 207 (31) contained an abundance of shallow pool habitats, and represents the first documented occurrence of longear sunfish in a stream in Minnesota. Paul Radomski, MN DNR, reported the single individual from Ten Mile Lake in 2006.

Eagle Lake #2 Big Fork Watershed), and Junction Bay, Staege Bay and Feldt Channel Rainy River [Lake] Watershed) fall within the Lake of the Woods Basin. Six lakes are in the Upper Mississippi River Basin and include Potato (Crow Wing Watershed); Balsam (Mississippi R/Grand Rapids); Movil & Turtle (Mississippi R/Headwaters); and Ten Mile & Girl (Leech Lake). Many Point Lake (Otter Tail River) is in the Red River of the North Basin.

Lakes with Historic Records. Longear sunfish were found in 11of the 18 historic waters that were sampled. Specimens from the following lakes confirm historic records for those lakes: Little Bemidji (Site #3), Eagle #1 (11), Bertha (17), Rush (20), Cross (21), Three Island (27), Baby (62), Little Thunder (75), Pine (78), Grassy Bay (109), and Brown's Bay (110).

Sites within the Lake of the Woods Basin include Pine Lake (Big Fork Watershed), and Grassy Bay and Brown's Bay (Rainy River [Lake] Watershed). Seven lakes are in the Upper Mississippi River Basin and include Eagle #1 (Crow Wing Watershed); Little Thunder

(Mississippi R/Grand Rapids); Three Island (Mississippi R/Headwaters); Baby (Leech Lake); and Bertha, Rush, and Cross lakes (Pine River). Little Bemidji Lake (Otter Tail River) is in the Red River of the North Basin.

Lakes where Longear Sunfish were not found. Sixty-eight lakes were sampled satisfactorily but did not produce longear sunfish. One lake (Pleasant, Site #61) possessed what appeared to be suitable shallows, but extensive sampling by MN DNR personnel and P. Ceas & crew failed to produce any longear sunfish. None of the remaining lakes possessed the combination of habitat traits that longear sunfish seem to require. These lakes (see Figure 9 for representative photos) can be divided into one of seven simplified, yet useful, descriptive categories based on depth and/or substrate:

- **Sand Lakes.** Fourteen lakes were characterized as “sand lakes” (as described in the Introduction), and although they often had extensive shallows, longear sunfish were not found in these lakes even though other species of sunfishes were abundant. Lakes included in this category are Round Lake (5), Julia (24), Gull (30), Kitchi (37), Big (39), Moose (47), Deer (48), Steamboat (54), Inguadona (68), Mabel (71), Arrowhead (77), Jessie (80), Round (93), and Wabana (98),
- **No Shallows.** Thirty lakes lacked the extensive shallows that longear sunfish seem to require. These lakes can be further divided into two basic categories. The first category includes lakes that were ringed with emergent cattails/bog-like vegetation, so by the time open water exists the depths were "excessive" for longear sunfish: Norway (16), Emily (23), Big Rice (35), Little Rice (36), Pug Hole (38), Grace (40), Pughole (48), Sugar (53), Shingobee (56), Island (57), Portage (59), Blackwater (64), Boy (69), and Dora (76).

  The second category includes lakes in which the depth increased rapidly just a few feet from shore: Elbow (3), Mule (65), Turtle (82), Maple (83), Burns (85), Clubhouse (86), North Star (88), Grave (90), Lost (91), Owen (92), Burnt Shanty (94), Lost Moose (95), Echo (117), Jeanette (118), Big (119), and Fenske (120).
- **Bottomland Lakes.** Two lakes (Little White Oak #50, and White Oak #51) can be characterized as Mississippi River bottomland lakes that also lacked the shallow spawning habitat.
- **Bog-Stain Lakes.** The following lakes in the Red River of the North Basin (Otter Tail Watershed) contain waters with high levels of organic (humic) substances, which are not waters in which longears have been found (P. Ceas, pers. observ; J. Lyons, WI DNR, pers. comm.). These lakes include Tamarack (7), Pine (8), Height of Land (9), and Rice (10) lakes. Crane Lake (Sites 39-41, Lake of the Woods Basin) is also fairly bog-stained lake.
- **Poor Water Quality.** Rabideau (33) and Moose (34) lakes were characterized by poor water quality and excessive blooms of filamentous algae (Figure 9). Tulaby Lake (1) also had extensive algal growth in the shallows.
- **Habitat Alterations.** Wolf Lake (41) and Fish Hook Lake (13) have had extensive shoreland modifications and removal of submerged aquatic vegetation.
- **Other Lakes.** Mitchell Lake (22) contained a deep muck substrate that did not appear to offer suitable habitat.

Incomplete Surveys / Lakes to Revisit

Lakes with Historic Records. Longear sunfish were not found in seven lakes that contain historic records. These lakes include Whitefish (Site #18, record from 2001), Big Trout (19, 1990), Hen (20, 1995), Mukooda (38, 1997), Woman (63, 1987), Thunder (74, 2000), and Coon-Sandwick (79, 1992) lakes. Reasons for the lack of longears in these lakes are provided below. All seven lakes must be resampled to determine if longear sunfish maintain breeding colonies there.

- Coon-Sandwick and Thunder lakes do not appear to contain the extensive shallows that longears prefer.
- Woman, Whitefish, Hen, and Big Trout lakes each have an abundance of shallows, but it appears that much of the shoreland & shallows has been altered by continued upscale home development, and much of the original submerged and emergent vegetation has been removed. Paul Radomski, MN DNR, reported finding three individuals in Woman Lake during a multi-day sampling effort in September 2006; these fishes may represent waifs from the nearby and connected Girl Lake, which support an abundant population of longear sunfish.
- Mukooda Lake appears to be a clear gravel/boulder and “sand lake” that does not offer suitable habitat. The specimens connected to the 1997 record can not be located, and putative longear specimens collected in 2007 by MN DNR personnel are hybrid pumpkinseed x bluegill. However, the lake needs to be sampled again since time constraints did not allow us to sample it thoroughly.

Lakes without Historic Records. In addition to the seven historic lakes that need to be resampled to determine if longear sunfish maintain breeding colonies occur there, 18 lakes were visited but were not sampled effectively due to poor weather conditions or limited access to the lake. All 18 lakes may contain suitable habitat and need to be sampled at a later date. These lakes are listed below.

- **Inclement Weather.** Unusually strong winds and inclement weather kept us from sampling Beltrami (Site #28), Turtle River Lake (29), Andrusia (42), Cass (43), Winnibigoshish (44), Portage (55), and Little Turtle (81) lakes.
- **Limited Access.** Low water levels caused by the ongoing drought exposed completely the boat ramps at Little Winnibigoshish (45), Ball Club (46), and Trout (97) lakes, making it impossible to launch our boat, so these lakes could not be sampled. Five lakes (South Twin, 32; Vermillion, 52; Laura, 73; Jack-the-Horse, 84; and Big Island, 89) had sand boat ramps as public access points; unfortunately our 12-passenger field van is not equipped with 4-wheel drive, so launching our 18’ jon boat proved to be an exercise in digging out a stuck van rather than sampling these lakes. We hope to revisit these lakes with a 4WD vehicle to launch and retrieve the boat. All five lakes appear superficially to have suitable habitat for longear sunfish.
- **Reconnoitered Only.** Waboose (6). Potential habitat exists in this lake.
- **Other Lakes.** Both Pine Mountain (14) and Big Portage (15) lakes contain extensive shallows, but both also qualify somewhat as “sand lakes.” Due to time constraints these lakes were not sampled as extensively as other lakes, and it would be worth revisiting these lakes at a later date.

Namakan Lake and Other Border Lakes Within/Near VNP. Much of the shoreline along the southern shore of Namakan Lake (VNP) was reconnoitered or surveyed (Sites 99-104, 106-108; Map 9). In general, the southern shores of Namakan Lake do not offer the combination of extensive shallows, emergent vegetation beds, and submerged “carpets” of aquatic vegetation. The one location that had all three habitat characteristics (Junction Bay, Site 105) did contain longear sunfish. This location is immediately downstream of the Johnson River waterfalls; Little Johnson Lake, which is drained by Johnson River, contains a historical record for longear sunfish. There is much shoreline habitat within Namakan Lake and the other VNP border lakes that needs to be sampled to determine the distribution of longears in the border lakes.

Abundance and Distribution within a Water Body

Abundance. The term "Abundant" is relative; in no lakes did the abundance of longears ever approach that found for bluegills or pumpkinseeds, even in lakes where we found longears without much effort. In the shallows where longears were found, bluegills and pumpkinseeds still tended to be the more plentiful species in any given seine haul; the exceptions were when we would seine directly over a tightly clustered colony of longear nests. The species was considered "abundant" if numerous breeding colonies were located within a lake, "common" if only a few breeding colonies were located, and "present" if only one/a few individuals were located.

Longears Abundant. Longear sunfish were deemed abundant in the appropriate shallows in lakes within the following Watersheds:

- **Otter Tail.** Little Bemidji Lake and Many Point Lake (Map 3).
- **Crow Wing.** Eagle Lake #1 (Map 3).
- **Turtle River - Mississippi R (Headwaters).** Movil Lake, Turtle Lake, Turtle River @ CR 307 (Map 7).
- **Mississippi R (Grand Rapids).** Balsam Lake (Map 7).
- **Big Fork River.** Eagle Lake #2 (Map 7).
- **Rainy River.** Grassy Bay, Staege Bay, Brown's Bay (all Sand Point Lake). Map 9.
- **Boy River - Leech Lake River.** Baby Lake and Girl Lake (Map 7).
- **Pine River.** Bertha Lake (Map 5).

Longears Common. Longear sunfish were deemed common in the appropriate shallows in lakes within the following Watersheds:

- **Crow Wing.** Potato Lake (Map 3).
- **Turtle River - Mississippi R (Headwaters).** Three Island Lake (Map 7).
- **Mississippi R (Grand Rapids).** Little Thunder Lake (Map 7).
- **Pine River.** Rush Lake (Map 5).

Longears Present. Longear sunfish were deemed present in the appropriate shallows in lakes within the following Watersheds:

- **Big Fork River.** Pine Lake (Map 7). Pine Lake is a difficult lake to work with bog-like edges. Limited sampling in Pine Lake (due to the onset of dusk combined with rain and hordes of mosquitoes) produced only a few individuals; additional sampling is necessary.
- **Rainy River.** Junction Bay (Namakan Lake). Map 9.
- **Boy River - Leech Lake River.** Ten Mile Lake (Map 7).
- **Pine River.** Cross & Rush Lake (Map 5).

Distribution within a Lake. Longear sunfish were never generally distributed along the shoreline but instead were very localized. These locations corresponded to the limited occurrence of the proper combination of the species' specific habitat requirements. It was not unusual for the species to be abundant at these locations, but their habitat-specific needs appear to be a primary limiting factor in the distribution of the species within a given lake. See the middle photo in Figure 7 for an example of just how localized this species can be.

Distribution within a River. The Turtle River (31) @ CR 207 contained shallow pools along its margin, and had extensive stands of bulrush/wild rice alongside the pools (such habitat did not exist at the Hwy 22 location). Longear sunfish were abundant within these pools, but its distribution within the river appears to be severely restricted to these pools. The Turtle River at the CR 207 & Hwy 22 locations were the only riverine environments sampled. Further sampling throughout Turtle River and other rivers (see "Future Field Surveys" on p. 20) needs to be conducted to determine the extent to which longears normally occur in rivers.

Other Notable Species

Two additional SGCN were found in several lakes during this project: least darter, *Etheostoma microperca*, and pugnose shiner, *Notropis anogenus*. Other species that are considered indicators of high-quality waters were regularly found with longear sunfish. The species most commonly found included the blacknose shiner, *Notropis heterolepis*, blackchin shiner, *Notropis heterodon*, and banded killifish, *Fundulus diaphanus*, with the Iowa darter, *Etheostoma exile*, being found less frequently. Although we did not conduct quantitative sampling/analysis, anecdotal evidence suggested that if the bluntnose minnow, *Pimephales notatus*, was more abundant than the blackchin shiner then the odds of finding longear sunfish in a particular lake decreased considerably. Locality data for these species has been provided to K. Schmidt, MN DNR.

General Observations on Habitat and Species Abundance

Given that this project was limited in scope in the number of lakes sampled, we do not yet fully understand the complete range and full distribution of the longear sunfish within Minnesota. We do, however, have a good understanding of the species' habitat requirements. We also have a better understanding of the relative abundance of the species within a lake even though we did not conduct quantitative assessments of population densities (which was beyond the scope of this project).

During the course of this initial sampling effort the following general observations on habitat and abundance were made during both the 2006 & 2007 field seasons:

(1) When present, longear sunfish were quite specific in their habitat preferences (see Figures 6-9). These preferences, which were observed both during the spawning season and afterwards, included a combination of:
   - shorelines with relatively undisturbed stretches of emergent aquatic plants (e.g., bulrush beds),
   - extensive shallows (e.g., the water may be only 3' deep at distances of 150' from shore), and
   - a firm substrate, which was usually "carpeted" submerged plants and often contained a fine layer of organic debris.

(2) This habitat specificity, whether in a lake or the Turtle River, made it relatively easy to capture longears once the habitat was located, but the time-consuming component was often searching the perimeter of a lake for these particular habitats since there may be fewer than five such shallows in a lake. Not unexpectedly, many public boat ramps have been built in sections of lakes that contain extensive shoreline shallows, so when longear sunfish are present within a lake it is not unusual to find them around the boat ramp.

(3) Although we characterized some populations as being "abundant" in terms of the numbers of individuals within a lake, "abundant" is a relative term. This species is still a fairly rare fish even in lakes where we found them without much effort. For example, even when we occasionally hauled up 50-60 individuals in a single brief seine haul, in no lakes did the density of longears ever approach that found for bluegills or pumpkinseeds, which were always more abundant and widespread.

(4) Unlike longears, bluegills and pumpkinseeds were found in a variety of habitats and depths. These two species also were caught regularly in the same seine hauls with longears.

(5) Longear sunfish can live and reproduce in streams, provided that good backwater/pool habitat exists. It does not seem to be a regular inhabitant of flowing waters in Minnesota.

GENETIC DIVERSITY

Mitochondrial DNA Analysis. We analyzed 645 base pairs (bp) of the 1140 bp cytochrome *b* gene for 108 *L. megalotis* individuals; the sequence began at nucleotide position 40 (amino acid position 14) and ended at nucleotide position 684 (amino acid position 228). Out of the 49 variable nucleotide positions, 33 were at the third codon position (29 transitions, two transversions, two both), 14 were at the first codon position (11 transitions, three transversions), and 2 were at the second codon position (one transition, one transversion). No stop codons were found in any sequence, and 19 of the 215 amino acids were variable among the sequences. The variation resulted in 20 haplotypes (designated A-T); 14 of the 19 variable amino acid positions were autapomorphic (were different in just one haplotype). These nucleotide and amino acid substitution patterns are consistent with the evolution of mitochondrial protein-coding genes (e.g., greater substitution rate at the third position, large number of silent substitutions).

Because phylogenetic and network analyses both uncovered two distinct groups of samples (see further discussion below), pairwise distance calculations were conducted not only for all 108 sequences as a whole, but also for the two groups separately (Missouri and Tennessee sequences versus everything else). The average pairwise uncorrected p-distance among all 108 sequences was 1.17% (transitions 0.98%, transversions 0.186%), among the 12 Missouri/Tennessee sequences was 0.378% (transitions 0.27%, transversions 0.065%), and among the 96 remaining sequences was 0.212% (transitions 0.15%, transversions 0.065%). Both intra-group average distances are much lower than the overall average distance, revealing that latter value is larger due to inter-group comparisons with large genetic distance (about 5%).

Six haplotypes (G, H, J, L, Q, R) are unique to the Missouri/Tennessee samples, while nine haplotypes (A, B, C, E, F, I, K, M, O) were sampled from Minnesota; the other five haplotypes (D, N, P, S, T) are unique to other geographic areas in the Midwest and Southeast (see Table 4 for more details about haplotype distribution). Thus, when haplotypes are grouped in to the two groups from above (Missouri & Tennessee versus everything else), six haplotypes were sampled from 12 individuals in Missouri and Tennessee (0.5 haplotype/sample ratio) while 14 haplotypes were sampled from the other 96 individuals (0.15 haplotype/sample ratio) including the nine haplotypes from the 82 Minnesota fish (0.11 haplotype/sample ratio). This pattern is consistent with the pattern seen in the pairwise distance comparisons, where greater genetic diversity is seen in the unglaciated south versus the recently glaciated north.

Phylogenetic Relationships (Mitochondrial Data). Phylogenetic analysis of the 20 haplotypes (Figure 10) and network analysis of the 96 non-Missouri/Tennessee sequences (Figure 11) also support the findings of lower-diversity northern populations, and of closer genetic relationships between haplotypes of the upper Midwest with the southeast haplotypes instead of with the Ozark haplotypes. The unrooted tree in Figure 10 shows that the two groups of haplotypes are quite divergent (28 nucleotide substitutions between them), while haplotypes within the two groups are quite similar (no internal branch lengths longer than one, terminal branch lengths no more than three). The network diagram in Figure 11 provides a better look at the geographic areas associated with haplotypes of the larger group (n=96) of sequences. Most of the sequences are either haplotype A (n=39, including 29 Minnesota) or haplotype B (n=19, all Minnesota), which differ from each other by just one nucleotide. All of the other haplotypes differ from one of these two (A or B) by only one, or in one case each, two or three nucleotides, and there is some structure in Movil and Three Island lakes (haplotypes E and F).

A closer look at the non-Minnesota sequences reveals that their haplotypes are not localized in any one place on the network. In the Wisconsin sequences, two haplotypes (D and N) were uniquely sampled from the state, but four out of the seven Wisconsin fish sequenced have haplotype A (the most common Minnesota haplotype). Similarly, one of the Kankakee River (Illinois) fish possesses a unique haplotype

(T), but the other two fish from that drainage as well as the fourth Illinois fish (Bay Creek in southern Illinois) have haplotype A. Of the three more geographically distant fish (sequenced by Harris et al. 2005), the Maryland haplotype (S) is one nucleotide different from common Minnesota haplotype B, the Alabama haplotype (P) is three nucleotides different from common Minnesota haplotype A, and the Kentucky fish actually has haplotype A. Haplotype A's widespread geographic distribution, both within Minnesota and across the eastern states, and the location of the unique eastern haplotypes spread throughout the network (rather than clustering together) suggest that Minnesota's *L. megalotis* mitochondrial haplotypes sampled largely represent ancestral diversity introduced during postglacial recolonization. Of course, seven less common haplotypes unique to Minnesota were sampled, including E and F which have a nested relationship with respect to its putative ancestral haplotype B, suggesting the evolution of some local variation. Table 5 depicts haplotype frequencies at each collection site, again showing that there are haplotypes not only unique to specific sites, but also unique to Major Watersheds and Basins in the state. Obviously a haplotype unique to a particular collection site will also be unique to its Major Watershed and basin (haplotypes C, I, M and O), but haplotypes E and F are found at multiple sites all within the Upper Mississippi River Watershed, and haplotype K is found at two sites, both in the Crow Wing River Watershed.

While the overall genetic variation within Minnesota as measured by mitochondrial DNA sequences is low (especially compared with its southern conspecifics), the variation that does exist seems to exhibit some geographic structure. The obvious caveat is sample size; the <u>average</u> number of individuals sequenced per Minnesota site is only 4.55 (range 1-10), so in the above discussion we emphasized that the data are haplotypes sampled. Because of their sampled endemism in two or more nearby sites as discussed above, haplotypes E/F and haplotype K are probably the best candidates for locally evolved haplotypes; the haplotypes that are unique to one site are just likely to be sampled low-frequency ancestral variation at these sample sizes. For this particular project, when we saw that the mitochondrial sequences were not exhibiting much overall variation within the state, we chose to allocate our resources instead toward work on the potentially more variable microsatellite data set.

Optimization of Microsatellite Loci. We achieved some amplification for all loci screened except for Lmar8, Lmar16, and Lma121. Up to the present we have been able to optimize reaction formulas and conditions to achieve genotypable results for five of these loci (Lmar10, Lmar11, Lmar12, Lmar14, Lma29), and analyzable data for the first four. Preliminary data were obtained for locus Lma29, but reliable genotype data are not available.

Microsatellite Loci Analyzed. The microsatellite data set analyzed here included genotypes for 301 fish at four loci: Lmar10 (247 individuals), Lmar11 (218 individuals), Lmar12 (247 individuals), and Lmar14 (278 individuals). Table 3 provides some information about each of these four loci's alleles; Lmar10 has the highest allele diversity while Lmar12 has the lowest (as measured with a Shannon-Weaver diversity index). Allele range and frequencies for each locus are presented in more detail in Figures 12-15, where frequency distributions separated by state are graphed. For all four loci, frequency distributions of Minnesota and Wisconsin (albeit a very small sample) are similar, there are few to no shared alleles between Minnesota/Wisconsin and Missouri fish, and the few samples from Illinois fall out within the ranges of both of the former two geographic areas. Also of note on these graphs is the proportionately much greater number of alleles sampled from Missouri fish compared with Minnesota/Wisconsin fish; for examples, for Lmar10 seven Missouri fish collectively possess nine alleles, while 231 Minnesota fish collectively possess only 13 alleles. Both of these observations (differentiation between Minnesota and Missouri samples, greater overall genetic diversity in the south) are consistent with patterns observed in the mitochondrial data.

Linkage Disequilbrium Tests. A linkage disequilibrium test on all 301 fish in the microsatellite data set resulted in a statistically significant linkage relationship between loci Lmar10 and Lmar11. There was no mention of linkage tests in the paper that provided these loci (Schable et al. 2002), but that study was focused on developing new microsatellite primer pairs rather than testing population genetics. Linkage disequilibrium can be affected by a number of different things, including actual physical linkage on a chromosome, population size, and population history. Both of these loci had a fairly large percentage of uncalled individuals due to non-amplification, or more rarely being unable to distinguish clear alleles (7.8% for Lmar10 and 11.4% for Lmar11); the presence of null alleles could be a confounding factor in a linkage analysis. A larger data set and a confirmation of allele-calling in both loci are advisable before publication of these data.

Hardy-Weinberg Tests. Test results for Hardy-Weinberg genetic equilibrium within populations defined at the collection site, Major Watershed, and Basin levels are presented in Table 6 (Minnesota) and Table 7 (other states, although sample sizes are usually too low to expect statistical significance). There are a variety of populations at all three levels that exhibited statistically significantly lower observed heterozygosity (H_{obs}). Lower H_{obs} in microsatellite data can be associated with a variety of factors, including population subdivision in the sample (the Wahlund effect), null alleles, allele-calling errors, or microevolutionary forces such as drift or selection at non-neutral loci linked to the microsatellite loci. Some of the significant tests were indeed at the level of Major Watershed or Basin (e.g., Rainy River Watershed for Lmar14, Big Fork River Watershed for Lmar11) so population subdivision within these watersheds might result in lower H_{obs}. An interesting note about the significant test for Movil Lake (Lmar14) is that this site's 44 samples include fish from each of two summers of collecting; when the two years are treated as two populations, the statistical significance goes away (although this could also be the result of decreased sample size). An instance like Girl and Baby Lakes exhibiting no heterozygotes for locus Lmar12 might be due to a null allele, such as a population-specific mutation in a priming site leading to no amplification of the second allele in a heterozygote. However, Lmar12 actually had the lowest rate of non-amplifying individuals (2.4%), suggesting that null alleles may not be significantly numerous. Allele-calling errors should also be considered, and as mentioned above, confirmation of allele-calling will be done again before publication. However, all alleles for all individuals were assessed by eye, from three different images of the data, by the same person, and a given locus was called within a fluid time frame, so all efforts were made to avoid allele-calling errors.

AMOVA Results. As with the mitochondrial data, most of the geographic structure in the microsatellite data set was among broader geographic groups. The AMOVA results (Table 8) show that as populations are defined from larger to more local scales, the F_{ST} value (a measure of population differentiation) decreases. AMOVAs were conducted on populations defined as states (subpopulations = Basins), Basins (subpopulations = Major Watersheds), and Major Watersheds (subpopulations = sites). In all three analyses, the majority of the total variation was due to within-subpopulation variation, suggesting that the total variation in the data set was fairly widespread across the whole range. However, the F_{ST} of 0.253 for Basins within states and the F_{ST} of 0.121 for Major Watersheds within Basins are considered roughly great to moderate levels of differentiation, while the F_{ST} of 0.061 for Minnesota sites within Major Watersheds represents a fairly low amount of population differentiation. So not only is there relatively less overall variation in Minnesota longear sunfish than in the unglaciated south, but that variation also tends to be shared among Minnesota sites rather than partitioned geographically.

Phylogenetic Relationships (Microsatellite Data). Another way to examine the distribution of microsatellite variation across Minnesota and compared with other areas is through phylogenetic trees representing taxon relationships based on pairwise distances. Figure 16 shows a neighbor-joining tree built from the chord distance matrix (Table 9) of the 13 Major Watersheds in the microsatellite data set. The recurring themes of large north/south differences and lower variation in the north appear again in this tree; the branch connecting the three southern watersheds with the ten northern watersheds is long, as are the branch lengths leading to the three southern watersheds. The ten more northern watersheds form a starburst pattern with very short internal branch lengths, which usually suggests a radiation. This is consistent with post-glaciation recolonization and differentiation, where recolonizing fish can become relatively quickly separated in to newly available habitats where local differentiation can begin occurring.

Figure 17 shows a neighbor-joining tree built from the chord distance matrix (Table 10) of the 18 Minnesota collection sites in the microsatellite data set. The starburst pattern seen among northern watersheds is seen again here among Minnesota sites. In some cases, all sites within a watershed are most closely related in the neighbor-joining tree (Potato and Eagle [#1] Lakes in the Crow Wing Watershed; Little Bemidji and Many Point Lakes in the Otter Tail River Watershed). In other cases, all sites within a watershed cluster together in the same group though they are not sister taxa (Junction Bay and Sand Point Lake in the Rainy River Watershed; Balsam and Little Thunder Lakes in the Mississippi-Grand Rapids Watershed; Bertha, Cross and Rush Lakes in the Pine River Watershed). In still other cases, some sites within a watershed cluster together while another is placed elsewhere on the tree (Movil and Three Island Lakes versus Turtle River, of the Mississippi-Headwaters Watershed), or the sites within a watershed are found in different places on the tree (Baby and Girl Lakes of the Leech Lake Watershed; Pine and Eagle [#2] Lakes of the Big Fork River Watershed). As in the mitochondrial data, there may be some local differentiation of haplotype frequencies, along with widespread distribution of ancestral variation that can make geographically distant populations appear more genetically related.

Genetic Diversity in Minnesota (Microsatellites). In terms of unique microsatellite alleles (Table 11), six alleles representing all four loci analyzed are unique to a collection site. The Lmar10 allele (323 bp) unique to Sand Point Lake will be investigated further by genotyping some Sand Point Lake pumpkinseeds as it is an outlier (the next largest sampled Lmar10 allele is 311 bp). Obviously these six alleles are also unique to the Major Watersheds and then Basins that contain them, but at the Basin level there are four more unique alleles (all in the Upper Mississippi River basin). However, as discussed with the sampling of mitochondrial haplotypes, unique alleles may represent either local evolution or sampling of low-frequency ancestral alleles.

Overall levels of genetic diversity were fairly similar across Minnesota collection sites (Table 12). Heterozygosity (the percentage of individuals heterozygous at a locus) averaged across all four loci ranged from 18.4% in Junction Bay to 44.3% in Potato Lake, and the average value for all 18 sites was 36.5%. No populations were fixed at locus Lmar10, only Junction Bay was fixed at locus Lmar14, four populations (including Junction Bay) were fixed at locus Lmar11, and ten populations (including Junction Bay) were fixed at locus Lmar12. The only populations fixed at more than one of the four loci were Junction Bay (three loci) and Sand Point Lake (two loci); these populations are the two sampled from Voyageurs National Park, and if this sampling accurately represents levels of genetic diversity in these populations, they may be relatively depauperate due to their northernmost location (possible founder effects in a more recent recolonization farther north). With the exception of Junction Bay and Sand Point Lake, levels of genetic diversity in Minnesota longear sunfish populations are good as measured with these four microsatellite loci. It is important to remember that microsatellite loci are neutral or near-neutral genetic markers and as such are not likely to represent evolutionary significant genetic variation per se, but they do serve as an estimate for how much historical processes (such as bottlenecks during recolonization) or other random genetic drift since recolonization may have affected genetic diversity at phenotypically relevant loci.

COMMENTS ON DISTRIBUTION, POPULATION CLUSTERS, AND FUTURE FIELD & GENETICS WORK

Distribution. The number of Minnesota lakes/stream segments from which the longear sunfish has been collected now stands at 37, twelve of which represent new distributional records documented by this study. Longears were found in all three of the historic basins, and in eight of the nine Major Watersheds that were sampled (Hustler Lake, the lone historic record from Rainy River/Headwaters, was not sampled). New records were found in each of the eight watersheds, which indicate that the species is more widespread within these watersheds and is likely to be found elsewhere within these watersheds.

Population Clusters. We recognize eleven geographic clusters of longear populations within Minnesota. These clusters, listed below by river system within their respective watershed, are subject to modification pending future surveys:

- **Otter Tail.** Little Bemidji and Many Point lakes, Otter Tail River System (Map 3, Sites 3 & 4). Little Bemidji Lake flows into Many Point Lake.
- **Crow Wing.** Eagle Lake #1 and Potato Lake, Crow Wing River System (Map 3, Sites 11 & 12). Eagle Lake flows into Potato Lake.
- **Turtle River - Mississippi R (Headwaters).** Turtle Lake, Movil Lake, Three Island Lake, and Turtle River @ CR 207 (Map 7, Sites 25, 26, 27, 31). Turtle Lake flows into Movil Lake, which flows into Beltrami and then Fox lakes (both unsampled). Fox Lake flows into Three Island Lake, which is drained by the Turtle River. The Turtle River flows into Turtle River Lake, and the CR 207 location is downstream of Turtle River Lake.
- **Prairie River - Mississippi R (Grand Rapids).** Balsam Lake (Map 7, Site 96) is the one known record.
- **Willow River - Mississippi R (Grand Rapids).** Thunder and Little Thunder lakes (Map 7, Sites 74 & 75).
- **Rice River - Big Fork River.** Pine Lake, Coon-Sandwick Lake, and Eagle Lake #2 (Map 7, Sites 78, 79, 87).
- **Rainy River (Rainy Lake).** Junction Bay (Namakan Lake); Grassy Bay, Brown's Bay Staege Bay, Feldt Channel (all Sand Point Lake); Map 9, Sites 105, 109-112.
- **Rainy River (Headwaters).** Hustler Lake (Map 8, Site 32) is the one known record.
- **Boy River - Leech Lake River.** Ten Mile Lake, and five lakes within the Woman Lake Chain: Baby, Woman, Girl, Kid, Cooper (Map 7, Sites 60, 62, 63, 67; Map 6, Sites 17 & 20).
- **Shingobee River - Leech Lake River.** Anoway Lake (Map 6, Site 16) is the one known record.
- **Pine River.** Nine lakes within the Whitefish Chain: Bertha, Whitefish, Pig, Big Trout, Island-Loon, Hen, Rush, Cross Lake, and Daggett (Map 4, Sites 3 – 11).

Future Field Surveys/Studies. Given the number of lakes within Minnesota that contain potentially "ideal" habitat for longear sunfish, we expect that continued sampling will result in the discovery of additional new populations of longear sunfish. Table 2 includes those lakes that were visited in 2006-07 but were not sampled (poor access, inclement weather). These and many other lakes and streams must be sampled to develop a true understanding/baseline of the distribution and relative abundance of the longear sunfish in Minnesota. Of particular interest are the lakes within the entire Lake of the Woods border region, since our records from those difficult-to-reach lakes are so limited. In addition to the border lakes listed in Table 1, we are aware of one unconfirmed report of longear sunfish in Basswood Lake.

Further sampling throughout the Turtle River and numerous other riverine systems need to be conducted to determine the extent to which longears normally occur in rivers in Minnesota. We suspect that longears could be abundant in the proper pool habitats throughout a number of rivers. Some of the river systems that need to be sampled include the watersheds that harbor confirmed longear records: the Boy, Fish Hook, upper Otter Tail, Pine, Prairie, Johnson (Rainy), Rainy (headwaters), Rice – Big Fork, Shingobee, and Willow systems. Other river systems that need to be examined include (but are not limited to) the upper Crow Wing (e.g., the Shell River), remainder of the Leech Lake watershed (e.g., Kabekona River), and Pelican River (Otter Tail River). Additional watersheds that have never been sampled while specifically looking for longears, such as the Gull River watershed in the Brainerd area, must also be sampled.

To support CWCS Goal II, Management Challenge 1, Strategy II B, Priority Conservation Actions for Research, it is imperative to "*Identify important patterns and distributions of key habitats to better support SGCN populations*." It is with this Strategy in mind that we have begun a collaborative effort with Dr. Brian Welch (St. Olaf College) and students in his Spring 2008 GIS course. Using our data and knowledge concerning what we consider to be the key habitat components, we are working to develop a method to predict/identify those lakes that possess the combination of habitat characteristics that are preferred by longear sunfish. The logical step, if additional funds are made available, would then be to sample a subset of predicted lakes within all nine watersheds, and to eventually expand the search area to other watersheds.

With the proper field vehicle (i.e., 4WD to launch/retrieve a boat from lakes with sand ramps) many lakes can be sampled rather quickly. However, it will take considerably more time to assess the species' presence and relative abundance in the larger lakes that fall within its known range, such as Cass and Winnibigoshish. Numerous bodies of water do not have public boat access, so obtaining permission from landowners is a priority.

Future sampling should also include snorkeling/SCUBA efforts in select lakes. This would increase our confidence that longear sunfish are not regularly found in near-shore waters that are too deep to seine/boatshock efficiently.

If funding is made available we would also conduct a (preferably) multi-year life history study of the species, simultaneously studying populations from a number of water bodies within the state (i.e., the Turtle River population, one Mississippi Basin population, and one Lake of the Woods Basin population). This valuable data would surely supplement and expand upon the observations that we have made during the limited sampling that we did in 2006-07.

Future Genetics Work. There is some additional work to do with the genetic data sets before submitting manuscripts for publication in peer-reviewed journals. We will use what remaining supplies we have in the lab to complete genotyping for all four of the loci analyzed already, and reanalyze the data from locus Lma29 to see if reliable genotypes can be called. More detailed analyses concerning correlations between genetic and geographic patterns can be done, including distance (genetic and geographic) matrix correlations; we anticipate that the GIS work mentioned above may help us best reflect geographic distances in these tests. One interesting broader scale issue is the hypothesized source of postglacial colonists – did longear sunfish colonize from refugia east or west of the Mississippi River? Fortuitously, we have a colleague who has been studying longear sunfish genetic variation with a focus on geographic regions that we did not sample, so we plan to pursue a collaboration to address broader scale relationships within *Lepomis megalotis*.

RECOMMENDATIONS CONCERNING THE SPECIES' CONTINUED LISTING AS A SGCN

The number of Minnesota lakes/stream segments from which the longear sunfish has been collected now stands at 37. This number, although likely to rise when additional collecting occurs, still represents a tiny fraction of the waters within Minnesota. Until such time that the longear sunfish is found to be widespread and common throughout its range, or that the species can be shown not to be under any danger of population decimation/extirpation, this species must remain listed as a Species in Greatest Conservation Need in Minnesota. Furthermore, we recommend that this species be listed as "Special Concern" by the MN DNR because of its limited numbers and because it has "highly specific habitat requirements and deserves careful monitoring of its status" (http://www.dnr.state.mn.us/ets/index.html). Continued habitat degradation would necessitate its listing (along with numerous other fish species) as "Threatened" in Minnesota.

COMMENTS ON IMPORTANT POPULATIONS AND CONSERVATION ISSUES

Important Populations. In the draft version of the CWCS (under which this project was funded), Priority Conservation Goal I, Problem 2, Action A calls for the management of "important" SGCN populations. The CWCS indicates that important populations could be those that are found in high-density clusters, contain large numbers of individuals, or are associated with high quality habitats. We added that genetic biodiversity and/or uniqueness is another key factor in identifying important populations for management purposes. Populations that exhibit overlap in both genetic and ecological categories should be ranked as "most important" populations.

We have recommended that the species remain listed as a SGCN. We further suggest that, until more is known about the species, all 37 populations should be considered important in terms of management decisions. We can offer some additional subdividing/ranking of these populations based on the current and perceived threats to the lakes/habitats, and below we divide the populations into the following four categories: "Most Secure," "Uncertain," and "Most at Risk."

Most Secure. Limited development on these lakes has resulted in the retention of much original and quality habitat. The species is considered "abundant" in these waters and occurs as a cluster of populations within each watershed.

- **Turtle River System – Miss (Headwaters).** Turtle Lake, Movil Lake, Three Island Lake, and Turtle River @ CR 207 (Map 7, Sites 25, 26, 27, 31) currently represent the largest population cluster of longear sunfish in Minnesota, and may exhibit some locally evolved genetic variation.
- **Otter Tail.** Little Bemidji and Many Point lakes (Map 3, Sites 3 & 4) also may exhibit some locally evolved variation.
- **Sand Point Lake.** Map 9, Sites 109-112. The sampled genetic diversity was relatively low.

Uncertain. Each represents an isolated population (i.e., no clusters of lakes). There do not seem to be large-scale pressures from shoreland development, but we simply don't know much about these populations. Additional sampling is needed.

- **Mississippi (Grand Rapids)** – both the Prairie & Willow River lakes. Balsam Lake (Map 7, Site 96); Thunder and Little Thunder lakes (Map 7, Sites 74 & 75).
- **Big Fork System.** Pine Lake, Coon-Sandwick Lake, and Eagle Lake #2 (Map 7, Sites 78, 79, 87).
- **Rainy River (Headwaters).** Hustler Lake (Map 8, Site 32).
- **Rainy River (Rainy Lake).** Junction Bay (Namakan Lake); Map 9 Site 105., Little Johnson Lake (Map 8, Site 23).
- **Shingobee R – Leech Lake.** Anoway Lake (Map 6, Site 16).

Concern. Development of the shorelands in the Park Rapids region already has likely had a negative effect on populations.

- **Crow Wing System.** Eagle Lake #1 and Potato Lake (Map 3, Sites 11 & 12).

Most At Risk.

- **Pine River System.**
  Hen, Cross, Whitefish, and Big Trout lakes each have an abundance of shallows, but it appears that much of the shoreland & shallows has been altered by continued upscale home development, and much of the original submerged and emergent vegetation has been removed. Secure breeding colonies of longear sunfish within these lakes may already have been lost. (Map 4, Sites 3 – 11). The other lakes with historical records (Pig, Island-Loon, Daggett, Table 1) need to be sampled.
- **Boy River – Leech Lake.**
  Woman Lake has an abundance of shallows, but it appears that much of the shoreland & shallows has been altered by continued upscale home development, and much of the

original submerged and emergent vegetation has been removed. Paul Radomski (pers. comm.), MN DNR, found only three individuals in Woman Lake during a multi-day sampling effort in September 2006. So small numbers indicates that this species is not common in Woman Lake, and perhaps can be found in the lake more commonly as waifs from the Girl Lake population. The shoreline of Woman Lake appears to have been modified significantly over the years. Other lakes within the Chain need to be sampled. While traveling through this area it appears that the Woman Lake Chain is undergoing a transition from smaller lake homes to more extensive shoreland development and habitat alterations similar to what has already happened on the Whitefish Chain of the Pine River System. (Map 7, Sites 60, 62, 63, 67; Map 6, Sites 17 & 20).

Genetic Perspective on Possible Management Practices. Given some of the habitat degradation and corresponding lower longear sunfish population sizes observed in this study, it may become advisable to supplement and/or restore ailing or extirpated longear sunfish populations in the future. Both the mitochondrial and microsatellite data sets show sampled ancestral variation to be distributed across Minnesota populations. However, both data sets also show some potential local variation, specifically in watershed-specific mitochondrial haplotypes and unique microsatellite alleles. So, even in the only 10,000 years since current longear sunfish habitats were unglaciated, some genetic differentiation at these neutral or near-neutral markers may have occurred. Whether local adaptive genetic differentiation at more slowly-evolving selected loci has occurred is not known, but it is prudent to preserve any potential local adaptive variation by supplementing from native populations. Our genetics results to date suggest that any genetic differentiation occurring is largely at the watershed level, so maintaining watershed fidelity when moving longear sunfish among populations should be sufficient.

Conservation Issues. Ultimately, the longear sunfish may not prove to be widespread in the state of Minnesota, and it may remain listed as a SGCN (Species in Greatest Conservation Need) within Minnesota. Given the continued pressures to develop lakeshore properties across the state combined with a lack of regard by many landowners for habitat protection (witnessed first-hand by the authors), this habitat-limited species is likely to experience a decrease in range, population size, and health as more and more lakeshores are altered. Lakes that have or are experiencing rapid alterations to the natural shorelines, such as Woman Lake, have likely already lost significant populations of this unique species. Unfortunately there do not exist sufficient historical records to conclusively support this statement; however, in our professional judgment we feel confident in making such statements.

Longear Sunfish as Indicators of Lake Quality. The longear sunfish is a species whose extirpation/decimation/decline in abundance (and health) can be used as an indicator of a water body's deteriorating health. Conversely, the species' high abundance can be used as an indicator of a water body's good health. Decreases in populations have already been observed in the Whitefish Chain, where the shorelines of most of the lakes continue to be modified extensively.

Once the baseline data have been completed for lakes throughout its range in Minnesota, long-term monitoring will identify any positive or negative trends in longear populations within a given water body. Negative trends can then be used to inform federal, tribal, and state natural resource managers that a particular lake/stream may be experiencing a decrease in water/habitat quality. Positive trends can help to signal that a water body is maintaining its health or showing improvement.

ACKNOWLEDGMENTS

A Merck/AAAS grant and a Howard Hughes Medical Institute grant to St. Olaf College provided funding for one additional summer research student each in the summers of 2006 and 2007 respectively, and the Behrents Fund (administered through the St. Olaf College Department of Biology) provided some extra genetics supplies in the falls of 2006 and 2007. The genetics work benefited from conversations with L. Miller (University of Minnesota) and T. Near (Yale University), and M. Jensen (LiCor) was very helpful with equipment and software troubleshooting. We gratefully acknowledge St. Olaf College Department of Biology facilities, equipment, and colleagues, as well as St. Olaf College staff for administrative help.

Konrad Schmidt, MN DNR, provided historical records and offered suggestions for choosing new lakes to sample. J. Norris, MN DNR, provided helpful advice and answers to budget-related questions throughout the study. L. Kallemeyn provided locality data within VNP and offered his thoughts on where to sample. We were assisted in the field at various times by the following persons: C. Cook, E. Onuma, and D. Schackman (USFS); K. Moen, A. Plain (MN DNR); J. Brown, C. Eggebroten, S. Ellingson, D. Gruner, J. Morrison, and J. Rolfes (St. Olaf College summer research students); and P. Jackson (St. Olaf College Dept. Environ. Studies). Frozen Minnesota specimens were provided by K. Moen (Bemidji Area Fisheries Office), Jeff Eibler (International Falls Fisheries Office), and the Grand Rapids Area Fisheries Office. Specimens from Missouri were collected by K. Schmidt, R. Weitzel (MPCA), and R. Hrabik (MO Dept. Conserv.); Wisconsin specimens were collected by K. Schmidt and J. Lyons (WI DNR). Illinois specimens were collected by U. Thomas (aquarist). We thank the MN DNR Fisheries Area Offices & personnel for reporting all longear-like sunfishes during the past two seasons. We also thank L. Pfannmuller , J. Wingate, Ed Quinn, K. Schmidt (MN DNR); C. Cook (CNF); and B. Commins, S. Windels, and R. Maki (VNP) for their enthusiastic support throughout this study.

Literature Cited

Bailey, RM, WC Latta and GR Smith. 2004. An atlas of Michigan fishes with keys and illustrations for their identification. *Miscellaneous Publications - Museum of Zoology, University of Michigan* 192: iv + 215 pp.

Cavalli-Sforza LL and AWF Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics* 19:233-257.

Clement M, D Posada and K Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657-1659.

Colbourne JK, BD Neff, JM Wright and MR Gross. 1996. DNA fingerprinting of bluegill sunfish (*Lepomis machrochirus)* using $(GT)_n$ microsatellites and its potential for assessment of mating success. *Canadian Journal of Fisheries and Aquatic Science* 53: 342-349.

Glaubitz J. 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* 4:309–310.

Guo S and E Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361-372.

Harris PM, KJ Roe and RL Mayden. 2005. A mitochondrial DNA perspective on the molecular systematics of the sunfish genus *Lepomis (Actinopterygii: Centrarchidae)*. *Copeia* 2005(2): 340-346.

Maddison W and D Maddison. 1992. MacClade version 3.03. Sinauer Associates, Sunderland, MA.

Neff BD, P Fu and MR Gross. 1999. Microsatellite evolution in sunfish (*Centrarchidae)*. *Canadian Journal of Fisheries and Aquatic Science* 56: 1198-1205.

Schable NR, RU Fischer and TC Glenn. 2002. Tetranucleotide microsatellite DNA loci from the dollar sunfish (*Lepomis marginatus*). *Molecular Ecology Notes* 2:509-511.

Schneider S, D Roessli and L Excoffier. 2000. Arlequin: A software for population genetics data analysis (Version 2.000). Genetics and Biometry Lab, Department of Anthropology, University of Geneva.

Slatkin M and L Excoffier. 1996. Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity* 76:377-383.

Song C-B, TJ Near and LM Page. 1998. Phylogenetic relationships among percid fishes as inferred from mitochondrial cytochrome b DNA sequence data. *Molecular Phylogenetics and Evolution* 10:343-353.

Swofford D. 2000. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Thompson JD, DG Higgins and TJ Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Weir B. 1996. *Genetic Data Analysis II: Methods for Discrete Population Genetics Data*. Sinauer Associates, Sunderland, MA.

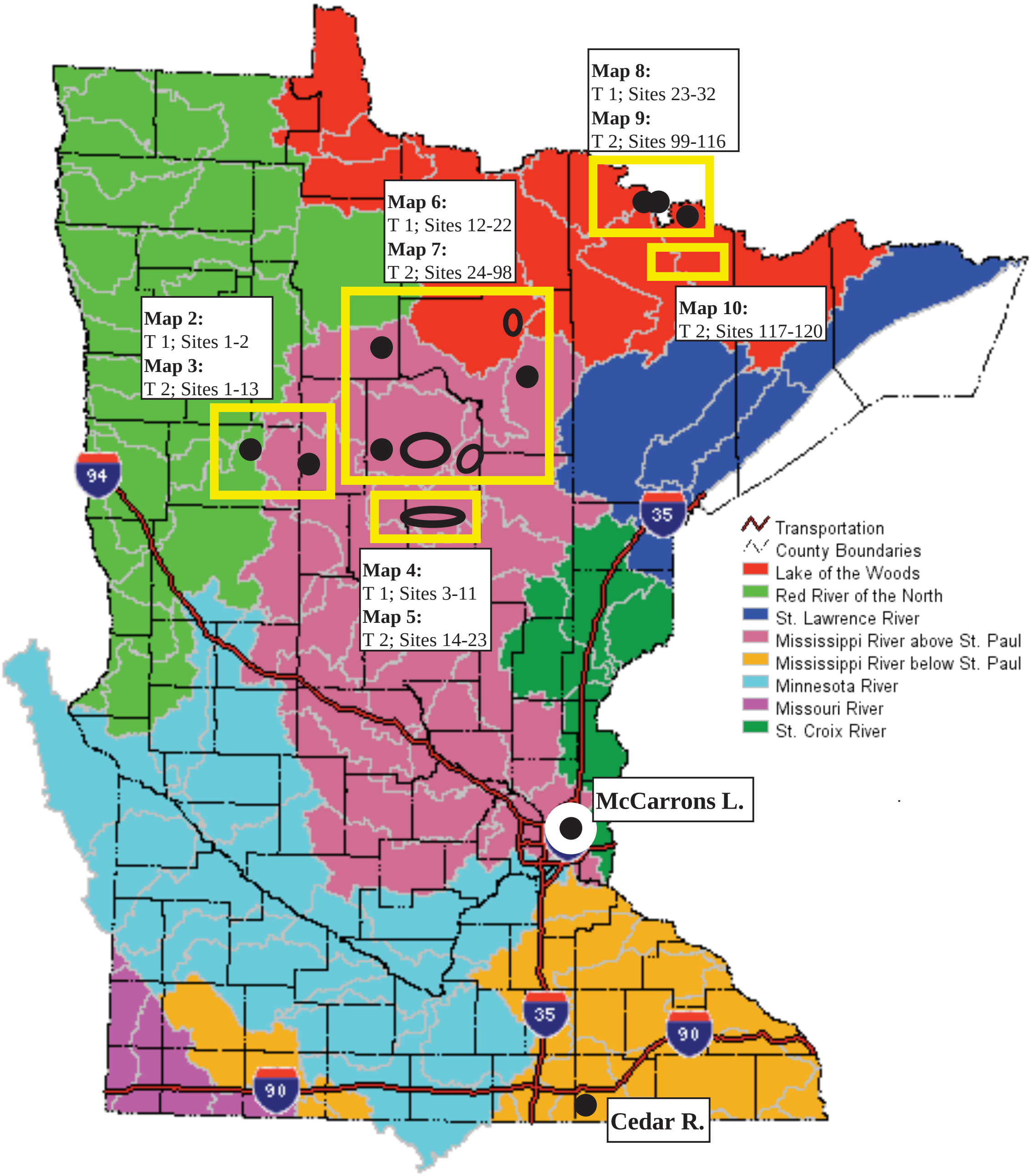
Appendix A – Maps

Maps 2–10 created with Google Earth images.

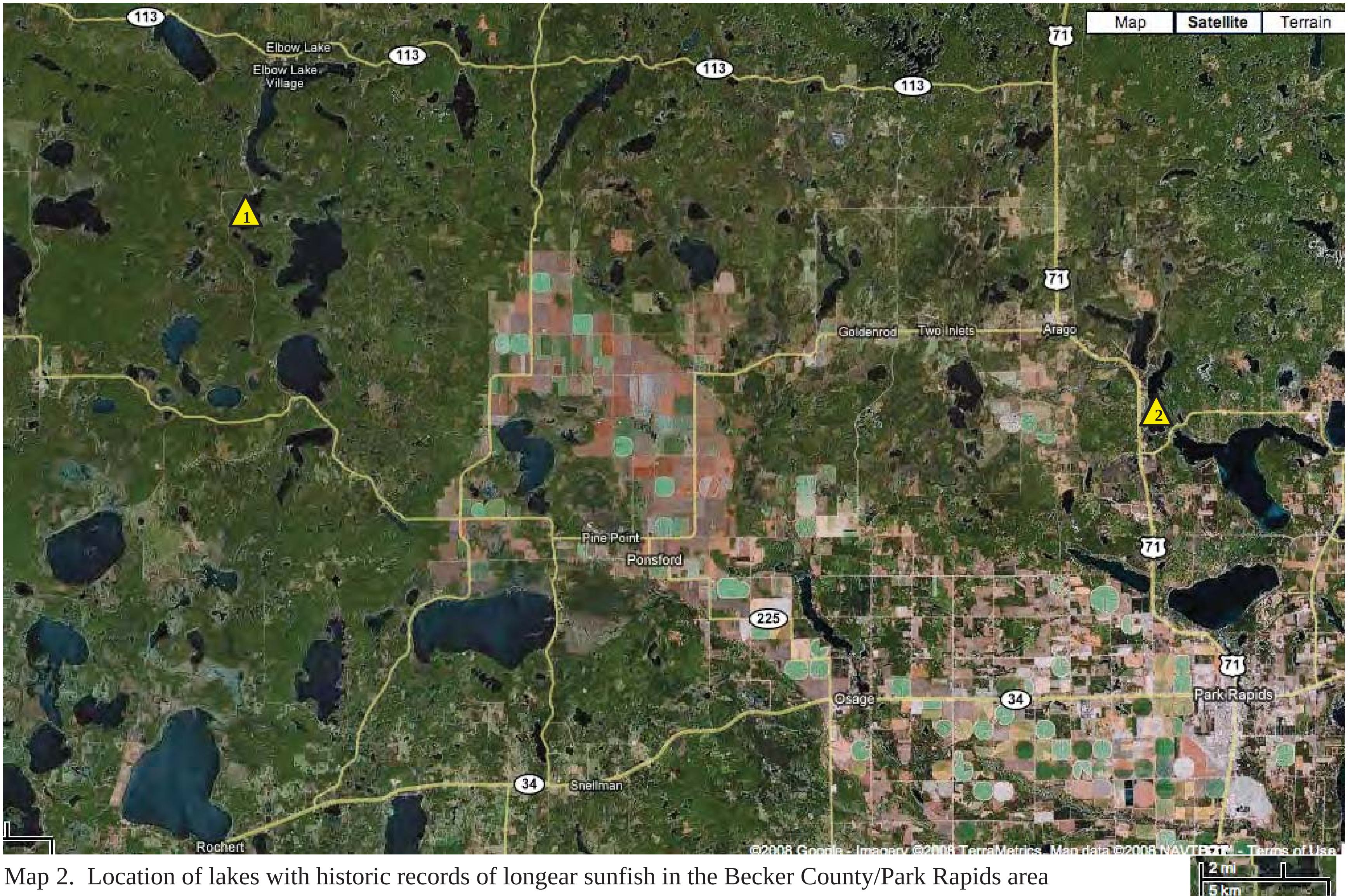
Map 1. General locations of all historic sites of longear sunfish in Minnesota (data from K. Schmidt, MN DNR).

Maps 2, 4, 6, 8.
Enlarged sections of Map 1 showing all historic sites of longear sunfish in Minnesota (data from K. Schmidt, MN DNR). The numbers correspond to Table 1, Appendix B.

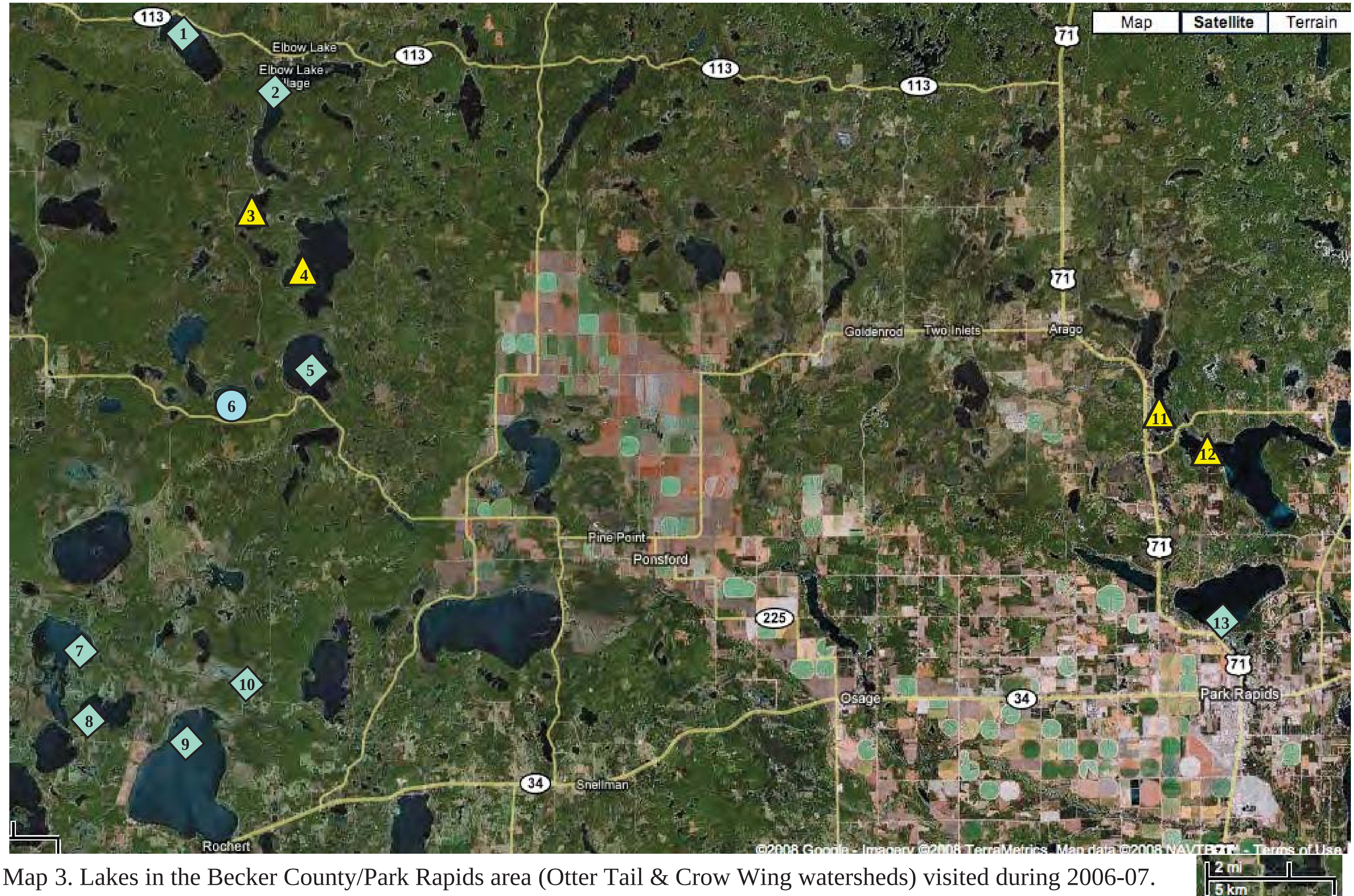
Maps 3, 5, 7, 9, 10.
Locations of all sites visited during 2006-2007. The yellow triangles indicate sites where longears were found; diamonds indicate sites where longears were not found; circles indicate lakes that were sampled incompletely and need to be visited in the future. The numbers correspond to Table 2, Appendix B.



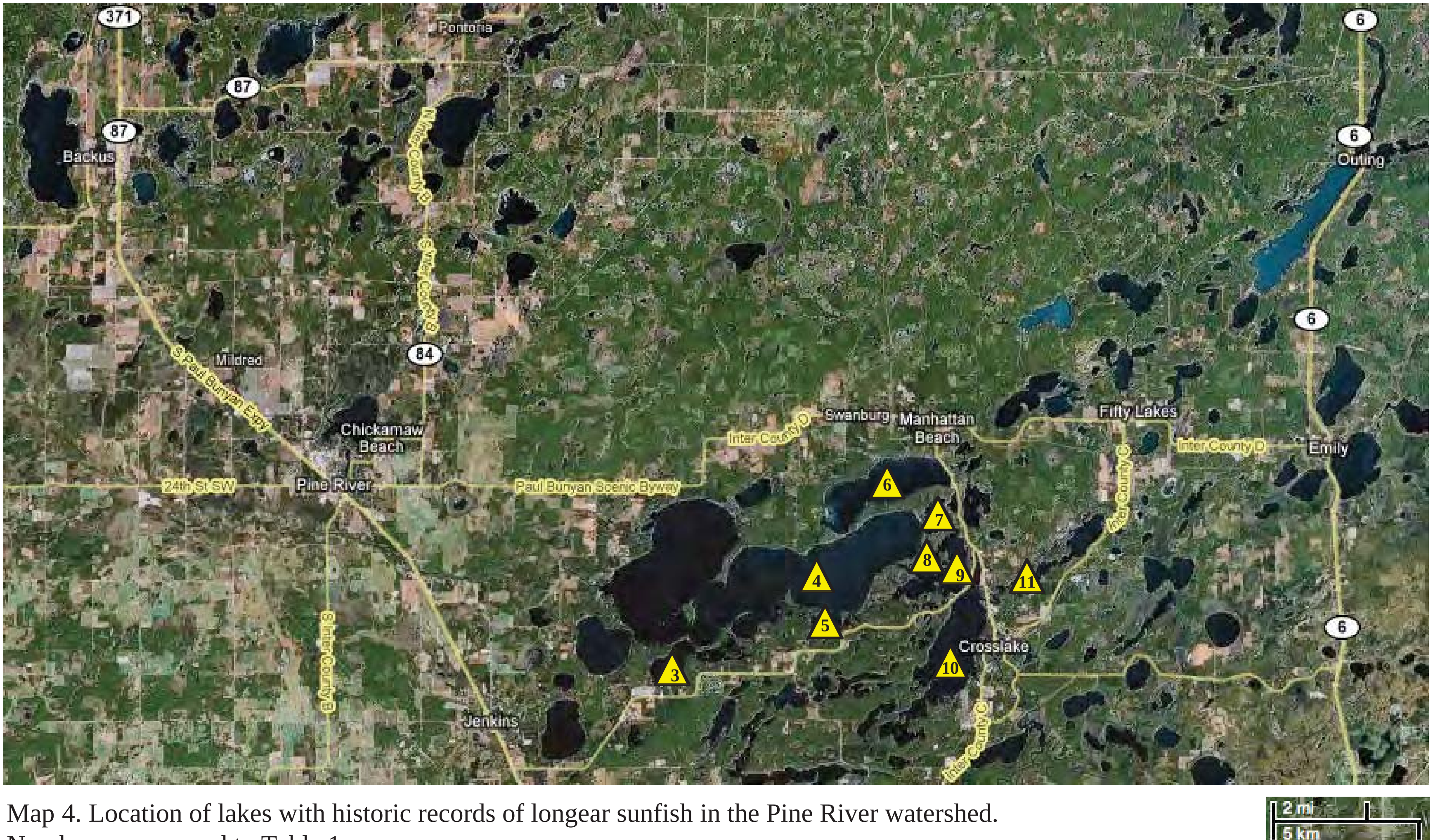
Map 1. General locations (black circles/ovals) of the historic records of longear sunfish within Minnesota. The areas enclosed by the yellow boxes are enlarged in Maps 2-10. T1 = Table 1, T2 = Table 2. The map, which also identifies the eight major basins and outlines the major watersheds in Minnesota, is modified from the following website: (http://gisdmnspl.cr.usgs.gov/cgi-bin/mapserv.exe?map=c:/apache2/htdocs/watershed/major_basins.map).



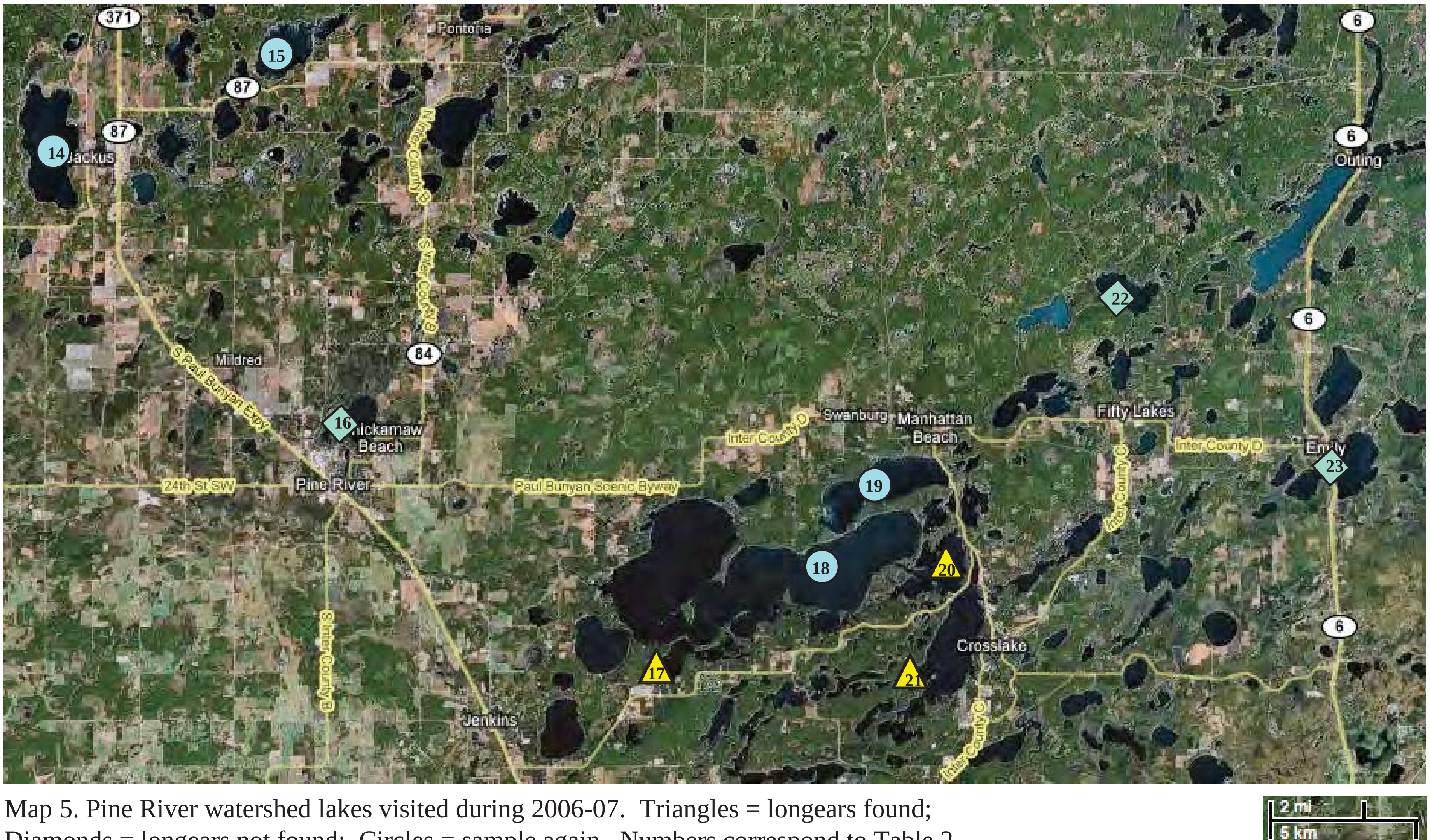
Map 2. Location of lakes with historic records of longear sunfish in the Becker County/Park Rapids area (Otter Tail River & Crow Wing River watersheds). Numbers correspond to Table 1.



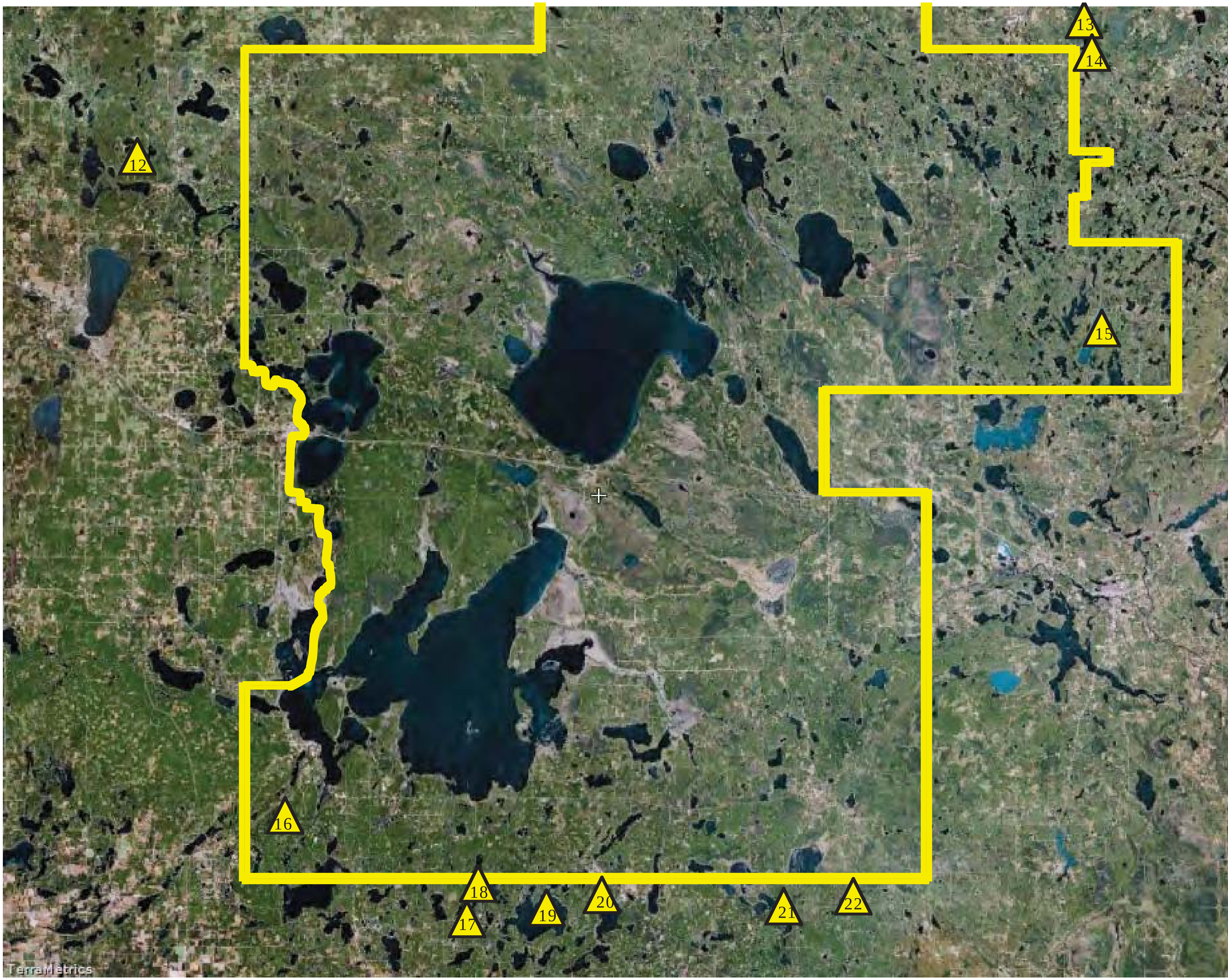
Map 3. Lakes in the Becker County/Park Rapids area (Otter Tail & Crow Wing watersheds) visited during 2006-07. Triangles = longears found; Diamonds = longears not found; Circles = sample again. Numbers correspond to Table 2.



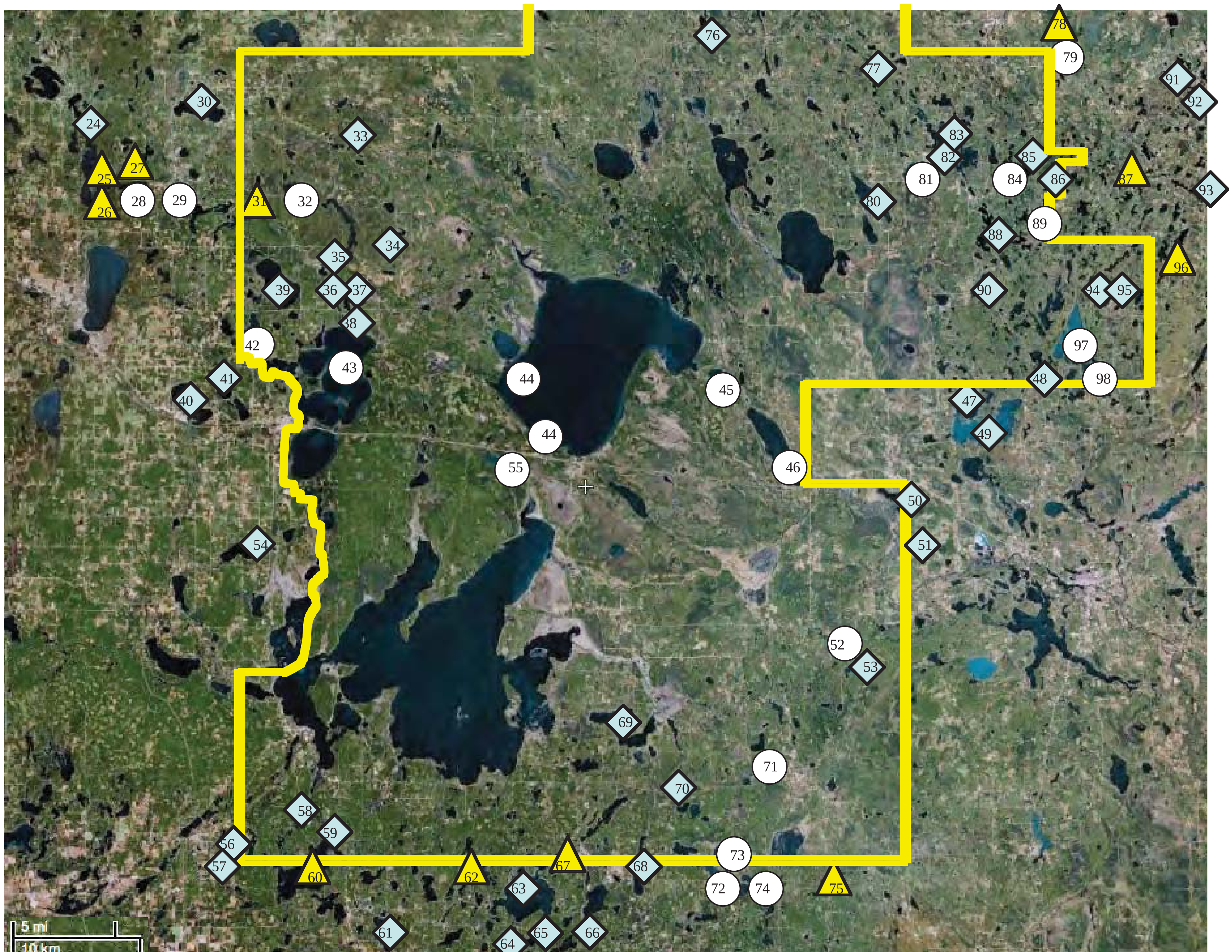
Map 4. Location of lakes with historic records of longear sunfish in the Pine River watershed. Numbers correspond to Table 1.



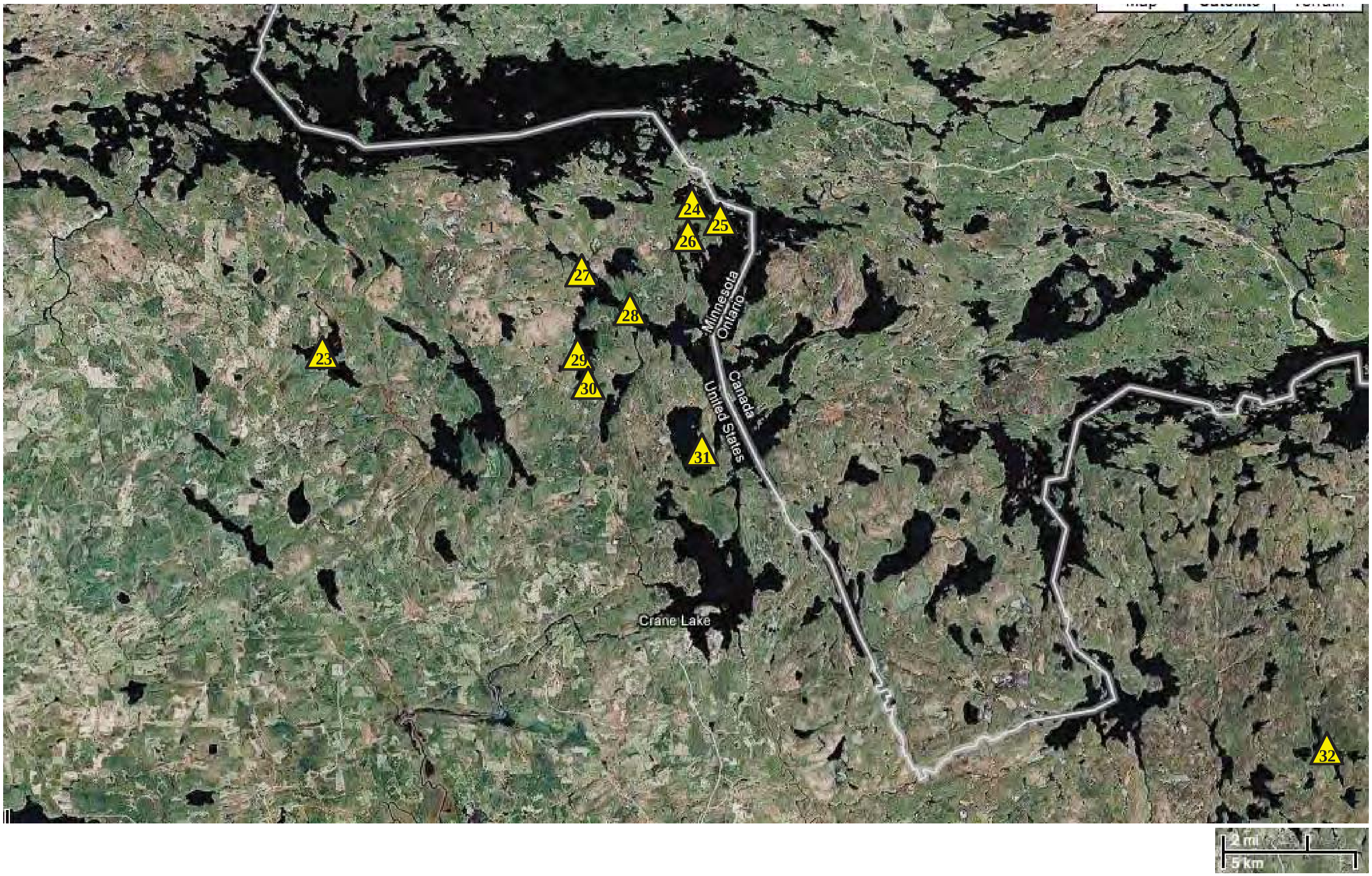
Map 5. Pine River watershed lakes visited during 2006-07. Triangles = longears found; Diamonds = longears not found; Circles = sample again. Numbers correspond to Table 2.



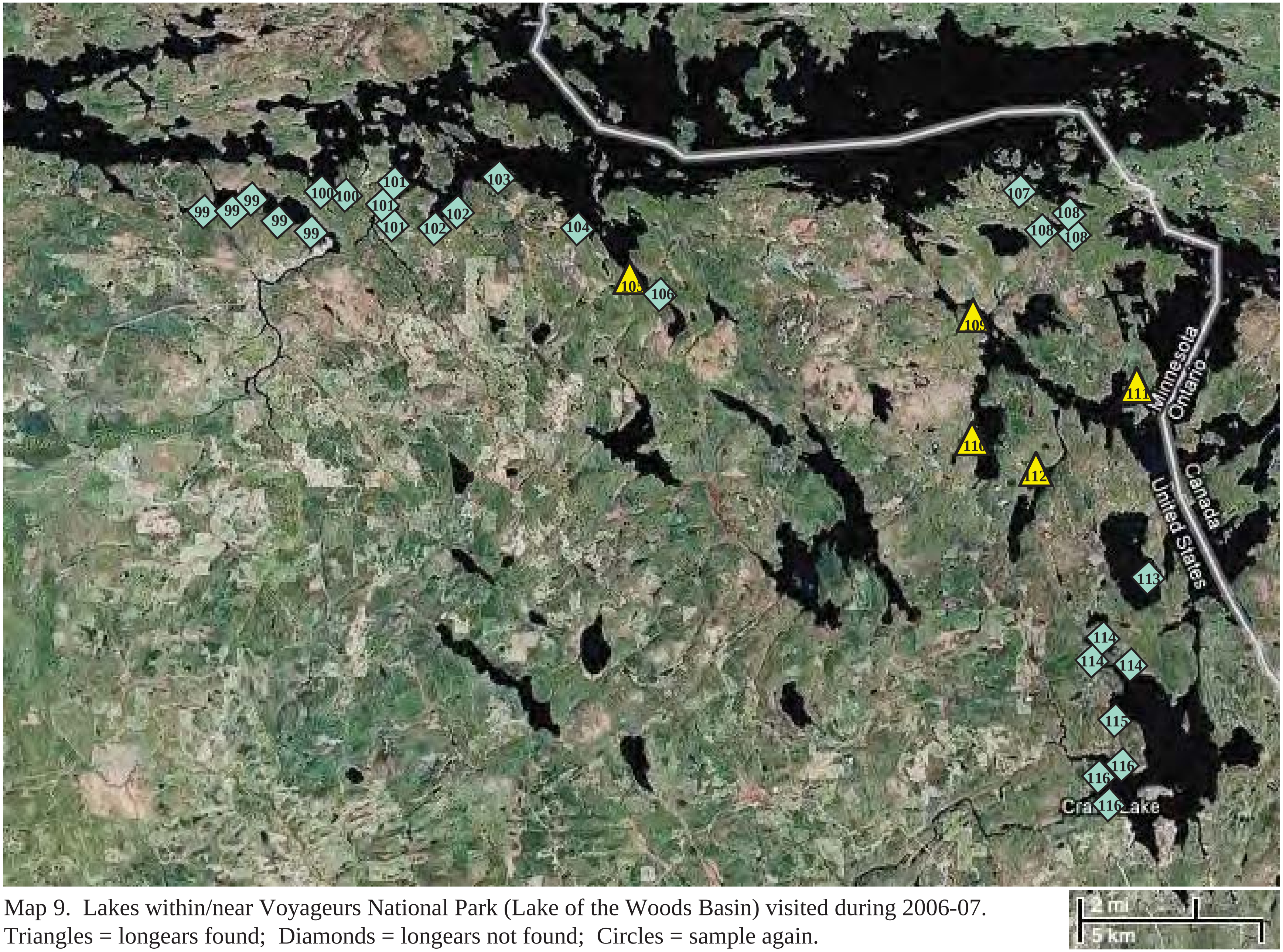
Map 6. Location of lakes with historic records of longear sunfish within/near the CNF (boundary outlined in yellow). Numbers correspond to Table 1.



Map 7. CNF & area lakes visited during 2006-07. Triangles = longears found; Diamonds = longears not found; Circles = sample again. Numbers correspond to Table 2.



Map 8. Historic records of longear sunfish within Voyageurs National Park and the Boundary Waters Canoe Area Wilderness. Numbers correspond to Table 1.



Map 9. Lakes within/near Voyageurs National Park (Lake of the Woods Basin) visited during 2006-07.
Triangles = longears found; Diamonds = longears not found; Circles = sample again.
Numbers correspond to Table 2.

Map 10. Echo Trail Lakes (Lake of the Woods Basin) visited during 2006-07. Triangles = longears found; Diamonds = longears not found; Circles = sample again. Numbers correspond to Table 2.

Appendix B – Tables

Table 1. List of lakes that contain historic records of the longear sunfish. The numbers in the left hand column correspond to Maps 2, 4, 6, and 8, Appendix A.

Table 2. Locations visited during 2006-07, with general notes on lake habitat, sampling results, and whether a lake needs to be sampled further. The numbers in the left hand column correspond to Maps 3, 5, 7, 9, and 10, Appendix A.

Table 3. Information about and some protocol specifics for the four microsatellite loci used in this study. All four loci were originally developed by Schable et al. (2002) for *L. marginatus*. "d" represents the Shannon-Weaver diversity index.

Table 4. Mitochondrial cytochrome *b* haplotype (A-T) frequencies at each site sampled (n = number of individuals sampled). A shaded box indicates a haplotype unique to that site. "*" indicates a sequence from Harris et al. (2005). See Figures AA and BB for hypotheses of genetic relatedness among haplotypes.

Table 5. Mitochondrial cytochrome *b* haplotype frequencies in Minnesota by collection site (italics), Major Watershed (bold) and Basin (bold caps), where n = number of individuals sampled and an "*" indicates a haplotype unique to that group. Mitochondrial cytochrome *b* haplotype frequencies in Minnesota by collection site (italics), Major Watershed (bold) and Basin (bold caps), where n = number of individuals sampled and an "*" indicates a haplotype unique to that group.

Table 6. Summary of Hardy-Weinberg genetic equilibrium tests for Minnesota populations defined at all three levels (collection site in italics, Major Watershed in bold, and Basin in bold caps). Data reported include number of individuals genotyped (n), and observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) for each locus.

Table 7. Summary of Hardy-Weinberg genetic equilibrium tests for non-Minnesota populations defined at all three levels (collection site in italics, Major Watershed in bold, and Basin in bold caps). Data reported include number of individuals genotyped (n), and observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) for each locus.

Table 8. Analysis of molecular variance (AMOVA) results for microsatellite data. Three AMOVAs were run, each on a data set where populations were defined as Basins, Major Watersheds, and Minnesota collection sites. Percent of variation refers to the amount of total variation attributed to variation at a given level, and F_{ST} refers to the amount of genetic structure when the populations are defined in the given way in an analysis.

Table 9. Table of pairwise chord distances among the 13 Major Watersheds.

Table 10. Table of pairwise chord distances among populations from 18 Minnesota collection sites.

Table 11. Minnesota populations containing unique microsatellite alleles. The three levels of population designation (collection site, Major Watershed, Basin) are included. Alleles are given in total nucleotide length.

Table 12. Average heterozygosities by locus and then averaged overall for the 18 Minnesota collection sites.

Table 1.

Historic localities for longear sunfishes in MN (K. Schmidt, MN DNR database). Numbers in the left hand column refer to numbers on Maps 2, 4, 6, and 8, Appendix A. Township/Range/Section numbers are for quick reference and do not necessarily include all Section #'s.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Year of last record
Map 2: Becker Co./Park Rapids Area							
1	**Little Bemidji Lake** 4 mi S Elbow Lake Village						
	Becker	142	39	23, 36	3023400	Otter Tail R – Red River of the North	2005
2	**Eagle Lake** 6 mi N Park Rapids						
	Hubbard	141	35	15, 22	29025600	Crow Wing River – Mississippi R	2005
Map 4: Pine River System							
3	**Bertha Lake** 7 mi WSW Cross Lake (town of)						
	Crow Wing	137	28	20	18035500	Pine River – Upper Mississippi R	2002
4	**Whitefish Lake** 3 mi WNW Cross Lake (town of)						
	Crow Wing	137	28	13	18031000	Pine River – Upper Mississippi R	2001
5	**Pig Lake** 4 mi W Cross Lake (town of)						
	Crow Wing	137	28	14	18035400	Pine River – Upper Mississippi R	2001
6	**Big Trout Lake** 4 mi NW Cross Lake (town of)						
	Crow Wing	138	27	31	18031500	Pine River – Upper Mississippi R	1990
7	**Island-Loon Lake** 5 mi SSE Walker						
	Crow Wing	137	27	5	18026900	Pine River – Upper Mississippi R	2001
8	**Hen Lake** 3 mi NW Cross Lake (town of)						
	Crow Wing	137	28	7	-------	Pine River – Upper Mississippi R	1995
9	**Rush Lake** 2 mi NW Cross Lake (town of)						
	Crow Wing	137	28	8	18031100	Pine River – Upper Mississippi R	2001
10	**Cross Lake Reservoir** 2 mi SW Cross Lake (town of)						
	Crow Wing	137	27	30	18031200	Pine River – Upper Mississippi R	2001
11	**Daggett Lake** 2 mi NE Cross Lake (town of)						
	Cass	137	27	16	18027100	Pine River – Upper Mississippi R	2001

Table 1.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)						
County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Year of last record
Map 6: Chippewa National Forest & Surrounding Area (Mississippi R/Headwaters, Leech Lake R, Mississippi R/Grand Rapids, Big Fk R, Little Fk R)						
12 Three Island Lake 3 mi NW town of Turtle River						
Beltrami	148	33	24	4013400	Turtle R – Miss R (Headwaters) – Upper Miss R	1975
13 Pine Lake Scenic State Park						
Itasca	61	25	32	----	Big Fk R – Lake of the Woods	1992
14 Coon-Sandwick Lake Scenic State Park						
Itasca	60, 61	25	6, 32	31052400	Big Fk R – Lake of the Woods	1992
15 Trout Lake 11 mi SE Marcell						
Itasca	58	25	29, 32	31041000	Prairie R – Miss R (Grand Rapids) – Upper Miss R	1945
16 Anoway Lake 5 mi SSE Walker						
Cass	141	31	8	----	Shingobee – Leech Lake R – Upper Miss R	2005
17 Kid Lake 8 mi WSW Longville						
Cass	140	29	7, 8	11026200	Boy R – Leech Lake R – Upper Miss R	1975
18 Baby Lake 7 mi W Longville						
Cass	140	29	8, 9	11028300	Boy R – Leech Lake R – Upper Miss R	1995
19 Woman Lake 3 mi SW Longville						
Cass	140	28, 29		11020100	Boy R – Leech Lake R – Upper Miss R	1986
20 Cooper Lake 3 mi SW Longville						
Cass	140	28	3	11016300	Boy R – Leech Lake R – Upper Miss R	1987
21 Thunder Lake 3 mi SW Longville						
Cass	140	26	15	11006200	Willow R – Miss R (Grand Rapids) – Upper Miss R	2000
22 Little Thunder Lake 3 mi SW Longville						
Cass	140	25	7	11000900	Willow R – Miss R (Grand Rapids) – Upper Miss R	2000

Table 1.
Concluded.

	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Year of last record
Map 8: Voyageurs National Park & Area; Boundary Waters Canoe Area Wilderness							
23	**Little Johnson Lake** (outlet flows north into Voyageurs National Park)						
	St. Louis	68	18	20	69076000	Rainy R – Lake of the Woods	1993
	Sand Point Lake Voyageurs National Park, eastern boundary of Park						
	St. Louis				69076000	Rainy R – Lake of the Woods	
24		69	17	34	near South Island, VNP Seine Locality #1		1989
25		69	17	35	near South Island, VNP Seine Locality #3		1989
26		68	17	2	Swansons Bay, VNP Seine Locality #4		1989
27		68	17	8	Grassy Bay, VNP Seine Locality #10		1991
28		68	17	16	Grassy Bay, VNP Seine Locality #7		1989
29		68	17	20	Browns Bay, VNP Seine Locality #9		1991
30		68	17	20	Browns Bay, VNP Seine Locality #8		1989
31	**Mukooda Lake** Voyageurs National Park, SE corner of Park						
	St. Louis	68	17	35	69068400	Rainy R – Lake of the Woods	1997
32	**Hustler Lake** BWCA, 6 mi NE Lake Jeaneatte State Forest, Hwy 116						
	St. Louis	66	14	5	69034300	Rainy R (Headwaters) – Lake of the Woods	1974

Table 2.

Lakes that were sampled or reconnoitered for longear sunfishes during 2006 and 2007. Numbers in the left hand column refer to numbers on Map 3 (#'s 1-13), 5 (#'s 14-23), 7 (#'s 24-98), 9 (#'s 99-116), or 10 (#'s 117-120), Appendix A. Township/Range/Section numbers are for quick reference and do not necessarily include all Section #'s visited.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
Map 3: Becker Co./Park Rapids Area											
1	**Tulaby Lake** 3 mi W Elbow Lake Village										
	Mahnomen	143	39	34	44000300	Otter Tail R – Red River of the North	6/13/06	No	N	-	No
	Notes: Extensive growths of filamentous algal mats in the shallows.										
2	**Elbow Lake** 12 mi ENE White Earth (town of)										
	Becker	142	37	8	3015900	Otter Tail R – Red River of the North	6/13/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
3	**Little Bemidji Lake** 4 mi S Elbow Lake Village										
	Becker	142	39	23, 36	3023400	Otter Tail R – Red River of the North	6/13/06	Yes	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
4	**Many Point Lake** **6** mi S Elbow Lake Village										
	Becker	142	39	36	3015800	Otter Tail R – Red River of the North	6/13/06	No	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
5	**Round Lake** 9 mi S Elbow Lake Village										
	Becker	141	38	7, 18	3015500	Otter Tail R – Red River of the North	6/13/06	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears; excessive amounts of filamentous algae along northern shore; sampled again 6/6/07.										
6	**Waboose Lake** **10** mi SSW Elbow Lake Village										
	Becker	142	39	36	3015800	Otter Tail R – Red River of the North	6/13/06	No	-	-	Yes
	Notes: Reconnoitered only; extensive shallows present.										

Table 2.
Continued.

	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)								2006-07 Results		
	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	Y/N	Relative Abundance	Sample Again?
7	**Tamarack Lake** Tamarack National Wildlife Refuge; NE Detroit Lakes										
	Becker	140	39	18	3024100	Otter Tail R – Red River of the North	6/6/07	No	N	-	No
	Notes: Tamarack National Wildlife Refuge; waters with "bog-stain" coloration which are not waters in which longears are generally found.										
8	**Pine Lake** Tamarack National Wildlife Refuge; NE Detroit Lakes										
	Becker	140	39	30	3020000	Otter Tail R – Red River of the North	6/6/07	No	N	-	No
	Notes: Tamarack National Wildlife Refuge; waters with "bog-stain" coloration which are not waters in which longears are generally found.										
9	**Height of Land Lake** Tamarack National Wildlife Refuge; NE Detroit Lakes										
	Becker	140	39	27	3019500	Otter Tail R – Red River of the North	6/6/07	No	N	-	No
	Notes: Tamarack National Wildlife Refuge; waters with "bog-stain" coloration which are not waters in which longears are generally found.										
10	**Rice Lake** Tamarack National Wildlife Refuge; NE Detroit Lakes										
	Becker	140	39	23	3020100	Otter Tail R – Red River of the North	6/6/07	No	N	-	No
	Notes: Tamarack National Wildlife Refuge; waters with "bog-stain" coloration which are not waters in which longears are generally found.										
11	**Eagle Lake #1** 6 mi N Park Rapids										
	Hubbard	141	35	15, 22	29025600	Crow Wing R – Upper Mississippi R	6/12/06	Yes	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
12	**Potato Lake** 5 mi N Park Rapids										
	Hubbard	141	35	23	29024300	Crow Wing R – Upper Mississippi R	6/7/07	No	Y	Common	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
13	**Fish Hook Lake** 0.5 mi N Park Rapids										
	Hubbard	141	35	14	29024200	Crow Wing R – Upper Mississippi R	6/6/07	No	N	-	No
	Notes: Extensive shallows, but shoreline altered; most emergent vegetation beds have been removed										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

#	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
Map 5: Pine River System											
14	**Pine Mountain Lake** 12										
	Cass	139	30	24	11041100	Pine River – Upper Mississippi R	7/16/06	No	N	-	Yes
	Notes: Extensive shallows & bullrush beds, but somewhat of a "sand lake" that may suitable habitat for longears. Sample again.										
15	**Big Portage Lake** 12										
	Cass	139	30	24	11041100	Pine River – Upper Mississippi R	7/16/06	No	N	-	Yes
	Notes: Extensive shallows, but somewhat of a "sand lake" that may suitable habitat for longears. Limited emergent vegetation. Sample again.										
16	**Norway Lake** 13 mi										
	Cass	138	29	30	11030700	Pine River – Upper Mississippi R	7/16/06	No	N	-	No
	Notes: Weedy lake, not much open water habitat; no extensive shallows with emergent vegetation beds.										
17	**Bertha Lake** 7 mi WSW Cross Lake (town of)										
	Crow Wing	137	28	20	18035500	Pine River – Upper Mississippi R	7/19/06	Yes	Y	Abundant	No
	Notes: Much development of shoreline; limited sections of extensive shallows & bullrush beds that form ideal spawning habitat.										
18	**Whitefish Lake** 3 mi WNW Cross Lake (town of)										
	Crow Wing	137	28	13	18031000	Pine River – Upper Mississippi R	6/1/07	Yes	N	-	Yes
	Notes: Entire shoreline of lower Whitefish Lake surveyed. Much development of shoreline, nearly complete removal of emergent vegetation from the shallows. Upper Whitefidh Lake needs to be sampled.										
19	**Big Trout Lake** 4 mi NW Cross Lake (town of)										
	Crow Wing	138	27	31	18031500	Pine River – Upper Mississippi R	7/19/06	Yes	N	-	Yes
	Notes: Much development of shoreline, extensive removal of emergent vegetation.										
20	**Rush/Hen Lake** 2 mi NW Cross Lake (town of)										
	Crow Wing	137	28	8	18031100	Pine River – Upper Mississippi R	6/1/07	Yes	Y	Present	No
	Notes: Much development of shoreline; longears found in small cove on NE east side of Rush Lake.										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

#	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
21	**Cross Lake Reservoir** 2 mi SW Cross Lake (town of)										
	Crow Wing	137	27	30	18031200	Pine River – Upper Mississippi R	7/18/06	Yes	Y	Present	No
	Notes: Much development of shoreline, extensive removal of emergent vegetation.										
22	**Mitchell Lake** 5 mi NW Emily										
	Crow Wing	138	27	12	18029400	Pine River – Upper Mississippi R	6/2/07	No	N	-	No
	Notes: Very mucky substrate; not ideal for longears.										
23	**Emily Lake** 1 mi S Emily Lake (town of)										
	Crow Wing	138	26	34	18020300	Pine River – Upper Mississippi R	6/2/07	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
	Map 7: Chippewa National Forest & Nearby Areas										
24	**Lake Julia** 8 mi NW town of Turtle River										
	Beltrami	148	33	5	4016600	Turtle R – Miss R (Headwaters) – Upper Miss R	6/5/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
25	**Turtle Lake** 5 mi W town of Turtle River										
	Beltrami	148	33	33	4015900	Turtle R – Miss R (Headwaters) – Upper Miss R	6/28/07	No	Y	Abundant	No
	Notes: Extensive shallows with emergent vegetation offer ideal spawning habitat.										
26	**Movil Lake** 5 mi WSW town of Turtle River										
	Beltrami	148	33	28	4015200	Turtle R – Miss R (Headwaters) – Upper Miss R	6/14/06	No	Y	Abundant	No
	Notes: Extensive shallows with emergent vegetation offer ideal spawning habitat. This lake also visited 6/04/07, 6/28/07 and 8/9/07.										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
27	**Three Island Lake** 3 mi NW town of Turtle River										
	Beltrami	148	33	24	4013400	Turtle R – Miss R (Headwaters) – Upper Miss R	6/5/07	Yes	Y	Present	No
	Notes: Shoreline ringed with beds of emergent vegetation; lake levels artifically raised by spillway; longears found spawning in the only extensive area of shallows in the lake.										
28	**Beltrami Lake** 3 mi W town of Turtle River										
	Beltrami	148	33	33	4013500	Turtle R – Miss R (Headwaters) – Upper Miss R	6/4/07	No	N	-	Yes
	Notes: Strong winds/rough waters prevented from sampling effectively. Resample this lake.										
29	**Turtle River Lake** SE town of Turtle River										
	Beltrami	148	32	33	4011100	Turtle R – Miss R (Headwaters) – Upper Miss R	6/4/07	No	N	-	Yes
	Notes: Strong winds/rough waters prevented from sampling effectively. Resample this lake.										
30	**Gull Lake** NW Tenstrike										
	Beltrami	148	32	4	4012000	Turtle R – Miss R (Headwaters) – Upper Miss R	6/5/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
31	**Turtle River** 5 mi S Tenstrike @ CR 307 ("Three Culverts Rd")										
	Beltrami	148	31	31	----	Turtle R – Miss R (Headwaters) – Upper Miss R	8/10/07	No	Y	Abundant	No
	Notes: Rice beds within the shallow backwaters and along the banks provide ideal habitat, downstream (Hwy 22) without backwaters.										
32	**South Twin Lake** 7 mi SE Tenstrike										
	Beltrami	148	31	35	4005300	Turtle R – Miss R (Headwaters) – Upper Miss R	8/10/07	No	N	-	Yes
	Notes: Sand ramp too soft to launch boat; sampling limited to shoreline near ramp. Much potential habitat. Revisit this lake.										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	Relative Abundance	Sample Again?
33	**Rabideau Lake** 7 mi ESE Tenstrike										
	Beltrami	148	32	17	4003400	Turtle R – Miss R (Headwaters) – Upper Miss R	6/14/06	No	N	-	No
	Notes: Thick muck substrate, extensive growths of filamentous algal mats in the shallows.										
34	**Moose Lake** 4 mi N Pennington										
	Beltrami	147	30	22	4001100	Turtle R – Miss R (Headwaters) – Upper Miss R	6/14/06	No	N	-	No
	Notes: Thick muck substrate, extensive growths of filamentous algal mats in the shallows.										
35	**Big Rice Lake** 4.5 mi NW Pennington, access via Kitchi Lake										
	Beltrami	147	31, 30	25, 30	4003100	Turtle R – Miss R (Headwaters) – Upper Miss R	8/9/07	No	N	-	No
	Notes: Shoreline ringed with extensive beds of emergent vegetation mats/islands; no extensive shallows to provide spawning grounds.										
36	**Little Rice Lake** 3.5 mi NW Pennington, access via Kitchi Lake										
	Beltrami	147	30	31	4001500	Turtle R – Miss R (Headwaters) – Upper Miss R	8/9/07	No	N	-	No
	Notes: Shoreline ringed with extensive beds of emergent vegetation mats/islands; no extensive shallows to provide spawning grounds.										
37	**Kitchi Lake** 1 mi NW Pennington, access via Kitchi Landing Resort (N side of lake)										
	Beltrami	147	30	32, 33	4000700	Turtle R – Miss R (Headwaters) – Upper Miss R	8/9/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
38	**Pug Hole Lake** 2.5 mi WSW Pennington, access via Kitchi Lake										
	Beltrami	146	30	7, 8	----	Turtle R – Miss R (Headwaters) – Upper Miss R	8/9/07	No	N	-	No
	Notes: No extensive shallows to provide spawning grounds.										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
39	**Big Lake** 6 mi WNW Pennington										
	Beltrami	147	31	34	4004900	Miss R (Headwaters) – Upper Miss R	8/10/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
40	**Grace Lake** 8 mi WNW town of Cass Lake										
	Hubbard	146	32	33	29007100	Miss R (Headwaters) – Upper Miss R	8/8/07	No	N	-	No
	Notes: Depth drops quickly from shoreline; shoreline heavily developed with residences, most emergent plants removed.										
41	**Wolf Lake** 5 mi NW town of Cass Lake										
	Beltrami	146	32	35	4007900	Miss R (Headwaters) – Upper Miss R	8/8/07	No	N	-	No
	Notes: Shallows have been altered by extensive removal of emergent plants rip-rap and boulders installed along much of shoreline.										
42	**Lake Andrusia** 7 mi NNW town of Cass Lake										
	Beltrami	146	31	7, 8	4003800	Miss R (Headwaters) – Upper Miss R	10/23/07	No	-	-	Yes
	Notes: Reconnoitered only. Much potential habitat; needs to be sampled.										
43	**Cass Lake** N of town of Cass Lake										
	Cass	145-6	30, 31	--	4003000	Miss R (Headwaters) – Upper Miss R	10/23/07	No	-	-	Yes
	Notes: High winds did not allow for boating. Much potential habitat. This lake needs to be surveyed extensively.										
44	**Winnibigoshish Lake** West side of lake @ boat ramp north of Mississippi River; and Richards Campground on S shore of lake										
	Itasca/Cass	--	--	--	11014700	Miss R (Headwaters) – Upper Miss R	10/23/07	No	-	-	Yes
	Notes: High winds did not allow for boating. Much potential habitat. This lake needs to be surveyed extensively.										
45	**Little Winnibigoshish Lake** 11 mi NW Deer River										
	Itasca	146	26	31	31085000	Miss R (Headwaters) – Upper Miss R	6/17/07	No	-	-	Yes
	Notes: Low water; could not launch boat so the shoreline near the ramp was sampled; extensive shallows & bullrush beds; this lake needs to be sampled more extensively.										

Table 2.
Continued.

	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)								2006-07 Results		
	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	Y/N	Relative Abundance	Sample Again?
46	**Ball Club Lake** 6 mi W Deer River										
	Itasca	145	26	35, 36	31081200	Miss R (Headwaters) – Upper Miss R	6/17/07	No	-	-	Yes
	Notes: Low water; could not launch boat so the shoreline near the ramp was sampled; extensive shallows & bullrush beds; this lake needs to be sampled more extensively.										
47	**Moose Lake** 6 mi NE Deer River										
	Itasca	57	26	29	31072200	Miss R (Headwaters) – Upper Miss R	6/21/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
48	**Pughole Lake** 11 mi NE Deer River; 1 mi N Hwy 60 along Hwy 38										
	Itasca	57	26	13, 24	31060200	Miss R (Headwaters) – Upper Miss R	6/21/07	No	N	-	No
	Notes: No extensive shallows to provide spawning grounds.										
49	**Deer Lake** 6 mi NE Deer River										
	Itasca	57	26	31, 32	31071900	Miss R (Headwaters) – Upper Miss R	6/21/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
50	**White Oak Lake** 2 mi S Deer River										
	Itasca	144	25	1	31077600	Miss R (Headwaters) – Upper Miss R	6/17/07	No	N	-	No
	Notes: Mississippi River bottomland lake; no extensive shallows.										
51	**Little White Oak Lake** 5 mi SSE Deer River										
	Itasca	55	27	2, 3	31074000	Miss R (Headwaters) – Upper Miss R	6/17/07	No	N	-	No
	Notes: Mississippi River bottomland lake; no extensive shallows.										
52	**Vermillion Lake** 9 mi NNE Remer										
	Cass	143	25	20, 29	11002900	Miss R (Headwaters) – Upper Miss R	6/17/07	No	-	-	Yes
	Notes: Sand ramp too soft to launch boat. Revisit this lake.										
	Other spp:										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
53	**Sugar Lake** 7 mi NNE Remer										
	Cas	143	25	33, 34	11002600	Miss R (Headwaters) – Upper Miss R	6/17/07	No	N	-	No
	Notes: No extensive shallows to provide spawning areas.										
54	**Steamboat Lake** 8 mi S town of Cass Lake										
	Cass	144	31	20	11050400	Leech Lake River – Upper Miss R		No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
55	**Portage Lake** 1 mi SW Ryan Village										
	Cass	145	29	26	11020400	Leech Lake River – Upper Miss R	10/23/07	No	-	-	Yes
	Notes: Reconnoitered only. Much potential habitat; needs to be sampled.										
56	**Shingobee Lake** 2 mi E Akeley						7/16/06	No	N	-	No
	Hubbard	141	32	26	29004300	Shingobee R – Leech – Upper Miss R					
	Notes: Heavily vegetated, steep shores; no extensive shallows to provide spawning areas.										
57	**Island Lake** 3 mi SE Akeley						7/16/06	No	N	-	No
	Hubbard	140	32	2	29000700	Shingobee R – Leech – Upper Miss R					
	Notes: Heavily vegetated, steep shores; no extensive shallows to provide spawning areas.										
58	**Shingobee River** 5 mi S Walker @ Rte 50						7/16/06	No	N	-	Yes
	Cass	141	31	15	----	Shingobee R – Leech – Upper Miss R					
	Notes: Flowing current throughout; no calm backwaters to provide habitat. The Shingobee watershed needs to be sampled thoroughly, however, since longears are documented from Anoway Lake.										
59	**Portage Lake** 6 mi SSW Walker @ Rte 50						6/4/07	No	N	-	No
	Cass	141	31	25, 26	11047600	Boy R – Leech – Upper Miss R					
	Notes: No extensive shallows to provide spawning areas.										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
60	**Ten Mile Lake** 5 mi S Walker @ Rte 50						8/24/06	No	Y	Present	No
	Cass	140/141	31		11041300	Boy R – Leech – Upper Miss R					
	Notes: One individual collected by Paul Radomski, MN DNR.										
61	**Pleasant Lake** 2 mi E Haackensack						6/3/07	No	N	-	No
	Cass	140	30	21	11038300	Boy R – Leech – Upper Miss R					
	Notes: Although marginal habitat existed along the northern shores no longears were found.										
62	**Baby Lake** 7 mi W Longville						6/3/07	Yes	Y	Abundant	No
	Cass	140	29	8, 9	11028300	Boy R – Leech – Upper Miss R					
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat. Sampled again 10/24/07; longears still common.										
63	**Woman Lake** 3 mi SW Longville						7/17/06	Yes	N	-	Yes
	Cass	140	28, 29		11020100	Boy R – Leech – Upper Miss R					
	Notes: Most of the shallow shoreline have been substantially altered by property/home development; few bullrush beds remain, longear habitat has been nearly eliminated. This lake needs to be sampled again in an attempt to find longears.										
64	**Blackwater Lake** 7 mi SW Longville						7/17/06	No	N	-	No
	Cass	140	29	25	11027400	Boy R – Leech – Upper Miss R					
	Notes: Heavily vegetated, steep shores; no extensive shallows to provide spawning areas.										
65	**Mule Lake** 6 mi SSW Longville						7/17/06	No	N	-	No
	Cass	140	29	28, 29	11020000	Boy R – Leech – Upper Miss R					
	Notes: No extensive shallows to provide spawning areas.										
66	**Wabedo Lake** 5 mi S Longville						6/3/07	No	N	-	Yes
	Cass	140	28	27	11017100	Boy R – Leech – Upper Miss R					
	Notes: The NE basin is a "sand lake" that does not appear to have suitable habitat for longears. Did not sample SW basin. Sampling of the SW basin is desirable.										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
67	**Girl Lake** Longville						7/17/06	No	Y	Abundant	No
	Cass	141	28	33, 34	11017400	Boy R – Leech – Upper Miss R					
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
68	**Inguadona Lake** 4 mi E Longville						7/17/06	No	N	-	No
	Cass	140	27	5, 8	11012000	Boy R – Leech – Upper Miss R					
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
69	**Boy Lake** 9 mi NNE Longville						6/2/07	No	N	-	No
	Cass	142	28	24	11014300	Boy R – Leech – Upper Miss R					
	Notes: Heavily vegetated, steep shores; no extensive shallows to provide spawning areas.										
70	**Big Sand Lake** 7 mi W Remer, Hwy 200						6/2/07	No	N	-	No
	Cass	141	26	34	11007700	Boy R – Leech – Upper Miss R					
	Notes: Sand ramp too soft to launch boat. This lake has much potential habitat and needs to be sampled.										
71	**Mabel Lake** 7 mi W Remer, Hwy 200						6/2/07	No	N	-	No
	Cass	141	27	10	11012100	Boy R – Leech – Upper Miss R					
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
72	**Upper Trelipe Lake** 3 mi SW Longville						7/18/06	No	N	-	Yes
	Cass	140	27	1	11010500	Boy R – Leech – Upper Miss R					
	Notes: Heavily vegetated in the few shallows, difficult to sample. Further sampling needed.										
73	**Laura Lake** 3 mi SW Longville						6/2/07	No	N	-	Yes
	Cass	141	26	31	11010400	Boy R – Leech – Upper Miss R					
	Notes: Sand ramp too soft to launch boat. This lake has much potential habitat and needs to be sampled.										

Table 2.
Continued.

#	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	Relative Abundance	Sample Again?
74	**Thunder Lake** 3 mi SW Longville						7/18/06	Yes	N	-	Yes
	Cass	140	26	15	11006200	Willow R – Miss R (Grand Rapids) – Upper Miss R					
	Notes: No extensive shallows; this lake does not appear to offer good spawning habit. However, longears have been recorded, so further sampling is recommended.										
75	**Little Thunder Lake** 3 mi SW Longville						7/18/06	Yes	Y	Common	No
	Cass	140	25	7	11000900	Willow R – Miss R (Grand Rapids) – Upper Miss R					
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
76	**Dora Lake** 0.5 mi W town of Dora Lake										
	Itasca	149	27	12	31088200	Big Fk R – Lake of the Woods	6/20/07	No	N	-	No
	Notes: No extensive shallows to provide spawning grounds.										
77	**Arrowhead Lake** 8.5 mi WSW Big Fork										
	Itasca	149	25	22, 23	31080500	Big Fk R – Lake of the Woods	6/20/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
78	**Pine Lake** Scenic State Park										
	Itasca	61	25	32	----	Big Fk R – Lake of the Woods	6/20/07	Yes	Y	Present	Yes
	Notes: Difficult shoreline to work (floating bogs, many snags in water), but longears are present. Sample further to determine relative abundance.										
79	**Coon-Sandwick Lake** Scenic State Park										
	Itasca	60, 61	25	6, 32	31052400	Big Fk R – Lake of the Woods	6/20/07	Yes	N	-	Yes
	Notes: Sampled 8/16/2006 and again in 2007. Longears not found; extensive shallows not present. Sample again to determine if a substantial breeding colony eixsts in this lake.										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

#	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
80	**Jessie Lake** 6 mi WSW Marcell										
	Itasca	148	25	2	31078600	Big Fk R – Lake of the Woods	6/20/07	No	-	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears.										
81	**Little Turtle Lake** 3 mi W Marcell										
	Itasca	59	27	9, 10	31077900	Big Fk R – Lake of the Woods	6/20/07	No	-	-	Yes
	Notes: High winds, could not launch boat. Longears not found near boat ramp, but this lake needs to be revisited since appropriate habitat appears to be present.										
82	**Turtle Lake** 4 mi NW Marcell										
	Itasca	60	27	36	31072500	Big Fk R – Lake of the Woods	8/15/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
83	**Maple Lake** 4.5 mi NW Marcell										
	Itasca	60	27	36	31077300	Big Fk R – Lake of the Woods	8/15/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
84	**Jack-the-Horse-Lake** 3 mi NE Marcell										
	Itasca	59	26	9, 10	31065700	Big Fk R – Lake of the Woods	6/19/07	No	N	-	Yes
	Notes: Could not launch boat; no concrete ramp. Extensive shallows and bullrush beds; need to sample more extensively.										
85	**Burns Lake** 4.5 mi NE Marcell										
	Itasca	59	26	2	31065400	Big Fk R – Lake of the Woods	8/15/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
86	**Clubhouse Lake** 5 mi E Marcell										
	Itasca	59	25	7, 18	31054000	Big Fk R – Lake of the Woods	6/19/07	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										

Table 2.
Continued.

	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
	Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)										
87	**Eagle Lake #2** 10 mi E Marcell										
	Itasca	59	25	11	31045400	Big Fk R – Lake of the Woods	8/16/06	No	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
88	**North Star Lake** 3.5 mi SE Marcell										
	Itasca	59	26	32, 33	31065300	Big Fk R – Lake of the Woods	8/15/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
89	**Big Island Lake** 4.5 mi ESE Marcell										
	Itasca	59	26	25, 26	31067100	Big Fk R – Lake of the Woods	6/19/07	No	N	-	Yes
	Notes: Could not launch boat; no concrete ramp. Extensive shallows and bullrush beds; need to sample more extensively.										
90	**Grave Lake** 7 mi S Marcell										
	Itasca	58	26	17, 20	31062400	Big Fk R – Lake of the Woods	8/15/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
91	**Lost Lake** 12 mi ESE Bigfork										
	Itasca	60	24	9	31028900	Little Fk R – Lake of the Woods	8/16/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
92	**Owen Lake** 13 mi ESE Bigfork										
	Itasca	60	24	15, 16	31029200	Little Fk R – Lake of the Woods	8/16/06	No	N	-	No
	Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
93	**Round Lake** 12 mi E Marcell										
	Itasca	59	24	11	31026800	Miss R (Grand Rapids) – Upper Miss R	6/18/07	No	N	-	No
	Notes: A "sand lake" that does not appear to have suitable habitat for longears; most of original beds of emergent vegetation appears to have been removed.										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)								2006-07 Results		
County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	Y/N	Relative Abundance	Sample Again?
94 Burnt Shanty Lake 9 mi SE Marcell										
Itasca	58	25	16	31042400	Miss R (Grand Rapids) – Upper Miss R	6/19/07	No	N	-	No
Notes: Maps indicated numerous shallows, but lake depths dropped off rapidly; no prime habitat for longears.										
95 Lost Moose Lake 10 mi SE Marcell										
Itasca	58	25	15	31043200	Miss R (Grand Rapids) – Upper Miss R	6/19/07	No	N	-	No
Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
96 Balsam Lake 15 mi SE Marcell										
Itasca	58	24	9	31025900	Miss R (Grand Rapids) – Upper Miss R	6/18/07	No	Y	Abundant	No
Notes: The southern basin offers extensive shallows & bullrush beds that form ideal habitat.										
97 Trout Lake 11 mi SE Marcell										
Itasca	58	25	29, 32	31041000	Miss R (Grand Rapids) – Upper Miss R	6/17/07	Yes	-	-	Yes
Notes: Low water; could not launch boat.										
98 Wabana Lake										
Itasca	57	25	9, 16	31039200	Miss R (Grand Rapids) – Upper Miss R	8/17/06	No	N	-	No
Notes: A "sand lake" that does not appear to have suitable habitat for longears.										

Table 2.
Continued.

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

#	County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
Map 9: Voyageurs National Park & Area; Boundary Waters Canoe Area Wilderness											
	Namakan Lake VNP; Numerous locations along side south of lake from Ash River Visitor's Center to Junction Bay										
	St. Louis				69069300	Rainy R (Rainy Lake) – Lake of the Woods					
99		69	19	31, 32	Sullivan Bay -- 5 sites		6/26/07	No	N	-	No
100		69	19	28, 29	Old Dutch Bay -- 2 sites		6/26/07	No	N	-	No
101		69	19	27, 34	Moose Bay -- 3 sites		6/26/07	No	N	-	No
102		69	19	34, 35	Hoist Bay -- 2 sites		6/26/07	No	N	-	No
103		69	19	26	Unnamed Cove southeast of Stevens Island		6/26/07	No	N	-	No
104		69	19	31	Unnamed Cove west of Postage Island		6/26/07	No	N	-	No
		Notes: Reconnoitered & sampled throughout Sullivan Bay, Old Dutch Bay, Moose Bay, and Hoist Bay. No extensive shallows; lake depths dropped off rapidly; no prime habitat for longears. High winds, heavy rains throughout the day.									
	Junction Bay, Namakan Lake VNP; @ mouth of Johnson River										
	St. Louis	68	18	5	69069300	Rainy R (Rainy Lake) – Lake of the Woods					
105					@ mouth of Johnson River		6/26/07	No	Y	Present	Yes
106					@ Net Lake portage		6/26/07	No	N	-	No
		Notes: Longears found in shallows along east side of cove, downstream of Johnson R. Falls. High winds, heavy rains throughout the day. Additional sampling in Junction Bay must be done to determine longear population status.									
107	**Namakan Lake** VNP; Unnamed Bay, SW of Rusty Island										
	St. Louis	69	17	29	69069300	Rainy R (Rainy Lake) – Lake of the Woods	6/28/06	No	N	-	No
		Notes: Muck substrate in bay; Lake depths dropped off rapidly; no prime habitat for longears.									
108	**Namakan Lake** VNP; 3 sites in Hammer Bay										
	St. Louis	68	17	33	69069300	Rainy R (Rainy Lake) – Lake of the Woods	6/28/06	No	N	-	No
		Notes: No extensive shallows; lake depths dropped off rapidly; no prime habitat for longears.									

Table 2.
Continued.

No.	**Lake Name** & General Location (usually expressed as the approx. distance from public boat ramp) / County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
109	**Grassy Bay, Sand Point Lake** Voyageurs National Park, VNP Seine Locality #10										
	St. Louis	68	17	8	69076000	Rainy R (Rainy Lake) – Lake of the Woods	6/27/06	Yes	Y	Abundant	No
	Notes: Longears limited to small cove that contained shallows, firm substrate, and bullrush beds that form ideal spawning habitat.										
110	**Brown's Bay, Sand Point Lake** Voyageurs National Park, VNP Seine Locality #9										
	St. Louis	68	17	20	69076000	Rainy R (Rainy Lake) – Lake of the Woods	6/27/06	Yes	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
111	**Staege Bay, Sand Point Lake** Voyageurs National Park										
	St. Louis	68	17	21	69076000	Rainy R (Rainy Lake) – Lake of the Woods	6/27/06	No	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
112	**Sand Point Lake** Voyageurs National Park; campsite NW of Feldt Channel										
	St. Louis	68	17	14	69076000	Rainy R (Rainy Lake) – Lake of the Woods	6/28/06	No	Y	Abundant	No
	Notes: Extensive shallows & bullrush beds that form ideal spawning habitat.										
113	**Mukooda Lake** Voyageurs National Park, SE corner of Park										
	St. Louis	68	17	35	69068400	Rainy R (Rainy Lake) – Lake of the Woods	6/27/06	Yes	N	-	Yes
	Notes: Water is crystal clear; depths increase rapidly. This is a "snd lake" with substrates of boulder, cobble, gravel, sand.										
	Crane Lake (outlet flows north into NE corner of Voyageurs National Park)										
	St. Louis				69061600	Vermillion R – Lake of the Woods					
114		67	17	2, 3	NW Arm -- 3 sites		6/26/06	No	N	-	No
115		67	17	11	west side		6/26/06	No	N	-	No
116		67	17	14	Rollick Bay -- 3 sites		6/26/06	No	N	-	No
	Notes: Waters have "bog-stain" coloration which are not waters in which longears are generally found.										

Table 2.
Concluded.

County	T (N)	R (W)	Sec.	MN DNR LakeFinder	Watershed - Basin	Date(s)	Historical Record?	2006-07 Results Y/N	2006-07 Results Relative Abundance	Sample Again?
Map 10: Lakes along Echo Trail Corridor (Hwy 116) through BWCA										
117 Echo Lake 7 mi NW Ely along Hwy 116 (Echo Trail)										
St. Louis	64	11	30	69061500	Vermillion R – Lake of the Woods	6/25/07	No	N	-	No
Notes: Lake depths dropped off rapidly; no prime habitat for longears. Algal blooms along shoreline.										
118 Jeanette Lake 10 mi E Buyck along Hwy 116 (Echo Trail)										
St. Louis	65	14	7	69045600	Rainy R (Headwaters) – Lake of the Woods	6/25/07	No	N	-	No
Notes: Lake depths dropped off rapidly; no prime habitat for longears.										
119 Big Lake 12 mi NW Ely along Hwy 116 (Echo Trail)										
St. Louis	65	12	27	69019000	Rainy R (Headwaters) – Lake of the Woods	6/25/07	No	N	-	No
Notes: Lake depths dropped off rapidly; no prime habitat for longears. Algal blooms along shoreline.										
120 Fenske Lake 5 mi NE Buyck along Hwy 116 (Echo Trail)										
St. Louis	66	16	26	69008500	Rainy R (Headwaters) – Lake of the Woods	6/25/07	No	N	-	No
Notes: Lake depths dropped off rapidly; no prime habitat for longears.										

Lake Name & General Location (usually expressed as the approx. distance from public boat ramp)

Table 3. Information about and some protocol specifics for the four microsatellite loci used in this study. All four loci were originally developed by Schable et al. (2002) for *L. marginatus*. Value “d” represents the Shannon-Weaver diversity index.

Locus	Repeat	Touchdown Temperatures (C)	Number of Alleles (MN only)	Allele Range (bp)	d (MN only)
Lmar10	AGAT	60 for 5 cycles 60 to 50 C in 20 cycles 53 for 10 cycles	19 (13)	227 – 323	7.5 (6.4)
Lmar11	ATCC	60 for 5 cycles 60 to 47.5 C in 25 cycles 55 for 10 cycles	15 (9)	187 – 255	2.3 (2.1)
Lmar12	ATCC	60 for 5 cycles 60 to 50 C in 20 cycles 53 for 10 cycles	11 (4)	258 – 326	1.5 (1.2)
Lmar14	AGAT	60 for 5 cycles 60 to 47.5 C in 25 cycles 55 for 10 cycles	13 (6)	267 – 323	3.1 (2.6)

Table 4. Mitochondrial cytochrome *b* haplotype (A-T) frequencies at each site sampled (n = number of individuals sampled). A shaded box indicates a haplotype unique to that site. An "*" indicates a sequence from Harris et al. (2005). See Figures 10 and 11 for hypotheses of genetic relatedness among haplotypes.

Locality	**n**	**A**	**B**	**C**	**D**	**E**	**F**	**I**	**K**	**M**	**N**	**O**	**P**	**S**	**T**	**G**	**H**	**J**	**L**	**Q**	**R**
Baby	5	0.6	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Balsam	5	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bertha	3	-	0.67	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cross	2	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eagle1	1	-	-	-	-	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-
Eagle2	5	0.6	0.2	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
Girl	3	0.67	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Junction	3	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Little Bemidji	5	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Little Thunder	3	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Many Point	2	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Movil	8	0.125	-	-	-	0.375	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pine	3	0.67	-	-	-	-	-	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-
Potato	7	0.14	-	-	-	-	-	-	0.86	-	-	-	-	-	-	-	-	-	-	-	-
Rush	4	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sand Point	9	0.89	-	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-
Three Island	4	-	-	-	-	0.75	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turtle R	10	-	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WI	7	0.57	-	-	0.29	-	-	-	-	-	0.14	-	-	-	-	-	-	-	-	-	-
IL	4	0.75	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-	-	-	-	-	-
KY	1	1.0*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AL	1	-	-	-	-	-	-	-	-	-	-	-	1.0*	-	-	-	-	-	-	-	-
MD	1	-	-	-	-	-	-	-	-	-	-	-	-	1.0*	-	-	-	-	-	-	-
TN	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0*
MO	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.46	0.09	0.27	0.09	0.09*	

Table 5. Mitochondrial cytochrome *b* haplotype frequencies in Minnesota by collection site (italics), major watershed (bold) and major basin (bold caps), where n = number of individuals sampled and an "*" indicates a haplotype unique to that group.

DRAINAGE	n	A	B	C	E	F	I	K	M	O
LAKE OF THE WOODS	**20**	**0.8**	**0.05**	**-**	**-**	**-**	**0.05***		**0.05***	**0.05***
Rainy River	**12**	**0.92**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.08***
Junction	3	1.0	-	-	-	-	-	-	-	-
Sand Point	9	0.89	-	-	-	-	-	-	-	0.11*
Big Fork River	**8**	**0.625**	**0.125**	**-**	**-**	**-**	**0.125***	**-**	**0.125***	**-**
Eagle2	5	0.6	0.2	-	-	-	-	-	0.2*	-
Pine	3	0.67	-	-	-	-	0.33*	-	-	-
RED RIVER NORTH	**7**	**-**	**1.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Ottertail River	**7**	**-**	**1.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Little Bemidji	5	-	1.0	-	-	-	-	-	-	-
Many Point	2	-	1.0	-	-	-	-	-	-	-
UPPER MISSISSIPPI	**55**	**0.27**	**0.2**	**0.02***	**0.29***	**0.09***	**-**	**0.13***	**-**	**-**
Headwaters	**22**	**0.04**	**-**	**-**	**0.73***	**0.23***	**-**	**-**	**-**	**-**
Movil	8	0.125	-	-	0.375	0.5	-	-	-	-
Three Island	4	-	-	-	0.75	0.25	-	-	-	-
Turtle R	10	-	-	-	1.0	-	-	-	-	-
Grand Rapids	**8**	**1.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Balsam	5	1.0	-	-	-	-	-	-	-	-
Little Thunder	3	1.0	-	-	-	-	-	-	-	-
Leech Lake River	**8**	**0.625**	**0.375**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Baby	5	0.6	0.4	-	-	-	-	-	-	-
Girl	3	0.67	0.33	-	-	-	-	-	-	-
Crow Wing River	**8**	**0.125**	**-**	**-**	**-**	**-**	**-**	**0.875***	**-**	**-**
Eagle1	1	-	-	-	-	-	-	1.0	-	-
Potato	7	0.14	-	-	-	-	-	0.86	-	-
Pine River	**9**	**-**	**0.89**	**0.11***	**-**	**-**	**-**	**-**	**-**	**-**
Bertha	3	-	0.67	0.33*	-	-	-	-	-	-
Cross	2	-	1.0	-	-	-	-	-	-	-
Rush	4	-	1.0	-	-	-	-	-	-	-

Table 6. Summary of Hardy-Weinberg genetic equilibrium tests for Minnesota populations defined at all three levels (collection site in italics, major watershed in bold, and major basin in bold caps). Data reported include number of individuals genotyped (n), and observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) for each locus.

		Lmar10		Lmar11		Lmar12		Lmar14	
POPULATION	**n**	**H_{obs}**	**H_{exp}**	**H_{obs}**	**H_{exp}**	**H_{obs}**	**H_{exp}**	**H_{obs}**	**H_{exp}**
LAKE OF THE WOODS	32	0.630	0.786	0.083**	0.233	-	-	0.286	0.408
Rainy River	19	0.688	0.778	-	-	-	-	0.176*	0.444
Sand Point Lake	12	0.778	0.712	-	-	-	-	0.300	0.521
Junction Bay	7	0.571	0.835	-	-	-	-	-	-
Big Fork River	13	0.545*	0.792	0.182*	0.463	-	-	0.455	0.437
Pine Lake	4	0..667	0.867	0.333	0.333	-	-	0.500	0.429
Eagle Lake 2	9	0.500*	0.800	0.125	0.592	-	-	0.429	0.648
RED RIVER NORTH	32	0.655	0.696	0.250*	0.454	0.074	0.108	0.600	0.535
Ottertail River	32	0.655	0.696	0.250*	0.454	0.074	0.108	0.600	0.535
Little Bemidji Lake	14	0.846	0.729	0.286	0.659	0.154	0.218	0.462	0.502
Many Point Lake	18	0.500	0.625	0.235	0.476	-	-	0.706	0.558
UPPER MISSISSIPPI RIVER	215	0.669**	0.816	0.252**	0.355	0.034**	0.093	0.417	0.481
Mississippi Headwaters	84	0.580**	0.780	0.226	0.251	0.068	0.106	0.443	0.509
Movil Lake	44	0.676	0.788	0.333	0.290	0.056	0.082	0.432*	0.563
Three Island Lake	20	0.526**	0.764	-	-	0.111	0.257	0.500	0.486
Turtle River	20	0.438	0.734	0.214	0.373	0.053	0.104	0.412	0.545
Mississippi Grand Rapids	34	0.813	0.725	0.267**	0.497	-	-	0.500	0.587
Balsam Lake	20	0.850	0.691	0.333**	0.622	-	-	0.550	0.556
Little Thunder Lake	14	0.750	0.739	0.167	0.304	-	-	0.429	0.635
Leech Lake River	43	0.848	0.790	0.193	0.322	0**	0.237	0.378	0.471
Girl Lake	20	0.667	0.802	0.286	0.328	0**	0.303	0.471	0.490
Baby Lake	23	1.00	0.786	0.118	0.324	0*	0.191	0.300	0.427
Crow Wing River	21	0.474	0.649	0.471	0.647	-	-	0.579	0.620
Potato Lake	16	0.571	0.690	0.462	0.655	-	-	0.500	0.598
Eagle Lake 1	5	0.200	0.733	0.500	0.607	-	-	0.800	0.822
Pine River	33	0.636	0.838	0.211	0.334	0.048	0.180	0.200	0.277
Bertha Lake	19	0.600	0.884	0.364	0.407	0.111	0.216	0.176	0.275
Cross Lake	6	0.500	0.758	-	-	0	0.455	0.333	0.318
Rush Lake	8	0.833	0.848	0	0.800	-	-	0.143	0.275

* = significantly lower at the p = 0.05 level, ** = significantly lower at the p = 0.01 level
\- = locus monomorphic

Table 7. Summary of Hardy-Weinberg genetic equilibrium tests for non-Minnesota populations defined at all three levels (collection site in italics, major watershed in bold, and major basin in bold caps). Data reported include number of individuals genotyped (n), and observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) for each locus.

POPULATION	n	Lmar10		Lmar11		Lmar12		Lmar14	
MISSISSIPPI RIVER/WI	8	0.800	0.889	-	-	-	-	0.500	0.642
Fox-Illinois River	1	1.00	1.00	-	-	-	-	1.00	1.00
Mukwonago Lake	1	1.00	1.00	-	-	-	-	1.00	1.00
Chippewa River	7	0.750	0.929	-	-	-	-	0.429	0.725
Grindstone Lake	3	-	-	-	-	-	-	0.333	0.933
Teal Lake	1	-	-	-	-	-	-	1.00	1.00
Trout River	3	1.00	0.867	-	-	-	-	0.333	0.600
MISSISSIPPI RIVER/IL	4	0.750	1.00	1.00	1.00	0.250	0.786	0.750	0.75
Illinois River	3	0.667	1.00	1.00	1.00	0	0.800	0.667	0.600
Bull Creek	1	-	-	-	-	-	-	-	-
Kankakee River	2	1.00	1.00	1.00	1.00	0	1.00	1.00	0.833
Ohio River	1	1.00	1.00	-	-	1.00	1.00	1.00	1.00
Bay Creek	1	1.00	1.00	-	-	1.00	1.00	1.00	1.00
MISSISSIPPI RIVER/MO	9	0.857	0.945	0.200*	0.867	0.778	0.804	0.667	0.948
White River	9	0.857	0.945	0.200*	0.867	0.778	0.804	0.667	0.948
Black River	9	0.857	0.945	0.200*	0.867	0.778	0.804	0.667	0.948

* = significantly lower at the p = 0.05 level, ** = significantly lower at the p = 0.01 level
- = locus monomorphic

Table 8. Analysis of molecular variance (AMOVA) results for the microsatellite data. Three AMOVAs were run, each on a data set where populations/subpopulations were defined as states/major basins, major basins/major watersheds, and major watersheds/Minnesota collection sites. Percent of variation refers to the amount of total variation attributed to variation at a given level, and F_{ST} correlates with the amount of genetic structure when the populations are defined in the given way in an analysis.

	% of Variation	F_{ST}
Major Basins within States:		
Among states	23.97%	F_{ST} = 0.253
Among basins within states	1.31%	
Within basins	74.72%	
Major Watersheds within Major Basins:		
Among basins	7.51%	F_{ST} = 0.121
Among watersheds within basins	4.61%	
Within watersheds	87.88	
Minnesota Collection Sites within Major Watersheds:		
Among watersheds	3.48%	F_{ST} = 0.061
Among sites within watersheds	2.64%	
Within sites	93.88%	

Table 9. Table of pairwise chord distances among the 13 major watersheds. See Figure 16 for the neighbor-joining tree constructed from these distances.

	1	2	3	4	5	6	7	8	9	10	11	12
1 Big Fork R												
2 Chippewa R	0.280											
3 Crow Wing R	0.286	0.241										
3 Fox (Illinois R)	0.260	0.367	0.309									
4 Illinois R	0.694	0.727	0.768	0.739								
5 Leech Lake R	0.250	0.215	0.308	0.393	0.682							
6 Mississippi (Grand Rapids)	0.189	0.275	0.298	0.363	0.684	0.266						
7 Mississippi (Headwaters)	0.259	0.201	0.233	0.381	0.724	0.161	0.281					
8 Ohio R	0.944	0.931	0.957	1.000	0.807	0.952	0.894	0.961				
9 Ottertail R	0.238	0.309	0.306	0.421	0.699	0.266	0.232	0.248	1.000			
10 Pine R	0.281	0.311	0.374	0.419	0.665	0.203	0.245	0.282	0.893	0.300		
11 Rainy R	0.244	0.197	0.297	0.284	0.692	0.247	0.253	0.245	0.935	0.307	0.292	
12 White R	0.864	0.804	0.857	0.910	0.663	0.829	0.841	0.834	0.787	0.865	0.834	0.846

Table 10. Table of pairwise chord distances among populations from 18 Minnesota collection sites. See Figure 17 for the neighbor-joining tree constructed from these distances.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Baby																	
2 Balsam	0.304																
3 Bertha	0.207	0.284															
4 Cross	0.223	0.293	0.245														
5 Eagle1	0.420	0.381	0.437	0.535													
6 Eagle2	0.268	0.206	0.321	0.321	0.337												
7 Girl	0.205	0.341	0.286	0.273	0.355	0.298											
8 Junction Bay	0.269	0.342	0.288	0.286	0.393	0.325	0.310										
9 Little Bemidji	0.286	0.264	0.325	0.371	0.287	0.229	0.280	0.312									
10 Little Thunder	0.215	0.226	0.308	0.287	0.358	0.158	0.245	0.276	0.264								
11 Many Point	0.279	0.236	0.314	0.331	0.325	0.187	0.281	0.274	0.189	0.194							
12 Movil	0.259	0.348	0.348	0.361	0.325	0.323	0.197	0.301	0.269	0.270	0.298						
13 Pine	0.245	0.344	0.360	0.307	0.452	0.286	0.331	0.297	0.376	0.257	0.319	0.282					
14 Potato	0.350	0.339	0.387	0.459	0.229	0.284	0.304	0.339	0.320	0.292	0.330	0.272	0.354				
15 Rush	0.317	0.275	0.260	0.329	0.374	0.240	0.377	0.274	0.334	0.293	0.306	0.350	0.269	0.310			
16 Sand Point	0.294	0.356	0.396	0.325	0.433	0.263	0.325	0.289	0.400	0.193	0.326	0.301	0.245	0.315	0.307		
17 Three Island	0.258	0.375	0.340	0.281	0.366	0.320	0.197	0.266	0.292	0.268	0.297	0.190	0.323	0.292	0.359	0.242	
18 Turtle	0.273	0.336	0.294	0.368	0.280	0.269	0.271	0.323	0.275	0.256	0.251	0.271	0.368	0.239	0.344	0.349	0.327

Table 11. Minnesota populations containing unique microsatellite alleles. The three levels of population designation (collection site, major watershed, major basin) are included. Alleles are given in total nucleotide length.

Population	**Locus**	**Allele**
By collection site:		
Girl Lake	Lmar14	267
Many Point Lake	Lmar11	187
Movil Lake	Lmar10	311
Potato Lake	Lmar11	207
Sand Point Lake	Lmar10	323
Turtle River	Lmar12	274
By major watershed:		
Crow Wing River	Lmar11	207
Leech Lake River	Lmar14	267
Mississippi Headwaters	Lmar10	311
	Lmar12	274
Ottertail River	Lmar11	187
Rainy River	Lmar10	323
By basin:		
Lake of the Woods	Lmar10	323
Upper Mississippi River	Lmar10	311
	Lmar11	195
	Lmar11	199
	Lmar11	207
	Lmar11	211
	Lmar12	258
	Lmar12	274
	Lmar14	267
Red River of the North	Lmar11	187

Table 12. Average heterozygosities by locus and then averaged overall for the 18 Minnesota collection sites.

Collection Site	Lmar10	Lmar11	Lmar12	Lmar14	Overall
Baby	0.764	0.216	0.095	0.335	0.353
Balsam	0.638	0.574	0	0.536	0.437
Bertha	0.820	0.318	0.105	0.265	0.377
Cross	0.667	0	0.278	0.292	0.309
Eagle1	0.500	0.375	0	0.660	0.384
Eagle2	0.711	0.430	0	0.439	0.395
Girl	0.764	0.258	0.231	0.476	0.432
Junction Bay	0.735	0	0	0	0.184
Little Bemidji	0.701	0.490	0.142	0.429	0.441
Little Thunder	0.691	0.153	0	0.541	0.346
Many Point	0.606	0.337	0	0.542	0.371
Movil	0.774	0.286	0.054	0.514	0.407
Pine	0.667	0.278	0	0.375	0.330
Potato	0.666	0.586	0	0.520	0.443
Rush	0.778	0.444	0	0.133	0.339
Sand Point	0.673	0	0	0.495	0.292
Three Island	0.722	0	0.202	0.472	0.349
Turtle	0.697	0.304	0.051	0.491	0.386

APPENDIX C – FIGURES

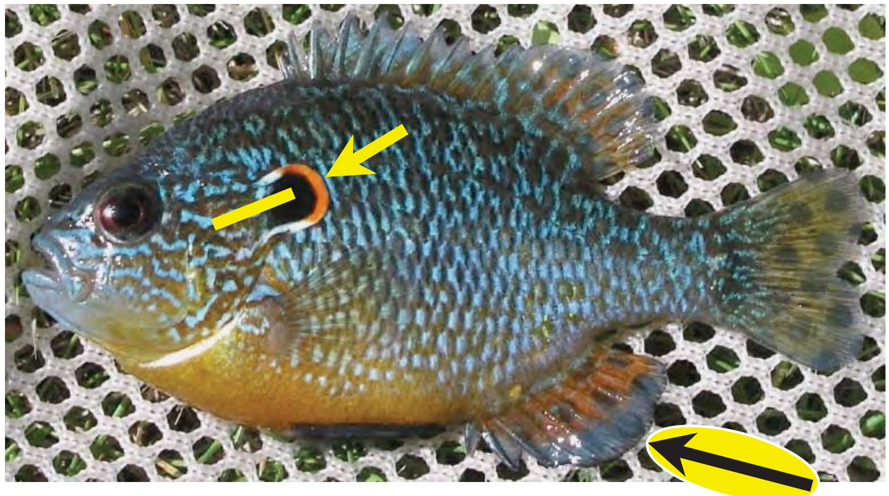
All fish/habitat photos by P. Ceas unless otherwise indicated.

Figure 15. Allele frequency distributions (by state) for locus Lmar14.

Figure 16. Neighbor-joining tree constructed from pairwise chord distance matrix of the 13 Major Watersheds represented in the microsatellite data set. Branch lengths are to scale, with longer branches representing larger amounts of genetic change hypothesized along those lineages.

Figure 17. Neighbor-joining tree constructed from pairwise chord distance matrix of the 18 Minnesota collection sites represented in the microsatellite data set. Branch lengths are to scale, with longer branches representing larger amounts of genetic change hypothesized along those lineages.

L. m. peltastes
- Opercle
  - red margin
  - 45° angle
- Anal Fin
  - blue margin



L. m. megalotis
- Opercle
  - dark margin, or with thin white line
  - horizontal
- Anal Fin
  - red margin

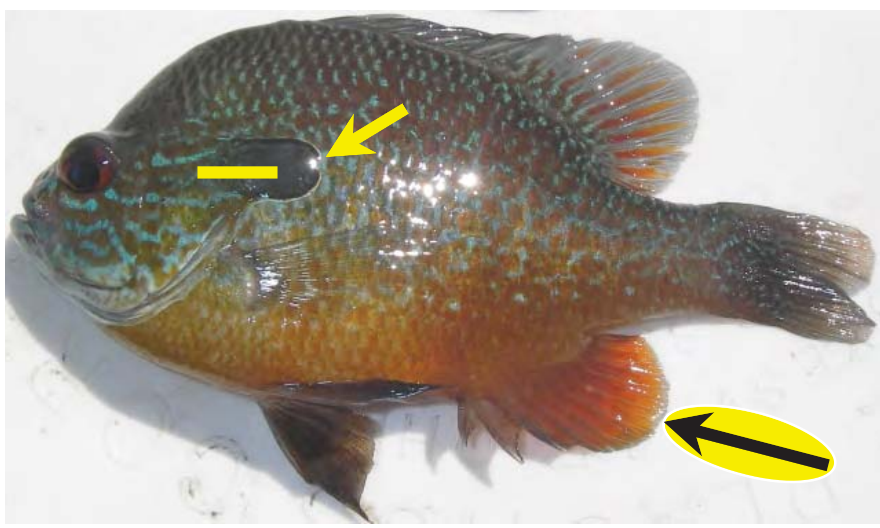
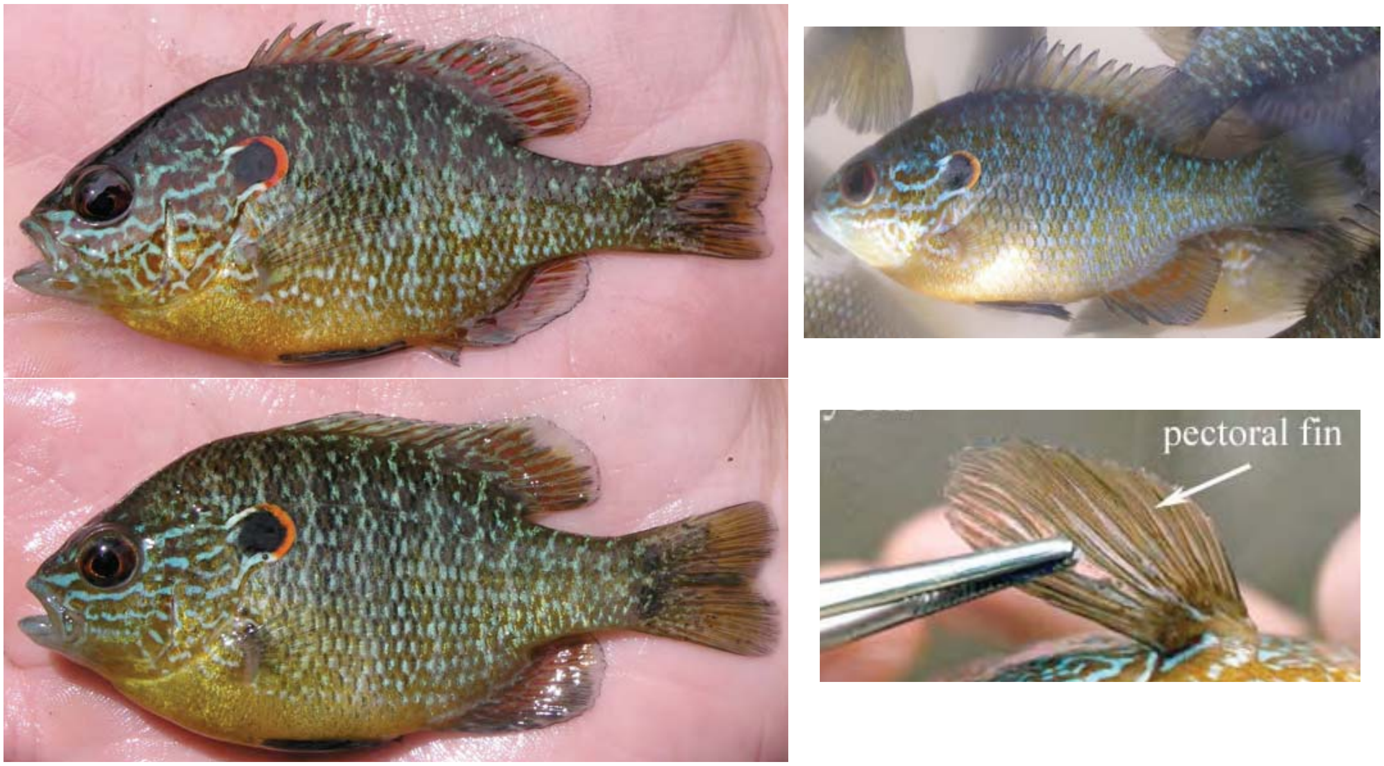


Figure 1. Photos of breeding male *Lepomis megalotis peltastes* and *Lepomis megalotis megalotis*, highlighting some of the diagnostic characteristics between the two taxa.

Longear Sunfish
- Pectoral fin shorter, tip rounded
- Opercle elongated; with red margin
- Anal fin with dusky blue margin



Pumpkinseed
- Pectoral fin longer, tip pointed
- Opercle small; with red spot
- Anal fin margin not dusky blue

Figure 2. Photos of breeding male longear sunfish and pumpkinseed, and of pectoral fin morphology for each species. Photos of fins by J. Lyons, WI DNR.

Longear Sunfish

Pumpkinseed

Bluegill

Green Sunfish

Figure 3. Photos of breeding male longear sunfish, pumpkinseed, bluegill, and green sunfish. Notice the relative size of each fish to the person's hand; longear sunfish breeding adults (individual in upper left is 6 yrs old) are considerably smaller than males of the other sunfishes. Photo of green sunfish by J. Lyons, WI DNR.

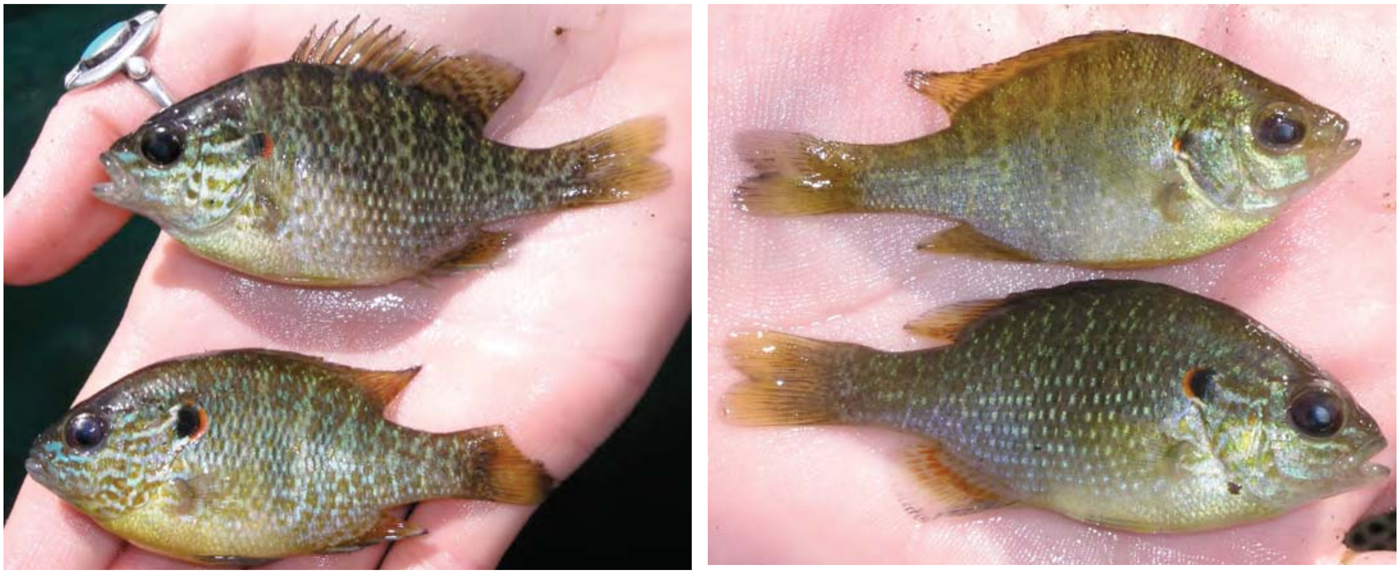


Figure 4. Photos of breeding male longear sunfish (bottom) and sub-adult pumpkinseed (top). Note the enlarged opercle ("ear flap") of the longear sunfish on these similar-sized fishes.

Figure 5. Photos of juvenile longear sunfish (top) and green sunfish (bottom). Note the proportionally larger mouth on the green sunfish.

Figure 6. Habitat photos of longear sunfish. Note the shallow water depth in the top photos (top left: Balsam Lake, Map 7, Site 96; top right: Grassy Bay, Map 9, Site 109), and the near-shore location of sunfish nests in the two bottom photos (Movil Lake, Map 7, Site 26). Middle photo: Little Bemidji Lake (Map 3, Site 3).

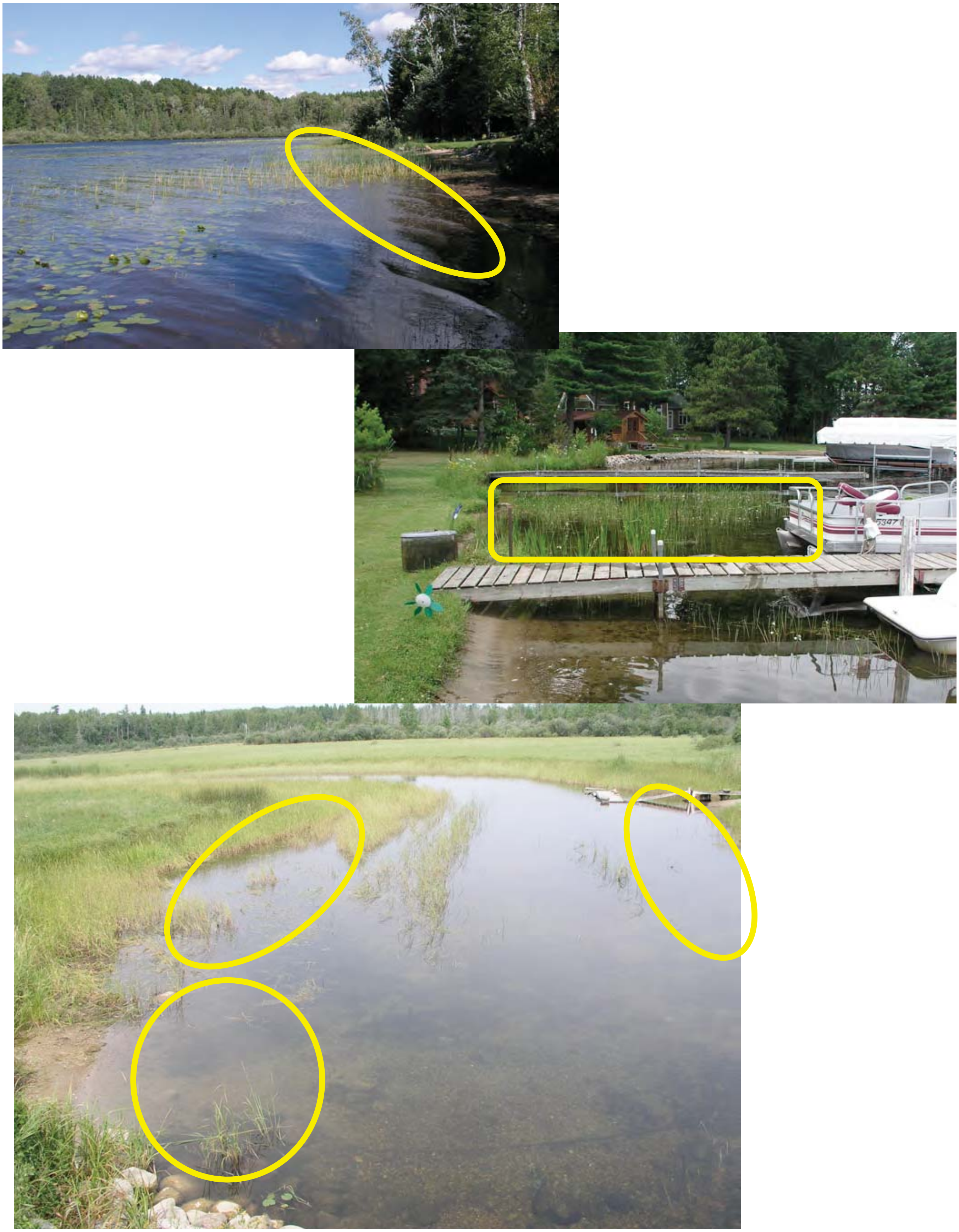


Figure 7. Habitat photos of longear sunfish. Top: Eagle Lake #2 (Map 7, Site 87). Middle: Bertha Lake (Map 5, Site 17). Bottom: Turtle River (Map7, Site 31). Longears were found in areas outlined by the yellow ovals.

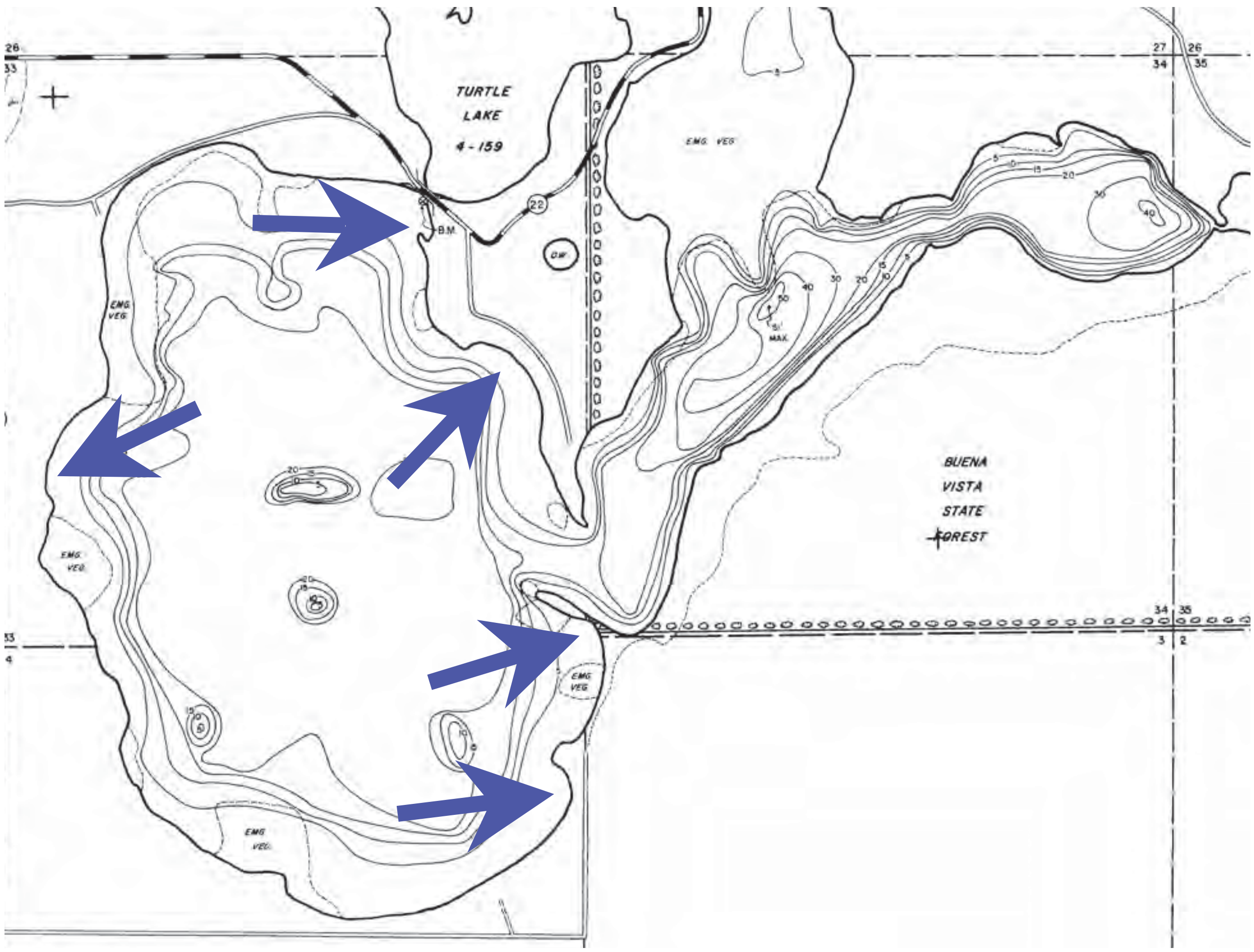


Figure 8. Bathymetric map of Movil Lake (Map 7, Site 26). Note the rapidly increasing depths near shore in the east basin vs. the extensive shallows of the west basin. The blue arrows indicate "prime" shallows for longear sunfish.

Figure 9. Examples of lakes that did not contain longear sunfish. Norway Lake (top; Map 5, Site 16) is representative of a “no shallows” lake; Moose Lake (middle; Map 7, Site 47) is representative of a “sand lake:” Rabideau Lake (bottom; Map 7, Site 33) is suffering from extensive algal blooms and high nutrient loads.

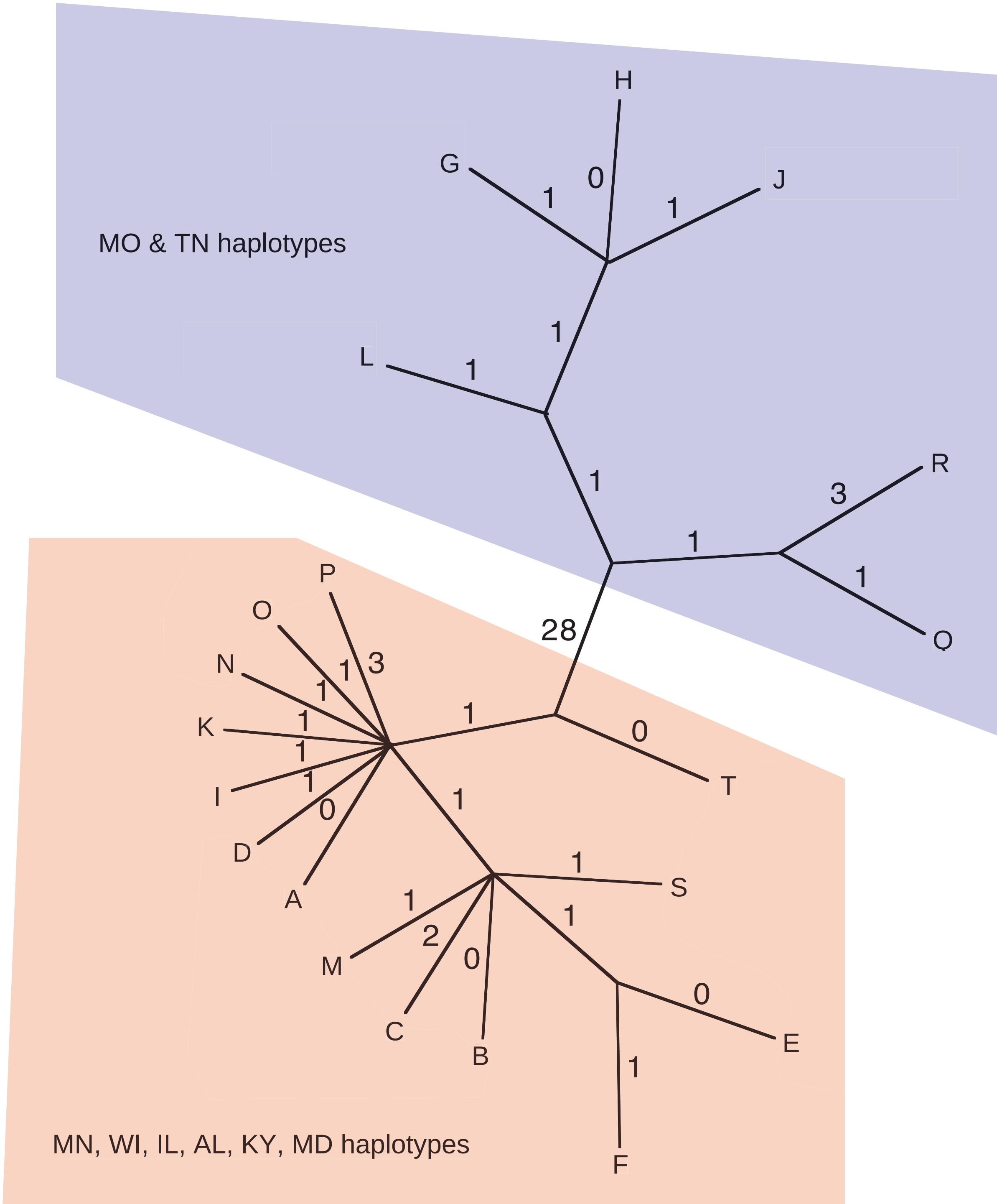


Figure 10. Unrooted phylogenetic tree of 20 *L. megalotis* cytochrome *b* haplotypes. Numbers along the branches represent the number of substitutions occurring along that lineage.

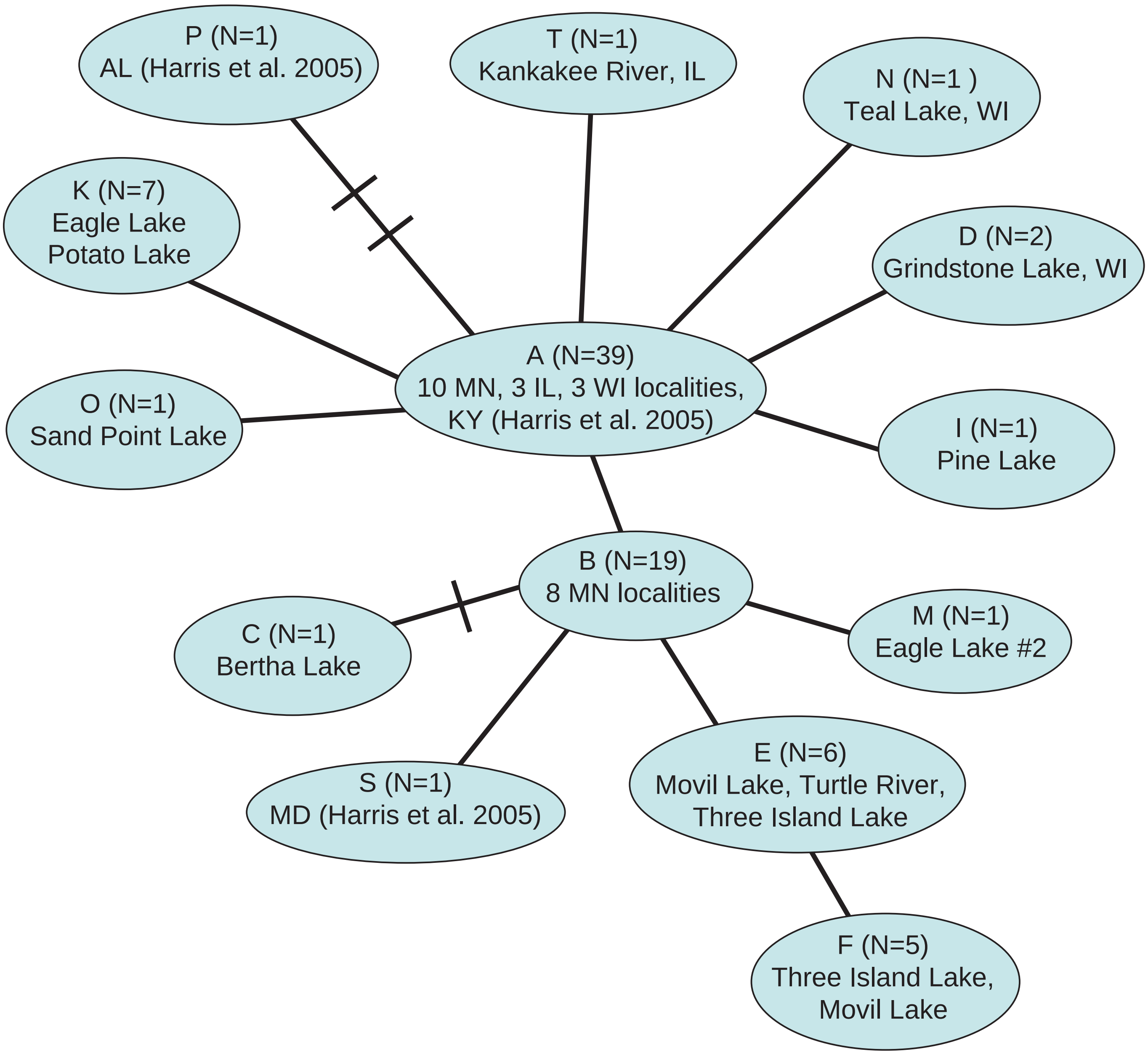


Figure 11. Haplotype network of the 96 *L. megalotis* cytochrome *b* sequences representing MN, WI, AL, MD, and KY. Hashmarks indicate hypothesized intermediate haplotypes not sampled. See Table 4 for information on haplotype frequencies at each locality sampled.

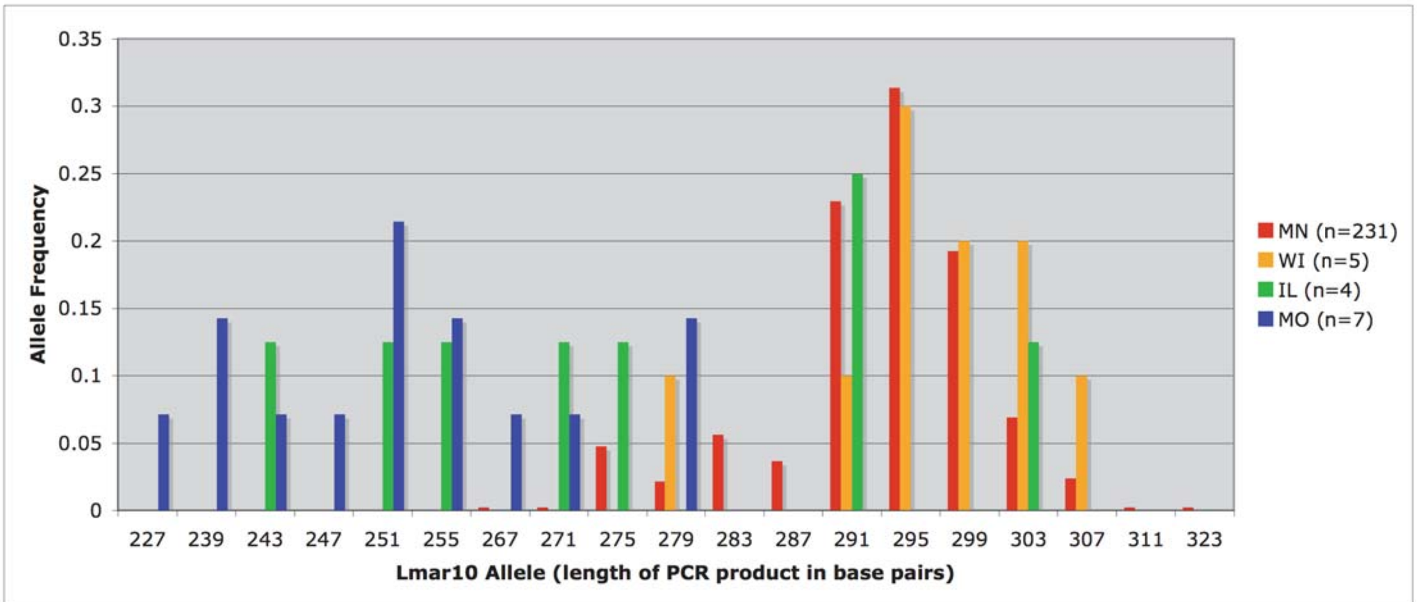


Figure 12. Allele frequency distributions (by state) for locus Lmar10.

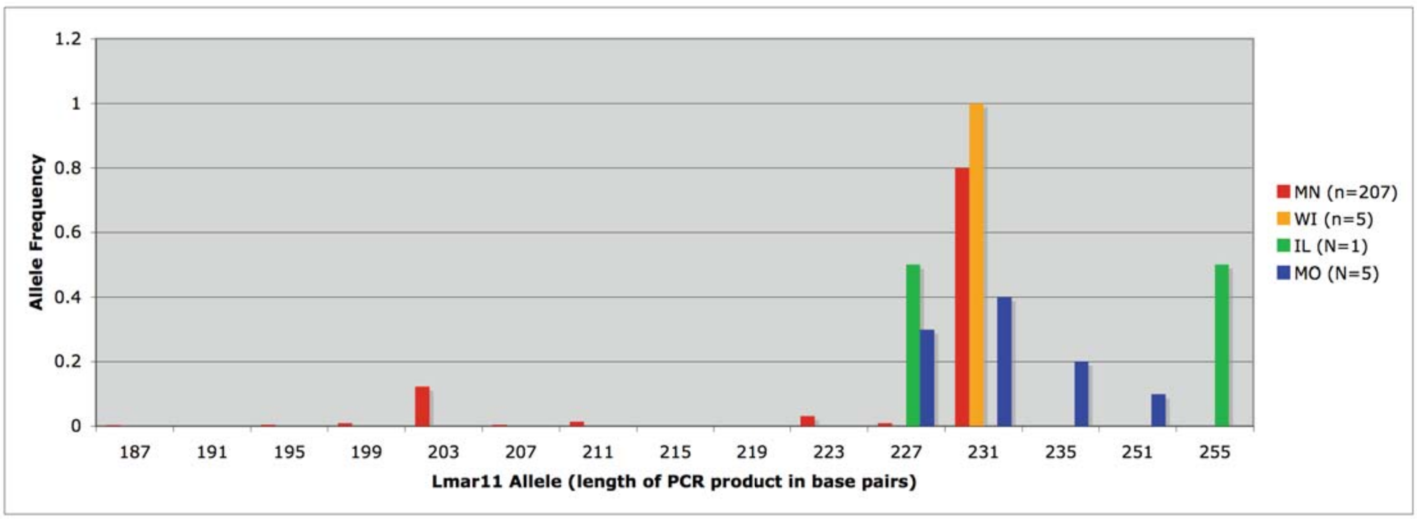


Figure 13. Allele frequency distributions (by state) for locus Lmar11.

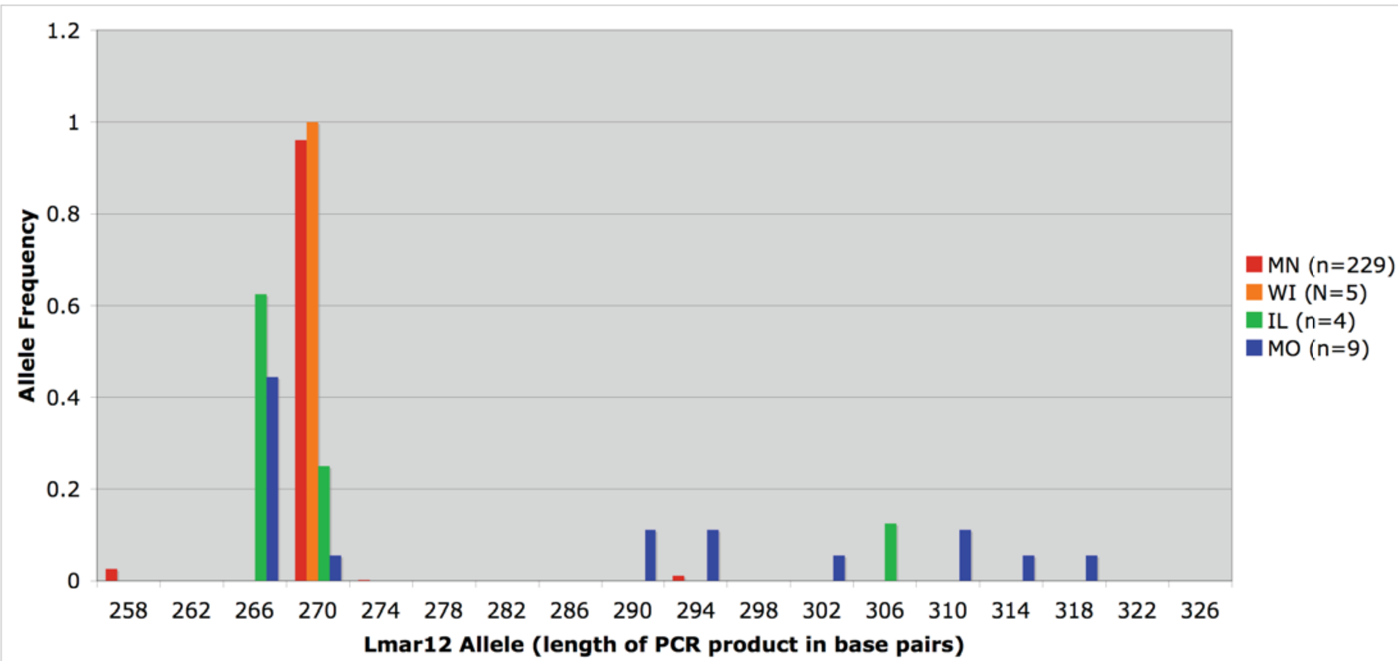


Figure 14. Allele frequency distributions (by state) for locus Lmar12.

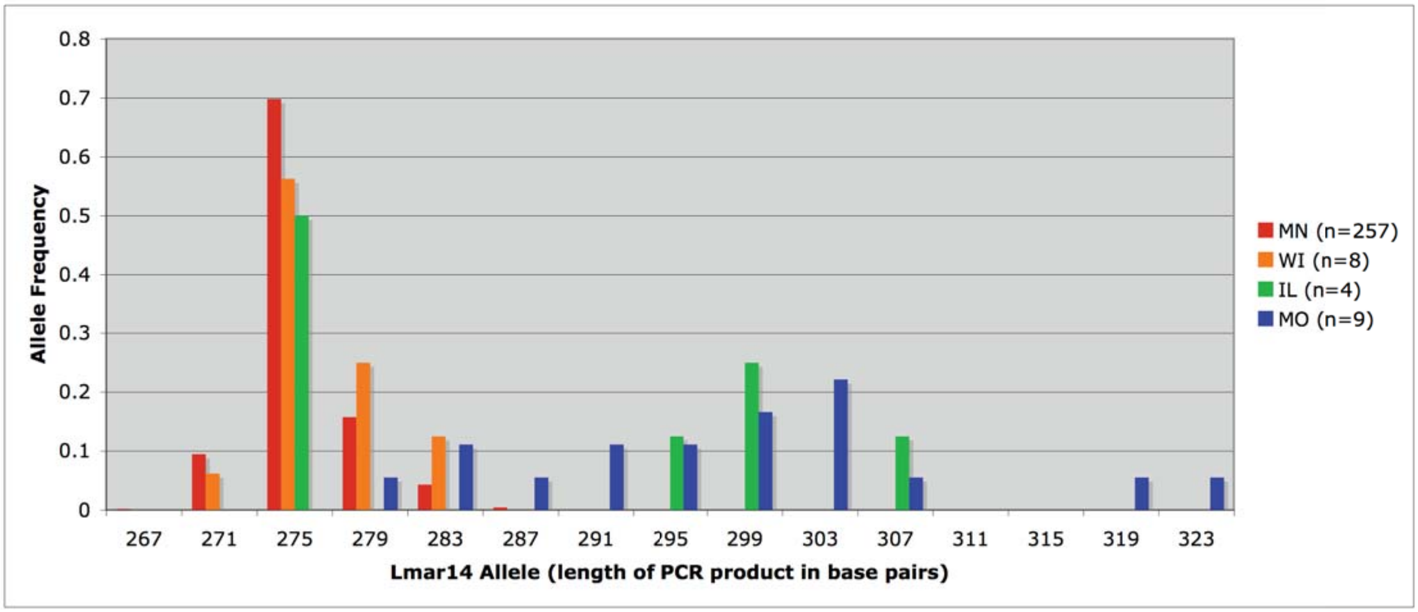


Figure 15. Allele frequency distributions (by state) for locus Lmar14.

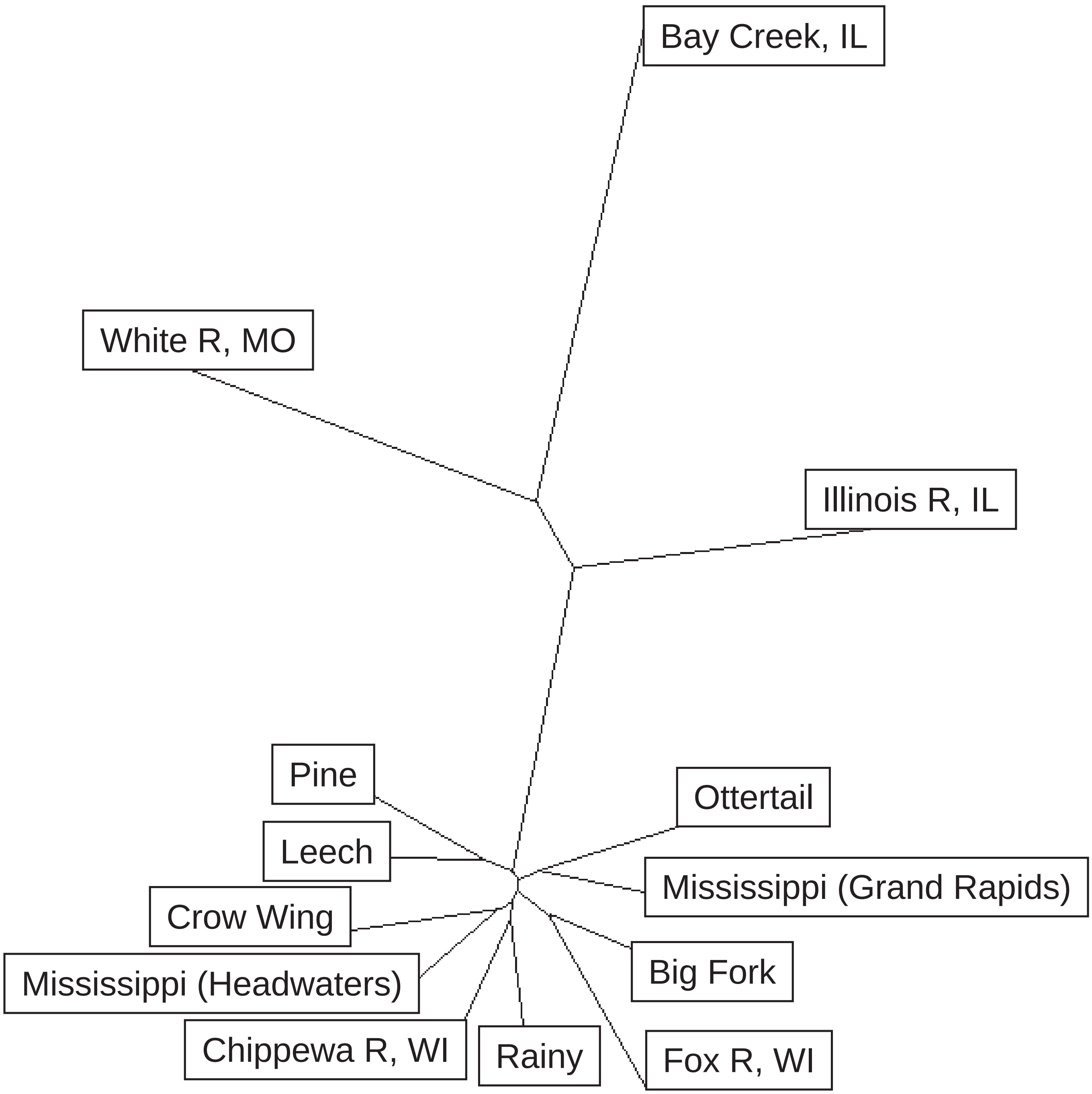


Figure 16. Neighbor-joining tree constructed from pairwise chord distance matrix (Table 9) of the 13 major watersheds represented in the microsatellite data set. Branch lengths are to scale, with longer branches representing larger amounts of genetic change hypothesized along those lineages.

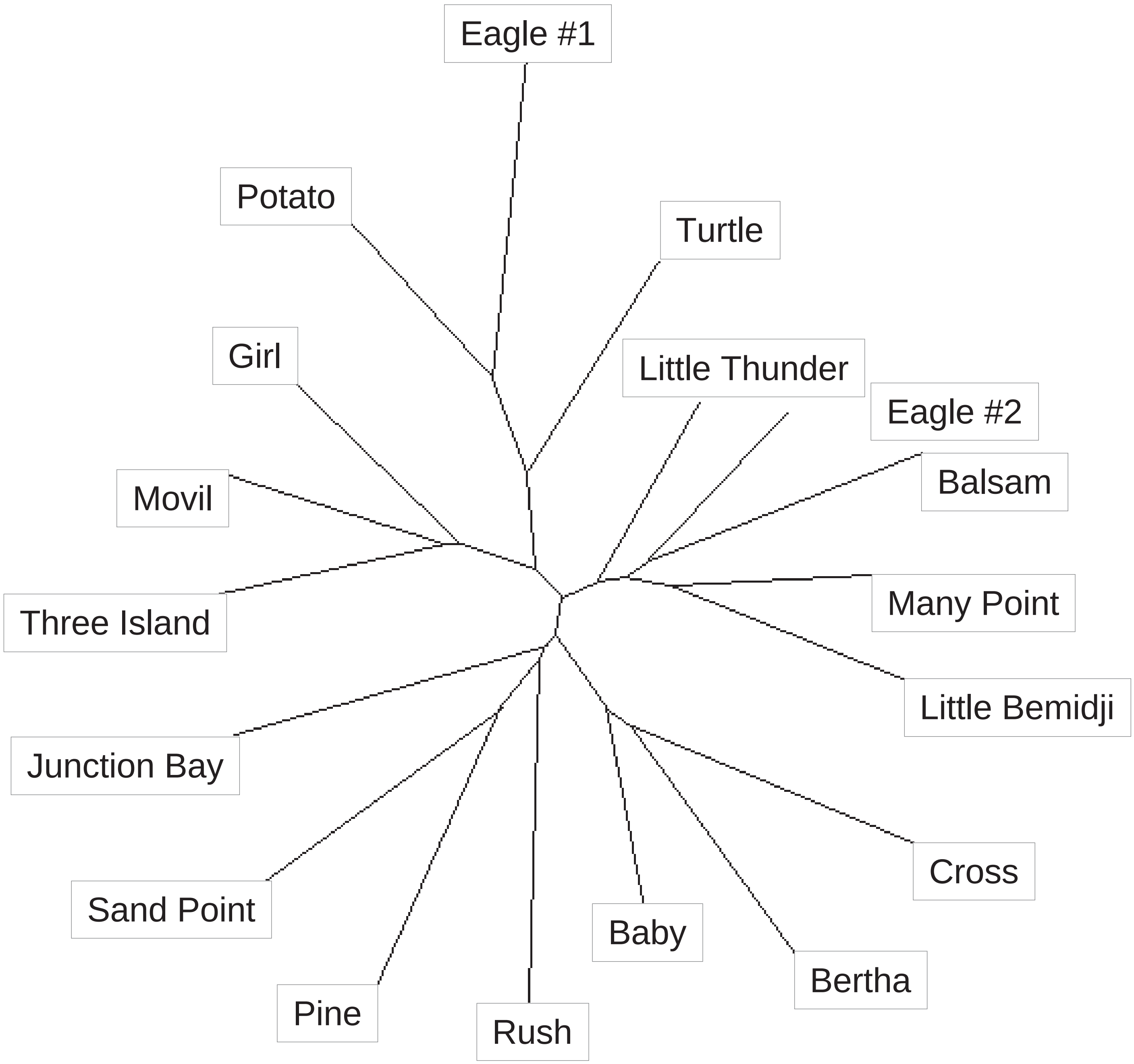


Figure 17. Neighbor-joining tree constructed from pairwise chord distance matrix (Table 10) of the 18 Minnesota collection sites represented in the microsatellite data set. Branch lengths are to scale, with longer branches representing larger amounts of genetic change hypothesized along those lineages.