CONTEMPORARY MUSKELLUNGE GENETIC RESOURCES IN NORTHERN WISCONSIN: IMPACTS OF SUPPLEMENTAL STOCKING AND GENETIC MANAGEMENT ZONES

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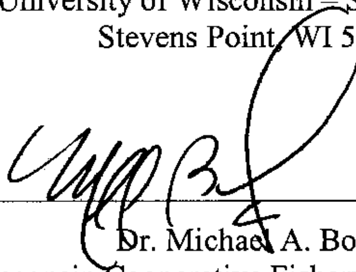

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ABSTRACT

Since the 1930s, muskellunge (*Esox masquinongy*) have been propagated extensively in Wisconsin as part of a comprehensive management plan aimed at providing a range of angling opportunities including trophy fisheries. Concerns exist about the effects of propagation on the genetic integrity of Wisconsin's muskellunge populations. Understanding and delineating contemporary stock structure is a prerequisite to refining propagation practices by selecting appropriate brood sources and defining operational management units to meet the goal of conserving muskellunge genetic integrity. The objectives of this study are to: 1) evaluate the temporal genetic dynamics of the Lac Courte Oreilles (LCO) muskellunge population to determine if two stocking events disrupted the genetic integrity of the population, and 2) determine if genetic population structuring occurs among muskellunge populations in northern Wisconsin and to provide an initial genetic stock model including measures of the degree of stock isolation.

Archived scale and spine samples from LCO, Big Spider Lake, and Mud-Callahan Lake were used to assess temporal changes in the LCO population following stocking of Big Spider Lake and Mud-Callahan Lake muskellunge (perceived small growth strain fish) into LCO. Significant genetic differences between the three populations were discerned yet no significant changes in LCO attributable to mixing of exogenous genes were observed over a 50-year timeframe (1953-2003). These findings suggest no significant impact of the two stocking events occurred in the genetic integrity of LCO muskellunge.

Twenty-four naturally recruiting and presumed native muskellunge populations with limited to no stocking history were genetically characterized at 14 microsatellite loci. Genetic stock identification, employing a hierarchical approach of cluster analyses and analysis of molecular variance (AMOVA), was used to delineate groupings of populations corresponding to genetic stocks and/or genetic management units. Basic diversity measures showed high levels of genetic variance between populations. The populations used in this study revealed significant genetic structure loosely corresponding to two major watersheds of Wisconsin: Wisconsin River and Chippewa River. The inclusion of additional populations outside these two watersheds showed that the structure was an approximate east/west geographic split. AMOVA tests showed little genetic significance between the currently employed management zones and the observed distribution of genetic diversity in muskellunge. The observed genetic structure is most likely explained by natural processes and muskellunge life history such as genetic drift, small population size, and low survival rates of stocked muskellunge.

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GENERAL INTRODUCTION

Muskellunge (*Esox masquinongy*) are apex predatory fish that are important to both the aquatic ecology and economy of Wisconsin. Within Wisconsin, there are over 700 recognized waters containing muskellunge; most are lakes, but some populations inhabit slow moving rivers and artificial reservoirs (Simonson 2002). Muskellunge are renowned as voracious feeders generally preying on yellow perch (*Perca flavescens*) and white sucker (*Catostomus commersoni*) (Bozek et al. 1999) but also known to opportunistically predate waterfowl and small mammals. Muskellunge are among the largest inland freshwater fish in North America reaching sizes in excess of 127 cm (>50 in).

Muskellunge are a favored species among North American anglers and are a significant part of Wisconsin's economy and tourism. They are targeted as a trophy fish among a select group of avid anglers and as a cultural component of Native American fisheries. The number of anglers fishing for Wisconsin's muskellunge has steadily increased since the 1950s, currently estimated at nearly 400,000 anglers (Simonson 2002). A majority of the revenue from muskellunge fishing occurs in the northern half of Wisconsin where ~90% of the muskellunge populations are found (Simonson 2002). With the high level of resource use, the muskellunge fisheries in Wisconsin are actively managed through a combination of harvest restrictions and propagation.

The Wisconsin Department of Natural Resources' (WDNR) muskellunge propagation program recently underwent a series of strategic modifications aimed at protecting the contemporary genetic integrity of populations (Sloss 2005). The focus of the modifications was to minimize the risk to naturally recruiting, native populations of

muskellunge. Among the key recommendations of Sloss (2005) were to: 1) restrict brood sources to native, naturally recruiting populations, 2) use a 3-5 population rotation of brood sources to account for alternate year stocking strategies, and 3) optimize effective population sizes by spawning 1 female to 3 males with a minimum of 19 females and 57 males per hatchery facility per year.

To meet the goals of this strategic plan, it is necessary to understand the contemporary genetic resources within and among muskellunge populations in the state and to determine, where possible, the impact of past and current management activities on the genetic integrity of muskellunge populations. Technological advances in molecular biology (Reading 2003; Sloss et al. 2008) have allowed more effective use of genetic techniques in resolving muskellunge spatial and temporal genetic diversity. These same advances have drastically reduced the amount of tissue required for genetic analysis. Non-lethal sampling (fin-clips) and the use of archived scales, spines, and other hard parts (cleithra and otoliths), once collected for aging, have made it logistically possible to analyze a wide distribution and large number of muskellunge populations as well as any temporal changes in response to natural and anthropogenic actions.

Taking into account the importance of the Wisconsin muskellunge resource to the state, and the goals of the muskellunge management plan, this project was focused on looking at the contemporary muskellunge genetic resources as they relate to past propagation practices and future propagation plans. The overall goals of this research were to provide a critical evaluation of suspect stocking events from the 1950s and 1980s into Lac Courte Oreilles (LCO) and to examine the contemporary genetic diversity within and among naturally recruiting Wisconsin muskellunge populations in the species' native

range. My first objective was to evaluate the temporal genetic dynamics of the LCO muskellunge population to determine if two stocking events that used perceived slow/small growth strains of muskellunge disrupted the genetic integrity of the population. This was achieved by comparing pre-stocking samples to post-stocking samples, specifically comparing Big Spider Lake and Mud/Callahan Lake samples (brood source populations) to Lac Courte Oreilles (recipient population) samples to determine if an admixture (i.e., introgression or gene flow) could be detected. My second objective was to determine if genetic population structuring occurred among muskellunge populations in northern Wisconsin.

Literature Review

Life history.—The native range of muskellunge is limited to lakes and slow moving rivers in the eastern portion of the United States, including the Mississippi River, Missouri River, and Ohio River drainages, and the Great Lakes (Crossman 1978; Dombeck 1986; Inskip 1986), and the Canadian provinces of Manitoba, Ontario, and Quebec (Becker 1983; Inskip 1986). Muskellunge have been introduced in other states outside their native range such as North and South Dakota, Nebraska, Texas, and California (Cook and Solomon 1987). In Wisconsin, the native range of muskellunge was identified by Becker (1983) as limited to the headwaters of the Chippewa, Wisconsin, and Flambeau Rivers (Figure 1). Muskellunge have been introduced outside their native range in Wisconsin and are now found throughout the state.

Muskellunge prefer, but are not limited to, large bodies of clear water containing both shallow areas with macrophyte beds and deep areas with abundant cover (Becker

1983). Cook and Solomon (1987) developed a habitat suitability index model that compiled optimal lake characteristics for muskellunge. Specific characteristics contributing to the model included water transparency, forage fish abundance, size diversity of forage fish, winter dissolved oxygen, water temperature, and ratio of spawning habitat to summer habitat. Secchi disk measurements have been correlated with muskellunge activity in numerous studies; as ambush hunters, muskellunge are believed to be more active when water transparency levels are higher (Miller and Menzel 1986). Oehmcke et al. (1965) found that muskellunge were also more tolerant of low oxygen conditions compared to other sport fish. It has also been shown that while muskellunge avoid areas where dissolved oxygen is low, they can over-winter at oxygen levels as low as 3.0 mg/L (Gilbertson 1986; Cook and Solomon 1987). The muskellunge has a preferred temperature range of 0.6-25.6ºC, but can survive maximum water temperatures of 32.2ºC (Dombeck 1979; Becker 1983; Cook and Solomon 1987).

Muskellunge typically spawn in shallow bays (<1 m) with a muck substrate and large woody structure (Nevin 1901; Oehmcke 1974; Becker 1984). Dombeck et al. (1984) classified spawning habitat while analyzing eight naturally recruiting muskellunge lakes in northern Wisconsin and found spawning took place over a variety of substrates near-shore. The commonly observed substrates included gravel, muck, emergent and submergent vegetation, and large woody structure (Dombeck et al. 1984). In some cases, muskellunge spawning areas were located near an inflowing stream (Dombeck 1979). Muskellunge have also been observed moving up and spawning in streams (Eddy and Underhill 1976). River-dwelling muskellunge usually spawn in slow moving portions of the river with similar substrate such as muck and large woody debris (Brewer 1969).

Scott and Crossman (1973) reported that muskellunge first spawn between 3-5 years of age. They spawn in the spring soon after ice-out as water temperatures reach 9.4-15.6°C. Generally, males home to spawning sites and are followed by females and may even cross deep, open water to get to shallow spawning sites (Becker 1983; Strand 1986). Typical spawning includes one female and two males isolating themselves from other spawning muskellunge. Males compete for position while releasing their gametes simultaneously with eggs released by the female and fertilized eggs are then dispersed over the substrate. Alternative spawning strategies have been observed where muskellunge and northern pike (*E. lucius*) are sympatric, specifically in the northern lakes of Minnesota and the Great Lakes. In these cases, offshore spawning in deeper water has been observed (Haas 1978; Strand 1986).

Muskellunge use a wide variety of habitat types depending on their life stage and the time of year. Eggs incubate 10-21 days based on the water temperature (Klingbiel 1986). After hatching, the fry (< 10 days) remain sedentary among aquatic vegetation and detritus until the yolk sack is absorbed (Scott and Crossman 1973; Craig and Black 1986). Muskellunge fry utilize both the previous summer's decaying aquatic vegetation and new emergent aquatic plants, such as arrowhead (*Alismaceae*), sedges (*Cyperaceae*), and water lilies (*Nymphaeaceae*), for cover while resting and foraging (Dombeck 1979; Craig and Black 1986). Young-of-year muskellunge remain in areas with abundant vegetation while feeding on small forage fish (Oehmcke et al 1958; Craig and Black 1986; Cook and Solomon 1987; Murray and Ferrell 2007). Miller and Menzel (1986) studied adult muskellunge habitat throughout the course of the year and found that after ice-out, muskellunge used both the littoral zone and deeper water portions of lakes. In

mid- to late-summer, habitat use was primarily restricted to the littoral zone, while muskellunge over-wintered in deeper water (Miller and Menzel 1986). From spring through fall, adult muskellunge established a large home range presumably to maximize predation activities (Strand 1986). Home ranges were often associated with submerged structures like weed beds, large woody debris, or rocky substrate (Miller and Menzel 1986; Strand 1986). Similar to the northern pike, muskellunge utilized habitat associated with the edges of macrophyte beds (Cook and Solomon 1987).

As apex aquatic predators, muskellunge forage on a variety of different prey species throughout the year. Muskellunge are voracious piscivores with common prey items including catostomids, cyprinids, and percids (Bozek et al. 1999). In addition, there have been anecdotal accounts of muskellunge feeding on water fowl, amphibians, and small aquatic mammals (Oehmcke 1965). Engstrom-Heg et al. (1986) studied the prey selection of esocids in laboratory experiments and found muskellunge were a "lurking" predator, remaining sedentary and making short, quick strikes. In their study, no prey species preference was observed, but rather prey selection was based on size (Engstrom-Heg et al. 1986).

Muskellunge have been observed to change feeding habits based on available cover and forage (Engstrom-Heg et al. 1986; Bozek et al. 1999). Bozek et al. (1999) studied muskellunge feeding habits in northern lakes of Wisconsin. They sampled muskellunge stomach contents from 31 lake populations from April to October. Muskellunge were concluded to be opportunistic predators with 31 different species of fish being consumed (Bozek et al 1999). These feeding habits and needs have become integral considerations in muskellunge management.

Taxonomy and intraspecific variation.—Muskellunge are members of Esocidae (Esociformes; Nelson 2006) which contains a single extant genus, *Esox.* Worldwide, five species of *Esox* occur: muskellunge, northern pike, Amur pike (*E. reicherti*), chain pickerel (*E. niger*), and two recognized subspecies of *E. americanus*: the redfin pickerel (*E. a. americanus*) and the grass pickerel (*E. a. vermiculatus*) (Casselman et al. 1986; Grande et al. 2004; Nelson 2006). North America contains three endemic species (muskellunge and the pickerels) and the circumpolar northern pike. The Amur pike is the only species that does not occur in North America (Siberia). The muskellunge fossil record goes back 25 million years and the species is thought to have evolved in North America most likely from a northern pike-like ancestor (Casselman et al. 1986). Phylogenetic evidence suggests the closest living relatives of muskellunge are the northern pike and the Amur pike (López et al. 2004).

Muskellunge and northern pike hybrids (collectively referred to as the tiger muskellunge; Inskip 1986) have been observed in nature (Oehmcke 1969; Casselman et al. 1986; Casselman and Crossman 1986; Wingate 1986) and employed in management of esocid fisheries. Background natural hybridization likely occurs wherever the two species are sympatric (Scott and Crossman 1973; Inskip 1986). The tiger muskellunge has been employed in fisheries management programs because of lower cost of propagation, better return to creel, higher growth rates (Casselman and Crossman 1986), and their assumed sterility (Wingate 1986). However, recent trends away from tiger muskellunge use, especially in areas with native muskellunge waters, have occurred for several reasons, including angler preferences for pure-strain trophy fish and concerns

over lower, but significant, levels of reproductive success posing a threat to the genetic integrity of native muskellunge populations (Wingate 1986).

Designation of subspecies of muskellunge has been suggested based on numerous morphological, ecological, meristic and partial genetic analyses (Scott and Crossman 1973; Crossman 1986; Lebeau 1992). However, these subspecies are currently not recognized by the scientific community (Koppelman and Philipp 1986). The presence of different muskellunge strains, a term most commonly used in the propagation/culture of fish species to designate a lineage of fish originating from either a single source (e.g., Spirit Lake strain of muskellunge) and/or exhibiting clear-cut physiological but not morphological differences from other strains (Merriam-Webster 2008), is commonly accepted among fisheries professionals and anglers. In Wisconsin and Minnesota, various muskellunge strains have been proposed. Based on several paired stocking studies, strains of muskellunge differing in morphology, performance, and/or physiological attributes have been shown including the Leech Lake (MN) strain, Shoepack (MN) strain, and multiple potential strains in WI (e.g., LCO, Mud-Callahan, etc.) are well-accepted (Johnson 1971; Lyons and Margenau 1986; Younk and Strand 1992; Margenau and Hanson 1996).

Minnesota's muskellunge management program has benefited greatly by identifying different strains in the state (Wingate and Younk 2007). During the 1900s, Minnesota saw a decline of trophy muskellunge (>127 cm). Minnesota Department of Natural Resources (MNDNR) fish biologists reacted by reducing bag limits, increasing the minimum length limit, and restricting the length of the season (Wingate and Younk 2007). Minnesota's muskellunge propagation program also responded by propagating

muskellunge exclusively from Shoepack Lake because, at the time, that population was an accepted representative of Minnesota muskellunge populations and egg collection quotas were easily met (Eddy and Surber 1943; Eddy and Underhill 1974; Younk and Strand 1992; Wingate and Younk 2007). However, after 30 years of stocking Shoepack Lake progeny in Minnesota, trophy-sized muskellunge had not increased and growth rates of stocked (Shoepack Lake) muskellunge were found to be slower compared to the naturally recruited muskellunge (Wingate and Younk 2007). This observation sparked concern that Shoepack Lake was not an ideal muskellunge brood stock population for the state's propagation program. Leech Lake was chosen as a potential alternative to Shoepack Lake because trophy muskellunge were still being caught there and the lake was a native muskellunge system (Wingate and Younk 2007). Genetic research supported the assumption that fish in the two systems were divergent (Hanson et al. 1983), and historical growth rates and paired stocking studies subsequently confirmed that Shoepack Lake muskellunge had a slower growth rate than Leech Lake muskellunge (Wingate and Younk 2007). After concluding that two strains were present, the MNDNR decided to switch brood sources and exclusively use Leech Lake muskellunge in their propagation program. Since the switch, Minnesota has become a major destination of anglers seeking trophy muskellunge.

Multiple strains of muskellunge are suspected to occur in Wisconsin. Johnson (1971) concluded that Big Spider Lake (Sawyer County) muskellunge stocked into Lac Courte Oreilles (LCO; Sawyer County) performed poorly because of an unknown heredity factor. In 1971, a life history study of muskellunge in Wisconsin showed that different stocks/strains performed differently when stocked in different waters (Lyons

and Margenau 1986). In addition, Margenau and Hanson (1996) observed that Mud/Callahan Lake (Sawyer County) strain muskellunge showed higher survival than LCO strain when both were stocked in Mud/Callahan Lake, suggesting local adaptation was, in part, responsible for strain-specific survival. The presence of perceived strains and local adaptation of muskellunge suggested more research was needed in Wisconsin to manage the genetic integrity of this historically and economically important resource.

Muskellunge management in Wisconsin.—Muskellunge harvest has a long and fabled history throughout its range as an important resource to anglers and the commercial fishing industry. Muskellunge were targeted by commercial anglers from the mid-1800s to the early-1900s, but commercial harvest was soon concentrated to small pockets in Ontario and Quebec (Crossman 1986). Angler pressure on the resource increased when northern expansion of the railroad made northern Wisconsin muskellunge lakes more accessible to the public (Nevin 1901; Crossman 1986) and the number of anglers using Wisconsin's muskellunge resources has steadily increased since the 1950s (Simonson 2002). Millions of dollars are spent on food, lodging, and equipment each year, making the muskellunge industry a significant part of Wisconsin tourism. Much of the revenue from muskellunge fishing is generated in the northern half of Wisconsin because nearly 90% of muskellunge populations are found there (Simonson 2002).

Habitat preservation is an important part of muskellunge management in Wisconsin. Key habitat types for muskellunge include shallow mucky bays, weed beds, and coarse woody structure (Cook and Solomon 1987). The main source of habitat disruption is human manipulation of lakeshore habitats, dredging wetlands, and weed removal (Margenau 2008). Jennings et al. (1999) measured cumulative effects of

shoreline development associated with fish assemblages. Their results suggested effective habitat preservation takes place at the landscape level; however, protecting small sections of habitat, such as private lake front properties, helps maintain habitat diversity and, subsequently, the species richness of a lake (Jennings et al. 1999). Jennings et al. (2003) also showed that shoreline development resulted in "simplified" habitat that is not conducive to fish, especially ambush predators such as muskellunge. These studies have helped government agencies educate lake associations and anglers in how to take an active role in maintaining critical habitats used by muskellunge and other fish (Margenau et al. 2008).

Over-exploitation of muskellunge resources has historically been a major concern addressed through regulations and encouragement of catch-and-release practices. Where over-exploitation has been observed in muskellunge populations, the likely cause has been a "relaxed" or non-existent regulation of size and bag limits (Nevin 1901; Graff 1986). In the 1970s, the WDNR instituted strict muskellunge regulations, including specific length limits, aimed at protecting mature fish of a minimum size and low bag limits aimed at ensuring a low annual harvest thus protecting Wisconsin's muskellunge fisheries (Simonson and Hewett 1999). In addition, the various state agencies and private angling groups, such as Muskies, Inc., dramatically changed muskellunge management by encouraging catch-and-release (Simonson and Hewett 1999). A study by Dent (1986) examined the impacts of catch-and-release on Pomme de Terre Lake, Missouri, and showed that catch-and-release could benefit muskellunge populations. The lake had been stocked since 1966 when a local chapter of Muskies, Inc. became active in maintaining the population through voluntary catch-and-release. Dent (1986) saw the number of legal

muskellunge caught increase, and the study showed that, with catch-and release, a muskellunge population can remain viable as angling pressure increases.

In Wisconsin, anglers and angling groups have enthusiastically adopted a catch-and-release ethic. The WDNR has published safe muskellunge release techniques both in the fishing regulations booklet and on their website to educate and encourage catch-and-release fishing (www.muskiesinc.org). Muskies, Inc. has also encouraged a catch-and-release ethic among its members by claiming that releasing muskellunge benefits both natural reproduction and supplemental stocking (www.muskiesinc.org). This attitude change has been perceived as benefitting Wisconsin's goal to produce trophy muskellunge (Simonson and Hewett 1999).

Despite the catch-and-release mentality, harvest of muskellunge does occur, which requires management regulations to protect and maintain the population's viability. Regulations are coupled with supplemental stocking to manage the resource. The WDNR's primary minimum size limit for muskellunge is 86.4 cm with a one fish total daily bag limit that is specifically aimed to help sustain natural recruitment and to protect large muskellunge until they reach trophy size (Simonson 2002). Recently, some lakes in northern Wisconsin have instituted large minimum length limits (101.6 – 127.0 cm) in an attempt to protect spawning fish (thus increasing the overall production the system) and to produce more trophy fish by minimizing mortality (Simonson 2002).

Muskellunge propagation is a prominent management tool used in conjunction with regulations by the WDNR and local fishing clubs to maintain or supplement populations throughout the state. Muskellunge stocking began in Wisconsin near the turn-of-the-century (Nevin 1901; Margenau 1999). The original intent of muskellunge

stocking in Wisconsin was to alleviate the impacts of overexploitation by simply augmenting the number of muskellunge available. In early fish management, it was thought that supplementing a muskellunge population with propagated fish would only enhance the resource (Margenau 1999). As propagation practices and efficiency became more refined, stocking was also used to restore extirpated populations, expand the natural range, and supplement populations without sufficient natural recruitment (Simonson and Hewett 1999).

Early muskellunge propagation primarily used fry and was mainly performed on a regional and convenience basis (Nevin 1901). Much of this early stocking was poorly recorded and evidence for success of the program was anecdotal at best. More recently, stocking using fingerlings (~11.6 cm) and extended-growth fingerlings (~22.9 cm) from two main hatcheries, the Governor Tommy G. Thompson State Fish Hatchery in Woodruff and the Art Oehmcke State Fish Hatchery in Spooner (Figure 2), has become standard practice in Wisconsin (Margenau 1999). These two hatcheries serve northeast Wisconsin and northwest Wisconsin, respectively.

Research in muskellunge propagation has included studies aimed at determining the effectiveness of stocking in relation to age at stocking (Hanson et al. 1986; Serns and Andrews 1986; Margenau 1992; Wahl 1999) and survival/performance of stocked fish (Hanson and Margenau 1992; Margenau 1992; Wahl 1999). Wahl (1999) found that survival rates increased when larger muskellunge were stocked into waters with similar temperature profiles. Predation, starvation, and stress have also been shown to be major factors in survival of stocked fish (Hanson and Margenau 1992).

As discussed previously, strain performance in Wisconsin has also been researched (Johnson 1971; Lyons and Margenau 1986; Margenau and Hanson 1996) to examine, in part, the potential influences of supplementally stocked fish on the genetic integrity and performance of a muskellunge fishery. Margenau and Hanson (1996) used paired-strain stockings to compare differences between lakes that are used as brood sources in Wisconsin by observing short term (<60 days) and long-term survival and growth rates based on length. Results showed that the Mud/Callahan muskellunge out-competed the LCO strain when both were stocked in Mud/Callahan Lake. The short-term survival of fingerlings showed that Mud/Callahan strain fingerlings likely outcompeted LCO fingerlings when both were present; however, the LCO strain grew to greater lengths faster than the smaller, slow growing Mud/Callahan strain (Margenau and Hanson 1996). However, by evaluating growth of tagged fish, Johnson (1971) was able to conclude that Big Spider Lake muskellunge stocked in LCO were outcompeted by the native muskellunge likely because of an unknown hereditary factor. Margenau and Hanson (1996) concluded these performance differences were the result of some combination of environmental and genetic factors.

In 2002, the WDNR established a muskellunge management plan outlining goals and measures that the state can use to monitor muskellunge resources. Two of the major goals of the current muskellunge management plan are to: (1) provide anglers with a variety of unique fishing opportunities, including trophy muskellunge (defined as >127 cm), and (2) "protect and enhance Wisconsin's naturally reproducing muskellunge populations" (Simonson 2002). Recently, the WDNR's muskellunge propagation program underwent a series of strategic modifications aimed at meeting the goal of

protecting the genetic integrity of local, naturally reproducing populations (Sloss 2004). The focus of these modifications was minimizing the risk to naturally recruiting, putatively native populations of muskellunge. To further these efforts and effectively manage Wisconsin's muskellunge populations into the future, a more resolved pattern of genetic stock structure is needed.

Muskellunge management and genetic resources.—A key concept in managing the genetic integrity of fishery resources is the stock concept (Kutkuhn 1981; STOCS 1981). A fishery stock is defined as an intraspecific group of randomly mating individuals that is reproductively isolated, shares a common gene pool, and has temporal and spatial stability (modified from Larkin 1972; Ihssen et al. 1981). The fishery stock concept is based on the idea that managing for the long-term sustainability of the component stocks will lead to long term stability of the entire resource (STOCS 1981). The use of the stock concept in fishery management programs allows multiple populations to be grouped for management purposes while still managing a biologically cohesive unit (Dizon et al. 1992). In recent years, a fishery stock in this sense has become synonymous with the management unit (MU). A MU is formally defined as the ecological component of larger evolutionary significant units that are diagnosed as a population(s) that exhibit significant allele frequency differences (Moritz 1999).

A critical assumption of any stock management approach is that the stocks being managed have been identified in a standardized, reliable, biologically relevant manner (Laikre et al. 2005). In practice, fisheries managers often recognize stocks as a group of organisms harvested in a particular area. This approach is dependent on what and how much information is available including data on age structure, life history, and other

phenotypic and demographic estimates. Collecting these data requires extensive field-based efforts that are often not feasible (Carvalho and Hauser 1994). Furthermore, meristic, morphological, and life history data can converge and/or diverge due to environmental effects, thereby not measuring isolation/separation of gene pools and/or populations (Shaklee and Currens 2003). However, it is preferable to base stock identification on more definable measures. Genetic data has become a favored method for stock identification due to the large amount of data that can be generated quickly from small, non-lethal amounts of tissue (Miller and Kapuscinski 1996) and the ability of genetic data to statistically assess the level of connectivity between populations, and thus, the degree of isolation between populations (Shaklee and Currens 2003).

Recently, a large body of research has emerged showing superior resolution of genetic data for stock delineation compared to geologic, geographic, and/or phenotypic approaches (Angers et al. 1995; Petit 1998; Potvin and Bernatchez 2001; Arnand-Haond et al. 2004; Laikre et al. 2005). For instance, Angers et al. (1995) looked at brook trout (*Salvelinus fontinalis*) population structure across five lakes in La Mauricie National Park, Quebec, Canada. Variation in microsatellite DNA showed large amounts of interpopulation genetic variation not seen with other genetic markers, such as mitochondrial DNA. This structure was resolved despite a geographically small region (536 km^2). The observed genetic diversity allowed Angers et al. (1995) to identify a quantitatively delineated stock structure for brook trout in the park.

Stock structure of northern pike has also been successfully studied using microsatellites. Miller and Kapuscinski (1996) tested newly developed northern pike microsatellite loci in hopes of finding greater genetic diversity than previous molecular

markers. They screened nine microsatellite loci using four northern pike populations (Miller and Kapuscinski 1996). The allele frequency distributions at these loci were able to distinguish populations (Miller and Kapuscinski 1996). Subsequently, microsatellite markers were used to delineate northern pike stock structure in the Baltic Sea (Laikre et al. 2005). Genetic diversity at five microsatellite loci showed that northern pike in the Baltic Sea were not one panmictic population (Laikre et al. 2005), but represented multiple management units based on genetic groupings.

The growing reliance on genetic data for stock delineation has spurred the development of a suite of powerful and accurate statistical methods commonly referred to as Genetic Stock Identification (GSI; Shaklee and Currens 2003). The goal of GSI is to test successively smaller combinations of samples (i.e., populations/spawning aggregates) to assess differences consistent with two or more gene pools in a sample. When non-significant combinations of samples are found among other significantly different groupings, a stock is generally identified (Shaklee and Currens 2003). This quantitative framework coupled with new, highly polymorphic molecular genetic techniques and expanded sample availability (i.e., noninvasive sampling and techniques with low DNA quality/quantity requirements) is responsible for the established reliance on genetic data for stock discrimination (Waples and Gaggiotti 2006).

Currently, the WDNR attempts to maintain the genetic integrity of their muskellunge resource using genetic management zones (GMZs) originally developed as part of a molecular genetic study conducted in the mid-1990s (Figure 3; Fields et al. 1997). These GMZs are used to delineate regions where stocking can occur within the zone with low risk of outbreeding depression but is prohibited across zones due to an

unacceptable risk of outbreeding depression. Fields et al. (1997) sampled ten muskellunge populations (n = 26-30 individuals/population) from four major watersheds in northern Wisconsin (Upper Chippewa River , St. Croix River, Upper Wisconsin River, and Lake Superior; Figure 4) and analyzed allozyme and mitochondrial DNA (mtDNA) diversity to describe stock structure. The mtDNA data showed no variability among populations; thus, no stock structure was resolved. The allozyme analysis showed only two polymorphic loci and resolved little to no genetic structure among Wisconsin muskellunge populations. Despite the overall lack of resolution, five stocks of Wisconsin muskellunge were conservatively suggested based on watershed boundaries within the state: Lake Superior, Chippewa River/St. Croix River, Lower (WI) Mississippi River, Upper Wisconsin River, and Lake Michigan; these five stocks represent the current GMZs used to manage Wisconsin's muskellunge. The Lower Mississippi River GMZ does not possess native muskellunge fisheries and as such, is currently given wide-latitude by the WDNR in terms of sources of stocked fish where lakes that have no immediate access to native range muskellunge populations are designated universal acceptor lakes. The Lake Superior GMZ is not managed with a unique brood source but instead is stocked with fish from the St. Croix/Upper Chippewa River GMZ (Tommy Thompson State Fish Hatchery, Spooner, WI). Despite the conservative resolution of five GMZs in Fields et al. (1997), a confidently resolved genetic structure among muskellunge populations was not recovered for several reasons. The study was confounded by low genetic variability among sampled loci (both allozyme and mtDNA), relatively low sample sizes (both number of populations and number of individuals/population), and related low statistical power. Only two allozyme loci (out of

61 originally surveyed for muskellunge variation) showed any polymorphism within Wisconsin populations, and the levels of polymorphism were low, resulting in limited resolution of genetic structure. Secondly, sample sizes employed by Fields et al. were below most contemporary standards for confident resolution of genetic structure. Ryman et al. (2006) showed that the use of a small number of loci, such as in Fields et al. (1997), coupled with low variability (number of alleles = 2-3 in Fields et al. 1997) requires sample sizes of at least 50 individuals to achieve even moderate power ($1-\beta \approx 0.5$). Further, Ruzzante (1998) found similar sample size and polymorphism requirements in simulations examining the ability of highly polymorphic loci to discern population differentiation.

The small number of Wisconsin muskellunge populations included in the study was a concern in delineating Wisconsin genetic structure; Wisconsin has >700 muskellunge populations throughout the state. Although their study focused on muskellunge throughout the Midwest, only 10 populations were sampled in the northern third of Wisconsin, representing a small proportion (<2.0%) of the total muskellunge populations in this region. Therefore, concluding no genetic structure exists based solely on this small population sampling, relatively small number of individuals/population, and a small number of genetic loci would risk missing underlying differences among populations within or between current watershed boundaries. For these reasons, the GMZs as outlined by Fields et al. (1997) should be considered conservative management zones. Recent developments in methods for conservation genetic studies, such as highly polymorphic microsatellite loci and non-lethal sampling techniques, could provide valuable data and potential clarity to the resolution of muskellunge GMZs in Wisconsin.

The long-term genetic integrity of Wisconsin muskellunge cannot be assured despite the use of these GMZs mainly because of past stocking practices. Unfortunately, much of the early stocking in Wisconsin was poorly documented and believed to have occurred across the current GMZs, raising questions about past impacts such as outbreeding depression and contemporary issues about current stock structure and propagation management. Each of the three Wisconsin hatcheries (Oehmcke = Wisconsin River, Thompson = St. Croix/Mississippi, and Wild Rose = Lake Michigan) mainly serves one GMZ and currently use brood fish only from their respective zones. However, known exceptions to this restriction have regularly occurred over the past 30-40 years. If current GMZs do not represent the natural genetic stocks, this approach could disrupt the genetic integrity it is trying to preserve.

Local adaptation is a genetic change that occurs in isolated populations because of natural selection driven by local environmental factors (Hallerman 2003). Fish populations in lakes, such as lake-dwelling muskellunge populations, are generally isolated from one another with limited or no migration between them. Isolation, caused by geological features or geographic distance between populations, can lead to genetic changes in the population via genetic drift (random events) and natural selection. Over time, selection pressures can result in populations that are locally adapted to their specific, isolated environment. Local adaptation often leads to coadaptations resulting in combinations of alleles from various loci that perform best (i.e., fitness) in the presence of other specific alleles. In time, coadaptations form coadapted gene complexes whereby translocation and/or recombination physically link the loci and the specific alleles in close proximity on a chromosome or a few chromosomes (Meselson and Radding 1975;

Chatti et al. 1999; Hallerman 2003; Swain et al. 2005). The result is a locally adapted population that exhibits, theoretically, higher fitness within its current, native environment.

Once local adaptation and gene complexes are formed, any disruption of local adaptations and/or gene complexes (i.e., disruption of the genetic integrity) can negatively affect fitness (Lynch and Walsh 1998). The most common fisheries practice that can disrupt genetic integrity is the stocking of genetically divergent fish potentially resulting in a phenomenon known as outbreeding depression (Philipp and Whitt 1991; Philipp and Claussen 1995; Hallerman 2003). Outbreeding depression is formally defined as the loss of fitness due to the disruption of locally adapted characteristics or coadaptive gene complexes (Dobzhansky 1948; Templeton 1986; Lynch 1991). Classic examples in fisheries science are studies of hybridization between the northern largemouth bass (*Micropterus salmoides salmoides*) and the Florida largemouth bass (*M. s. floridanus*; Philipp and Whitt 1991; Philipp and Claussen 1995; Philipp et al. 2002). Several crosses between the northern and southern strains were stocked into Midwestern waters to assess the performance of each strain and the crosses. Phillip and Whitt (1991) found that the southern bass and the various crosses involving southern bass had poor survival rates when compared to native bass in northern study in pond. They concluded their findings were caused by outbreeding depression in the various crosses and suggested that stocking Florida bass in northern climes would lower mean fitness of the receiving (northern) populations, contradicting sound, science-based management goals.

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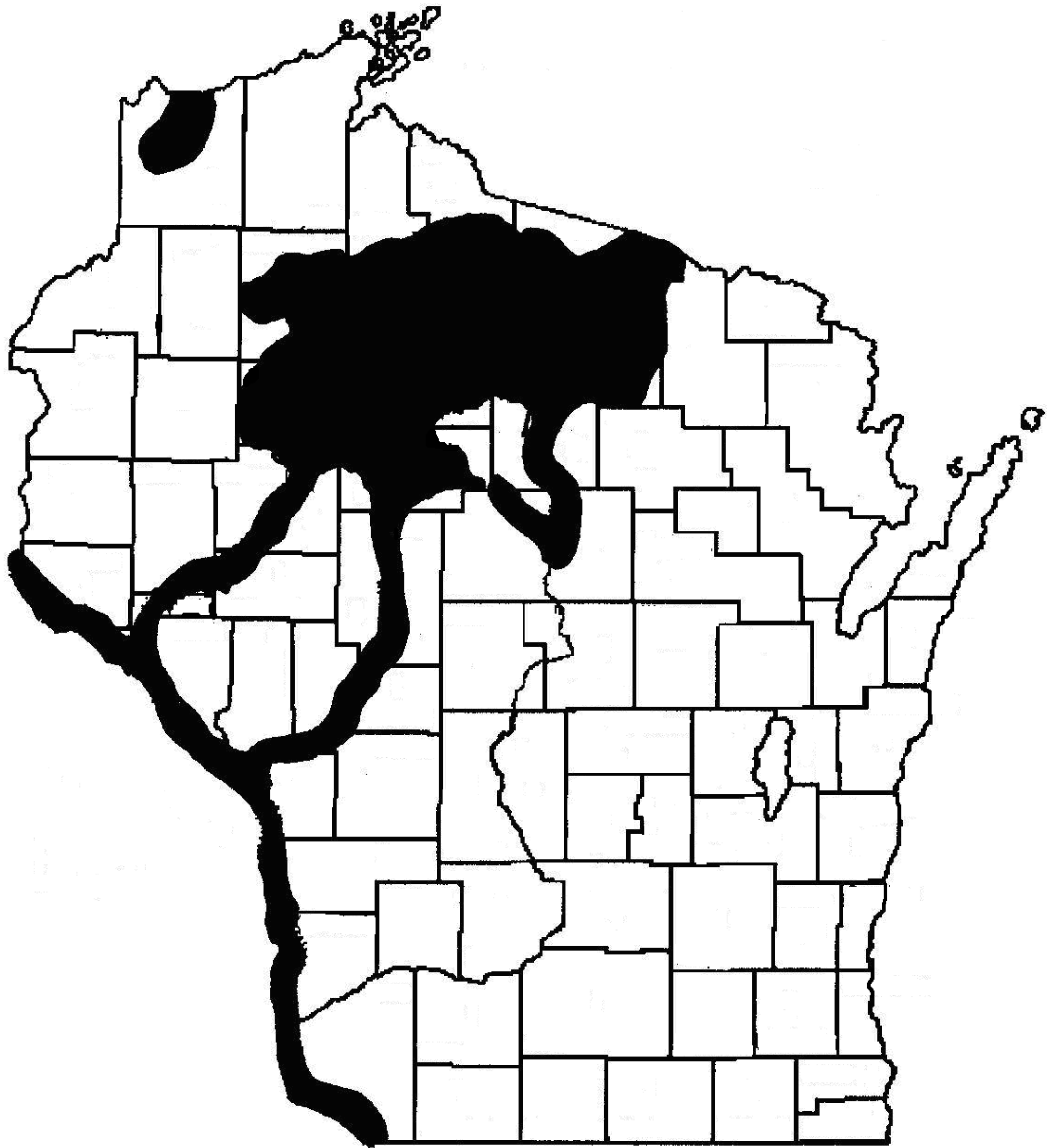

Figure 1. Native range of muskellunge in Wisconsin according to Becker (1983).

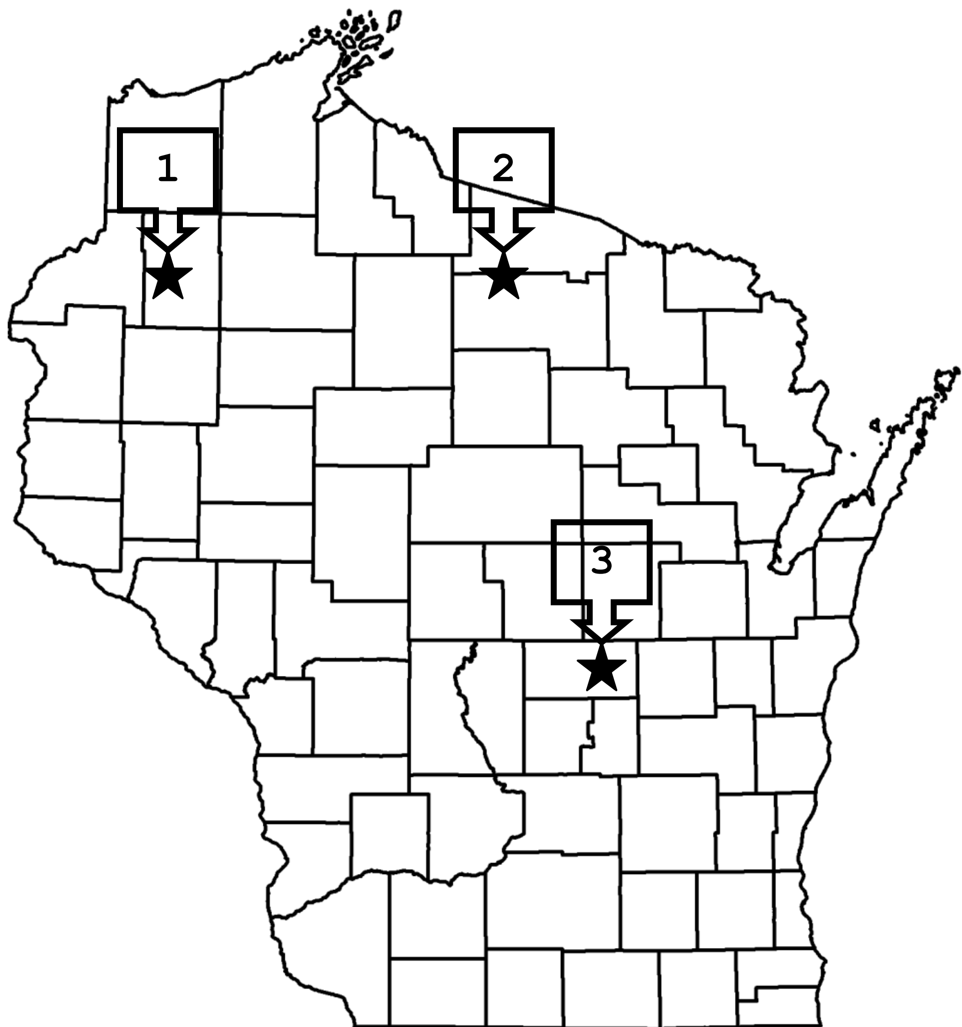

Figure 2. Three hatcheries involved with muskellunge propagation in Wisconsin and their relative location in the state. (1) Governor Tommy G. Thompson State Fish Hatchery, (2) Art Oehmcke State Fish Hatchery, and (3) Wild Rose State Fish Hatchery.

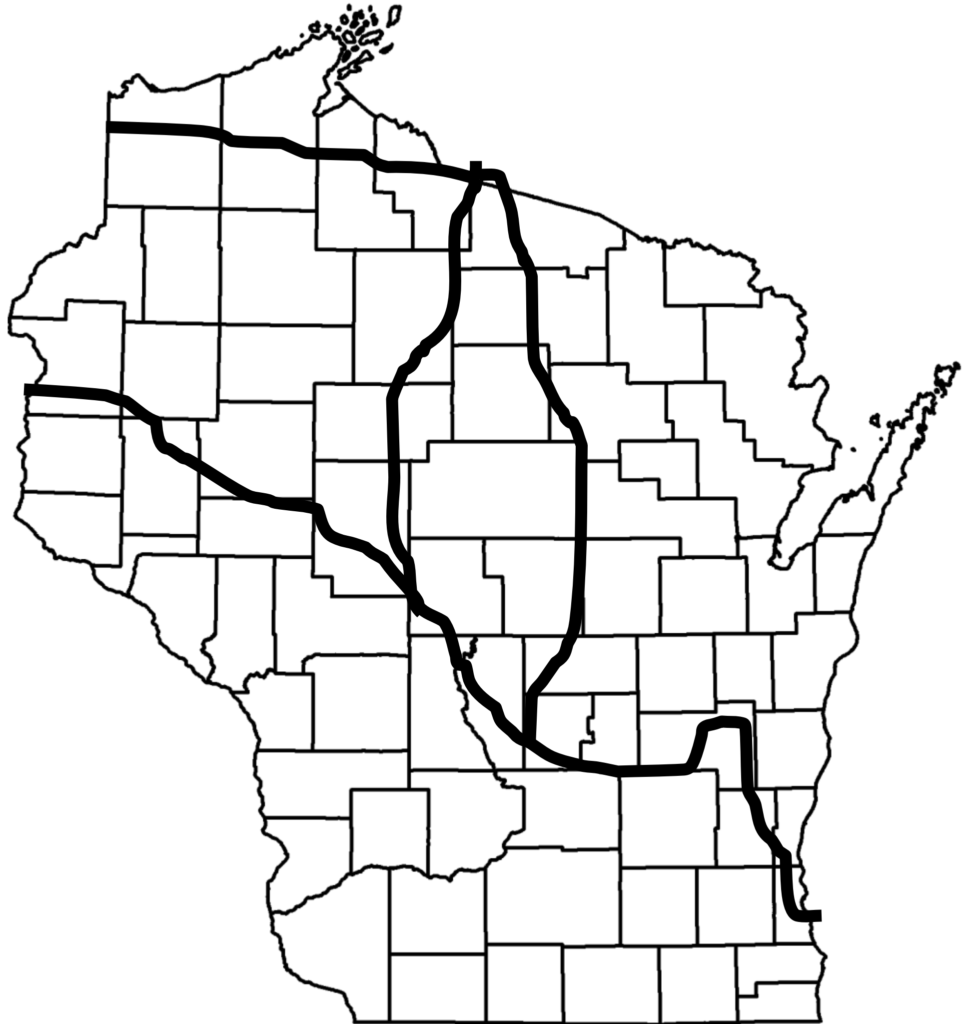

Figure 3. Current genetic management zones suggested by Fields et al. (1997) based partially on allozyme and mtDNA data.

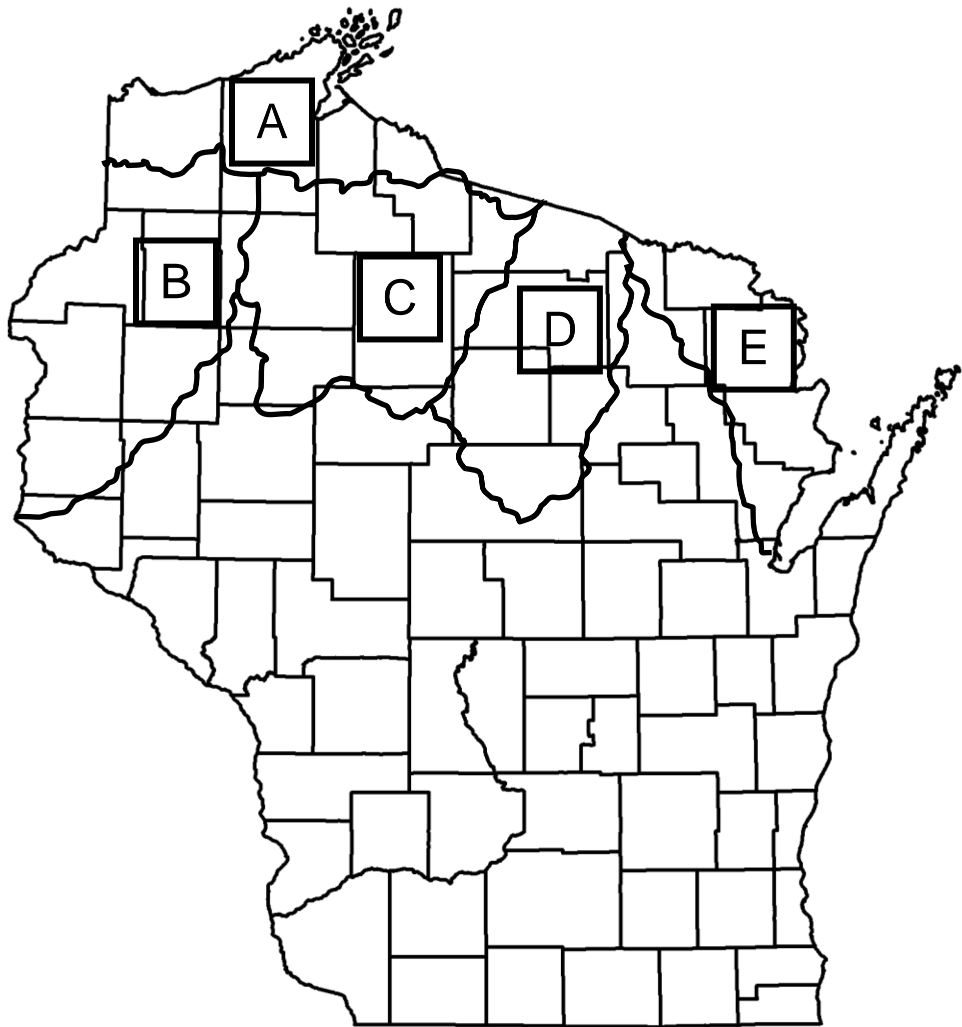

Figure 4. Major watersheds in northern Wisconsin: (A) Lake Superior, (B) Upper St. Croix, (C) Upper Chippewa, (D) Upper Wisconsin, and (E) Green Bay (Lake Michigan).

Chapter 1:

TEMPORAL GENETIC INTEGRITY OF LAC COURTE OREILLES' MUSKELLUNGE POPULATION: IMPLICATIONS FOR MUSKELLUNGE MANAGEMENT PRACTICES

Abstract–The muskellunge is the state fish of Wisconsin and plays a major role in Wisconsin's sport fishery. Since the 1930s, muskellunge have been propagated extensively in the state. The traditional brood source for northwest WI has been Lac Courte Oreilles (LCO), a 2,015.5 ha lake in the Chippewa River drainage favored because of its production of trophy-sized fish (>127 cm). However, stocking from other populations into LCO has occurred in the past. Concerns exist regarding the impacts on the genetic integrity of LCO muskellunge in light of these past stocking events and, subsequently, potential negative impacts of using LCO as a brood source for stocking in Wisconsin. The objective of this research was to determine if two suspect stocking events that used perceived slow/small growth strains of muskellunge disrupted the genetic integrity of the LCO population by evaluating the temporal genetic stability of LCO. Archived scale samples from all lakes (pre-stocking), from LCO 1966 and 1976, and contemporary samples from all lakes (LCO and both brood sources) were used to assess the genetic characteristics at nine microsatellite loci from each sampled population over time. The ability to distinguish among populations with microsatellite genetic diversity is critical to assessing stocking impacts; the assumption that sources of stocked fish (from waters within the Chippewa R. drainage) are from reproductively isolated populations has not been tested. Genetic and allelic diversity comparisons between the three muskellunge populations showed significant differences existed both pre-stocking and post-stocking. The muskellunge population in LCO changed through time but no

significant impact associated with introgression of genetic material from the two source populations or other admixture scenarios were observed. No observed impact from the two suspect stocking events was found suggesting these two events had no long-term genetic impact on the temporal integrity of LCO.

INTRODUCTION

Muskellunge propagation is a prominent management tool used by the Wisconsin Department of Natural Resources (WDNR) and local fishing clubs to maintain or supplement populations throughout the state. Muskellunge stocking began in Wisconsin near the turn of the 20th century (Nevin 1901; Margenau 1999) with the intent of alleviating the impacts of overexploitation by simply augmenting the number of available muskellunge. As propagation practices and efficiency have become more refined, stocking has been used to restore extirpated populations, expand the range of the species, and supplement populations without sufficient natural recruitment (Simonson and Hewett 1999).

In 1939, the Lac Courte Oreilles (LCO; Sawyer Co.) muskellunge population was first used as a brood source by the Wisconsin Department of Natural Resources (WDNR) for propagation. The lake contained a prominent muskellunge fishery renowned for large trophy fish. Recently, concerns have risen regarding a perceived lack of growth potential in LCO muskellunge. These concerns focus on potential genetic impacts of two suspect stocking events that have occurred in LCO over the past 50 years. In particular, LCO was supplementally stocked with fish from Big Spider Lake (SPI; Sawyer Co.) in 1956 and Mud/Callahan Lake (MC; Sawyer Co.) in the early 1980s. These two lake systems contain muskellunge that exhibit slow growth rates and/or small growth (i.e., size) potential (Lyons and Margenau 1986; Margenau and Hanson 1996). These studies implicated a genetic/hereditary factor as a contributing cause to the low growth potential in these populations.

Because growth attributes are, at least in part, heritable, the growth differences among these three populations may likely be the partial result of local adaptation to local conditions. Local adaptation is a genetic change (such as growth attributes) that occurs in isolated populations because of natural selection driven by local environmental factors (Shaklee and Currens 2003). Lake-dwelling muskellunge populations are generally isolated from one another (i.e., no migration between them); therefore, the populations are subjected to genetic changes via genetic drift (random events) and natural selection. Over time, selection pressures lead to populations that are locally adapted to their specific, isolated environment, and lead to coadapted gene complexes (Meselson and Radding 1975; Chatti et al. 1999; Hallerman 2003; Swain et al. 2005). Ultimately, this process results in a locally adapted population that is, theoretically, genetically superior within its current, native environment.

The supplemental stocking of LCO with SPI and MC muskellunge could have negatively affected the genetic integrity and, subsequently, the growth performance of LCO muskellunge if slow growing stocked fish introgressed with the native stock. Once local adaptation and gene complexes are formed, any disruption of local adaptations and/or gene complexes (i.e., disruption of the genetic integrity) can negatively affect fitness (Lynch and Walsh 1998). The most common fisheries practice that can disrupt genetic integrity is the stocking of genetically divergent sources. Two potential causes of disruption are: 1) outbreeding depression and 2) introgression. Outbreeding depression is formally defined as the loss of fitness because of disruption of locally adapted characteristics or coadaptive gene complexes (Dobzhansky 1948; Templeton 1986; Lynch 1991; Hallerman 2003). Diminished performance consistent with outbreeding

depression has been shown in largemouth bass (*Micropterus salmoides*; Philipp and Whitt 1991; Philipp and Claussen 1995). Introgression is the movement of alleles from one population/species to another through hybridization. As such, introgression has the can introduce genetic variants and, subsequently, heritable characteristics of one population into another. Since LCO, SPI, and MC have shown different growth attributes, the two supplemental stocking events in question could have resulted in heritable changes in the growth potential of LCO muskellunge.

The goal of this study was to examine the impacts of two supplemental stocking events on the genetic integrity of LCO. The specific objective was to evaluate the temporal genetic dynamics of the LCO muskellunge population to determine if significant genetic changes were observed in samples representing pre-stocking samples and post-stocking samples.

METHODS

Study Site

The primary study site for this project was Lac Courte Oreilles (LCO), a 2,015.5 ha soft water, drainage lake located in Sawyer County and connected to both Grindstone Lake and Whitefish Lake (Sather and Threinen 1968). Two secondary study sites were Mud Lake/Callahan Lake (MC) and Spider Lake (SPI), also located in Sawyer County. (Figure 1). Mud Lake and Callahan Lake are connected, soft water drainage lakes that collectively cover 218 ha (Sather and Threinen 1968) and are well known for their network of bays and inlets. Spider Lake is a 581.7 ha hard water drainage lake comprised of three main basins: Big Spider Lake, Little Spider Lake, and Clear Lake (Sather and Threinen 1968), and connected to Fawn Lake and North Lake (Sather and Threinen 1968).

Study Design

Archived scale and spine samples and contemporary fin clips preserved in 95% ethanol comprised the majority of samples in this study. The scales and spines were collected over the past 50+ years during spring lake surveys by the WDNR. Samples (target n = 50/temporal sample) were obtained from all three sites including multiple temporal samples from LCO (Figure 2). LCO samples consisted of individuals from 1956 (LCO56), 1966 (LCO66), 1976 (LCO76), 1990 (LCO90), and 2006 (LCO06). Samples for SPI were from 1956 (SPI56; the same year fish were taken from SPI for brood source) and the MC sample was from 1979 (MC79; just prior to the MC stocking event). These samples were specifically chosen to represent pre- and post-stocking event

samples and to characterize the genetic diversity of the historic SPI and MC populations that served as brood sources for the two supplemental stockings of concern (Figure 2). Tests were conducted in a linear time fashion such that LCO56 was compared to LCO66, LCO66 was then compared to LCO76, etc. All LCO samples were tested against the single SPI and MC samples to test for similarity in any temporal sample to the other populations. Three experimental assumptions/prerequisites were necessary for this study. First, it was necessary for genetic differences to be present between the LCO population at the time of supplementation and the two source populations (SPI and MC). Second, significant disruption of the genetic integrity of the LCO population was necessary for a change in performance characteristics to occur. Third, the suite of molecular markers employed was capable of detecting an admixture event as well as, any disruption in genetic integrity.

The first stocking event of interest occurred in 1956 and used brood stock from SPI. The LCO56 and SPI56 were used as pre-stocking samples and the remainder of the LCO samples acted as post-stocking samples. The two pre-stocking samples (LCO56 and SPI56) were compared to each other to measure genetic differences between the two populations. The samples were then tested in the linear temporal fashion described previously. If no differences were observed between the pre- and post-stocking samples, no impact of the stocking event would be inferred. The MC stocking event was evaluated by first comparing the LCO76 to MC79 samples to measure genetic differences between the two populations. Samples from LCO56, LCO66, LCO76, and MC79 were used as pre-stocking samples and post-stocking samples from LCO90 and LCO06 were evaluated to determine if genetic integrity was impacted. Differences between the pre-stocking

LCO samples and the post-stocking LCO samples were evaluated as potential signals of disruption of the LCO genetic integrity due to these two events.

Lab Methods

DNA was extracted from the fin-clip samples using the Wizard® Promega Genomic DNA purification kit (Promega Corporation, Madison, WI) and from the archived scale and spine samples using the QIAGEN DNeasy® extraction kit (QIAGEN Inc., Valencia, CA). Both extraction methods followed the manufacturer's suggested protocol except the final elution of extracted DNA, which was in 200 µl of Tris-low-EDTA buffer. DNA quality was evaluated by electrophoresing the DNA in a 1% agarose gel in the presence of ethidium bromide, visualized with UV light, and compared to a molecular weight ladder BioLine Hyperladder™ I (Bioline USA Inc., Randolph, MA). DNA quantities were measured with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples were normalized to equal DNA concentrations (25 ng/µl) to ensure consistent results between samples.

Nine microsatellite loci developed (Sloss et al. 2008) for the genetic analysis of muskellunge (Table 1) were used to genotype all sampled individuals. Multiplex PCR reactions (three loci/reaction; Table 1) were conducted to optimize time, effort, and cost while genotyping samples (Sloss et al. 2008). Loci were PCR amplified with fluorescently labeled primers and analyzed on an ABI Prism® 377XL automated DNA sequencer (Applied Biosystems Inc., Foster City, CA). Allele sizes were determined by comparison to an internal size standard (GeneFlo™ 625, Chimerx Inc., Milwaukee, WI) using GeneScan® (Applied Biosystems, Inc.).

Statistical Analysis

Basic diversity measures.—Basic genetic diversity measures were calculated for comparisons among temporal samples. Allelic richness (A_r), a measure of the number of alleles/locus, was estimated based on the rarefaction method of Kalinowski (2004) to account for unequal sample sizes using HP-Rare (Kalinowski 2005). The observed heterozygosity (H_o) and expected heterozygosity (H_e) of each temporal sample was calculated using GenAlEx v6 (Peakall and Smouse 2006). Each temporal sample was tested for conformance to Hardy-Weinberg Equilibrium (HWE) using the exact test of Guo and Thompson (1992) as implemented in GENEPOP 3.4 (Raymond and Rousset 1995) that uses a Markov Chain with 1,000 dememorization steps, 100 batches and 1,000 iterations. Due to known issues with low expected genotype frequencies and exact tests of HWE, loci/population tests showing significant deviation from HWE underwent pooling of rare genotypes (all genotypes with expected frequency <1% were pooled) and re-testing using a chi-square test (Crisp et al. 1978; Hedrick 2000). A gametic disequilibrium (GD) test for independence of each locus was conducted in GENEPOP 3.4 (Raymond and Rousset 1995) using 1,000 dememorization steps, 100 batches and 1,000 iterations. For both the HWE and the GD tests, alpha (0.05) was corrected for multiple pairwise tests using a sequential Bonferroni correction (Rice 1989).

Population differentiation.—Tests of differentiation between temporal samples were conducted using three primary approaches to ensure consistent findings. First, an analog of Wright's F_{ST}, theta (θ; Weir and Cockerham 1984), was calculated and tested for deviation from zero (i.e., no genetic difference) using Arlequin v3.1 (Excoffier et al. 2005). A sequential Bonferroni correction was used to correct alpha ($\alpha_1 = 0.05$) for

multiple pairwise comparisons. Second, a test of genic differentiation was used to test pairwise differences among all temporal samples. This test evaluates whether allele frequency distributions are significantly different among samples. This was conducted using the genic differentiation option in GENEPOP v3.4 (Raymond and Rousset 1995) with 1,000 dememorization steps, 100 batches and 1,000 iterations. A final test of population differentiation was performed using genetic distance measures and an unrooted neighbor-joining (NJ; Saitou and Nei 1987) clustering method. Cavalli-Sforza and Edwards (1967) chord distance was chosen because it is appropriate when looking at random changes caused by genetic drift (Shaklee and Currens 2003), it has been shown to be efficient and reliable in obtaining the correct tree topology (Takezaki and Nei 1996), and it is commonly used and accepted for microsatellite genetic data (Allendorf and Luikart 2007). A pairwise matrix of chord distance values was used to construct unrooted NJ trees in POWERMARKER (Liu and Muse 2005). Confidence in tree topology was inferred using 1,000 bootstrap pseudoreplicates performed in POWERMARKER. A majority rule consensus was constructed using the CONSENSE routine in PHYLIP v3.67 (Felsenstein 2007). A node was considered moderately resolved if the bootstrap values were ≥50% and highly resolved at bootstrap values ≥85%.

RESULTS

Basic Diversity Measures

In total, 321 muskellunge samples were genotyped to evaluate the temporal stability of LCO with the smallest sample (n = 36) from LCO06 and the largest (n = 50) from LCO76, LCO90, and MC79 (Table 2). Mean diversity measures were mostly consistent across populations with the LCO56 and SPI56 sample showing lower diversity than the other samples (Table 2). For example, allelic richness for LCO56 and SPI56 was 3.921 and 4.187, respectively, whereas the next lowest mean value was 4.317 (MC79). Initial tests of HWE showed 12 loci out of 63 tests (total of 9 loci and 7 populations with one locus monomorphic) were significantly different from HWE expectations. Following sequential Bonferroni correction and the pooling of rare genotypes, all loci and populations conformed to HWE.

Evaluation of Stocking Events

The 1956 Spider Lake stocking event.—The first step in evaluating the 1956-stocking event was to test whether significant population differences existed between the LCO56 sample and the SPI56 sample. The two samples were significantly divergent. Genic differentiation tests showed significant differences between LCO56 and SPI56 (p-value = <0.001; Table 3). The two samples had an $F_{ST} = 0.107$ that was significantly different from zero ($p < 0.0001$; Table 4). Therefore, a key assumption (divergence among source population and LCO) was met.

The 1980s Mud-Callahan Lake stocking event.—The same approach was taken when investigating the MC stocking event. Pairwise F_{ST} values comparing MC79 and

LCO66 showed significant divergence with an F_{ST} value of 0.0612 (p-value = <0.0001; Table 4). The NJ tree showed that MC was different from all LCO samples (Figure 3). This showed that MC and LCO were genetically different at the time MC was used as a brood source. Genic differentiation confirmed significant differences between MC and pre- and post- stocking LCO samples (Table 3). These three tests showed that LCO was different from MC both before and after the stocking event. In addition, pre- and post-stocking samples of LCO showed no genetic changes caused by stocking.

Lac Courte Oreilles genetic stability from 1950 to present.—The second step in evaluating the 1956 and 1980s stocking events was to test for temporal genetic change among pre-stocking samples of LCO and post-stocking samples of LCO. Tests of differentiation based on F_{ST} and genic differentiation appeared to yield different results. The only significant difference among the F_{ST} tests was between LCO66 and LCO76, a period with no stocking. Genic differentiation comparisons showed genetic stability of LCO; however, the LCO90 versus LCO06 comparison was statistically significant using genic differentiation. When the unrooted NJ tree is considered in this portion of the analysis, it appears there is relative stability among the LCO samples and no evidence of impact from the SPI56 stocking (Figure 3). The genic differentiation test used in this portion of the analysis examines the allele distribution of the study populations. Therefore, this test can be more sensitive to shifts in dominant alleles and/or small sample sizes that show less allelic diversity (Raymond and Rousset 1995).

DISCUSSION

This study examined the muskellunge population of LCO using archived scale samples to evaluate the temporal genetic dynamics in light of two stocking events that used perceived smaller, slower growing strains of muskellunge. The ability to distinguish among three populations in a relatively small geographic area using microsatellites was expected even with the stocking history of these lakes due to the relatively isolated nature of muskellunge populations, small population sizes, and the high levels of diversity observed at microsatellite loci. Studies similar to this have been successful in finding differences among populations on a micro-geographical scale (Adams and Hutchings 2003; Brunner et al. 1998; Beacham and Wood 1999; Angers et al 1995).

The genetic integrity of LCO's muskellunge population was unaffected by either the SPI stocking event or the MC stocking event. This could be due to low survival rates of stocked fish and/or low reproductive success of stocked fish that reach maturity. The apparent conflict between the results of F_{ST} and genic differentiation tests was likely a result of genetic drift and the sensitivity of genic differentiation to changes consistent with genetic drift. The results showed that LCO, SPI, and MC were genetically distinct before and after stocking. In addition, the genetic stability of LCO was shown through F_{ST} values, genic differentiation, and the distance-based NJ tree. These findings suggest that neither the SPI nor the MC stocking events affected the genetic stability of the LCO muskellunge population.

Many studies have looked at the survival of stocked fish, particularly sport fish such as muskellunge and walleye (Hanson and Margenau 1992; Margenau 1992; Wahl 1999; Parsons and Pereira 2001; Jennings et al. 2005). Wahl (1999) found that survival

rates increased when larger muskellunge were stocked into waters with similar temperatures. It was also shown that predation, starvation, and stress are major factors in survival of stocked fish (Hanson and Margenau 1992). In addition, Margenau (1992) found overwinter survival rates were low in stocked muskellunge. This suggests the suspect stocking events did not result in genetic impact because the probability of stocked fish reaching reproductive maturity was low, and if the stocked fish failed to reproduce and pass on their genetic material, the genetic integrity of the receiving population would not be affected by the supplemental stockings. Nevertheless, the potential genetic risk of such activities existed and continues to exist. For example, a current study in Minnesota has shown significant admixture in the Moose Lake muskellunge population with a minimum of three distinct genetic strains mixing over the past 40+ years (Loren Miller, Univ. of Minnesota, personal communication).

Philopatry may have played a major role in the failure of stocked fish to influence the long-term genetic integrity of LCO. Philopatry can cause reproductive isolation in large populations (Gharrett and Zhivotosky 2003) and natal philopatry (or homing) has been well documented in anadromous fish species, particularly the Pacific salmonids (*Oncorhynchus* spp.), but also documented in some freshwater species. For example, lake whitefish (*Coregonus clupeaformis*) are suggested to home to natal spawning grounds throughout Lake Michigan (Ebener 1980; Ebener and Copes 1985). Miller et al. (2001) found that northern pike returned to spawning sites based on a mark-recapture study done in Kabetogama Lake, in Voyageurs National Park, Minnesota. This study also found, using microsatellites, that the study lake had multiple spawning aggregates suggesting that northern pike show natal philopatry (Miller et al 2001). Crossman (1990)

found that muskellunge also return to the same spawning sites. In his study, nets were set every year during egg collection for muskellunge propagation in Stony Lake, Ontario, Canada. Fish were marked as they were caught and recaptures were recorded. Because of the large number of recaptured spawning fish in the same net year after year, Crossman (1990) concluded that the study population was philopatric. If muskellunge exhibit natal philopatry, stocking a self-sustaining population such as LCO may result in augmenting the population's numbers but failure of the adult, stocked fish to contribute to the gene pool of the population. In essence, the stocked fish may not have the correct cues to find the proper spawning location. Other studies have observed lack of homing ability in stocked and transplanted fish, which lead to low survival of stocked fish (Bams 1976; Gharrett and Smoker 1991; Gilk et al. 2004). The theory that stocked fish fail to home to spawning sites and thus fail to reproduce may be a factor for the lack of observed impact in this study.

It is possible some stocked fish reached a size that was sampled in the creel or population estimate surveys over the timeframe of this study. The 1976 LCO sample is a likely example of this. The LCO sample from 1976 was found to be genetically different from all other LCO samples. One hypothesis was there was an undocumented stocking event that essentially created a "put-grow-take" fishery that disrupted the 1976 LCO sample. These stocked fish could have grown to a size that was susceptible to the sampling gear (e.g., fyke nets) and were subsequently included in the current study. Over time, they would have slowly been removed through either natural mortality and/or angler harvest. More importantly, they failed to reproduce and, thus, had no lasting genetic effect on the LCO population's genetic integrity. This hypothesis was supported

when a stocking receipt confirmed a 1972-stocking event where surplus fish from the Art Oehmcke State Fish Hatchery (Woodruff, WI) were stocked into both Grindstone and Whitefish Lakes which have water connections with LCO. The Oehmcke State Fish Hatchery serves northeastern Wisconsin and represents a genetically different management zone in the state. Therefore, it was likely that genetically divergent fish (compared to LCO) were stocked into these two lakes. Additionally, this finding incidentally validated the approach used for this study since it identified a previously undocumented stocking event through genetic analysis and showed that it failed to have any long-term genetic effect on LCO's genetic integrity.

The findings in this current study are consistent with previous research conducted on a northern pike population by Larsen et al. (2005) to evaluate possible impacts or introgression from supplemental stockings over the past 50 years. Larsen et al. (2005) distinguished between brood sources, receiving populations, and archived samples. Their study showed no significant differences between archived and contemporary samples from the receiving populations; however, they did find significant differences between study populations (Larsen et al. 2005). By analyzing possible admixtures, they found no stocked fish were present in contemporary samples, which led the authors to conclude stocked fish failed to influence the receiving populations.

In conclusion, no genetic impact of either of the two documented LCO supplemental stocking events in question was found. Through genetic analysis, the two brood sources and LCO were distinguishable, implying discrete gene pools prior to stocking. Little genetic change was observed in LCO over the 50-year period of this study. The muskellunge population of LCO was not significantly impacted by

introgression or outbreeding depression likely due to low survival rates of stocked fingerlings and/or low reproductive success of stocked fish.

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Table 1. Microsatellite loci used in the current study and description of primer size range, and number of alleles for each locus (Sloss et al. 2008).

Locus	Primer Sequence (5'-3')	Number of Alleles	Allele Size (bp)
Ema A10	GCCAGATGTTCCTCTTCG TGGTCCAGAAAGCGTTATG	6	152-164
Ema A102	GGAACAGGTAGTGGGCAGAG CTTGGTGTGGGGTTTTGTG	4	131-139
Ema A104	TGCAGTCTGGAACGACATC TGCTCACAGCAATCTCATG	4	161-167
Ema B120	TGTTCCTGAAAGAGTTTTGTTG CGAGGGAGATGGAGACTG	2	234-236
Ema C1	CATTGTCTGCCTGAGGTATCT AAATCCAGTGTGACAGAAGTTG	4	205-221
Ema D5	CCGTAGACGCACAAAAAC TGGTTATCTGGCATCATTG	25	201-285
Ema D12a	CGTATGAACAGTAGGTTTTGTCTG GATGGTGGATTGTGCCTATC	11	181-229
Ema D116	GCAAAAGGACACAACACTG CGAGCAGAGGGAAACTAAG	14	239-295
Ema D126a	CCAATCAGAATGTGGCATTT AAAGGAACCCTGAAGTCAG	3	128-136

Table 2. Descriptive statistics for all sampled populations including sample size (n), number of loci genotyped (Loci), unbiased heterozygosity (H_e) and standard deviation (H_e SD), observed heterozygosity (H_o) and standard deviation (H_o SD), and allelic richness (A_r).

Population	n	Loci	H_e	H_e SD	H_o	H_o SD	A_r
LCO56	30	9	0.5221	0.0781	0.3486	0.0319	4.00
SPI56	18	9	0.5378	0.0941	0.3961	0.0439	4.22
LCO66	41	9	0.5502	0.0793	0.5213	0.0277	5.00
LCO76	50	9	0.5659	0.0862	0.5331	0.0246	5.11
MC79	48	9	0.5453	0.0915	0.5338	0.0262	4.89
LCO90	47	9	0.5635	0.0840	0.5823	0.0246	4.89
LCO06	35	9	0.5877	0.0799	0.5895	0.0278	5.33

Table 3. Genic differentiation p-values for all temporal pairwise comparisons.

	LCO56	SPI76	LCO66	LCO76	MC79	LCO90	LCO06
LCO56	*						
SPI76	<0.0001	*					
LCO66	0.0009	<0.0001	*				
LCO76	<0.0001	<0.0001	<0.0001	*			
MC79	<0.0001	0.0091	<0.0001	<0.0001	*		
LCO90	0.0006	<0.0001	0.0683	0.0040	<0.0001	*	
LCO06	0.0064	<0.0001	0.0842	0.0002	<0.0001	0.4125	*

Table 4. Pairwise F_{ST} values (above diagonal) and their corresponding p-values (below diagonal) for all temporal pairwise comparisons.

	LCO56	SPI76	LCO66	LCO76	MC79	LCO90	LCO06
LCO56	*	-0.0020	0.0136	-0.0028	-0.0069	-0.0060	-0.0479
SPI76	<0.0001	*	0.0402	-0.0040	-0.0046	0.0348	-0.0237
LCO66	0.9051	<0.0001	*	0.0264	0.0612	0.0131	0.0908
LCO76	0.8778	0.0099	0.0045	*	0.0142	0.0255	-0.0062
MC79	<0.0001	0.0792	<0.0001	0.0006	*	0.0628	-0.0081
LCO90	0.9990	0.0006	0.7909	0.8022	<0.0001	*	0.1074
LCO06	0.9998	0.7468	0.9212	0.6751	0.0113	0.5441	*

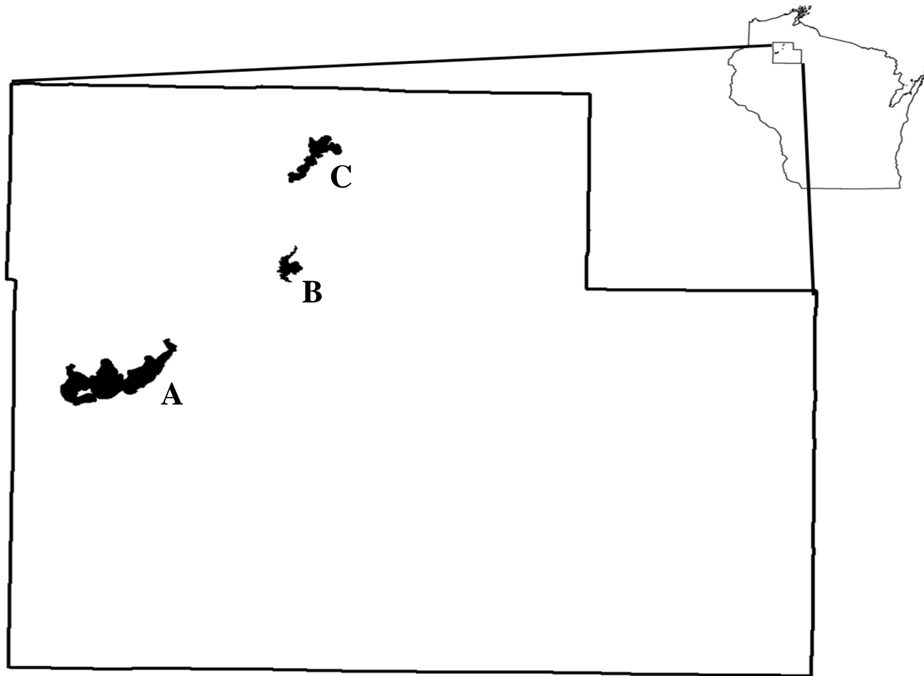

Figure 1. Geographic location of the three study sites in Sawyer County, WI. The lakes are (A) LCO, (B) MC, and (C) SPI.

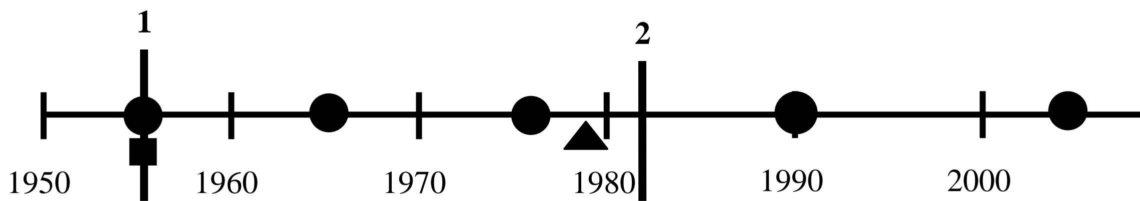

Figure 2. Timeline representing the sampling strategy for the project: circles represent LCO samples, the square is the SPI sample, and the triangle is the 1980 MC sample. The bars are the two documented stocking events 1) 1956 – SPI and 2) 1981 – MC.

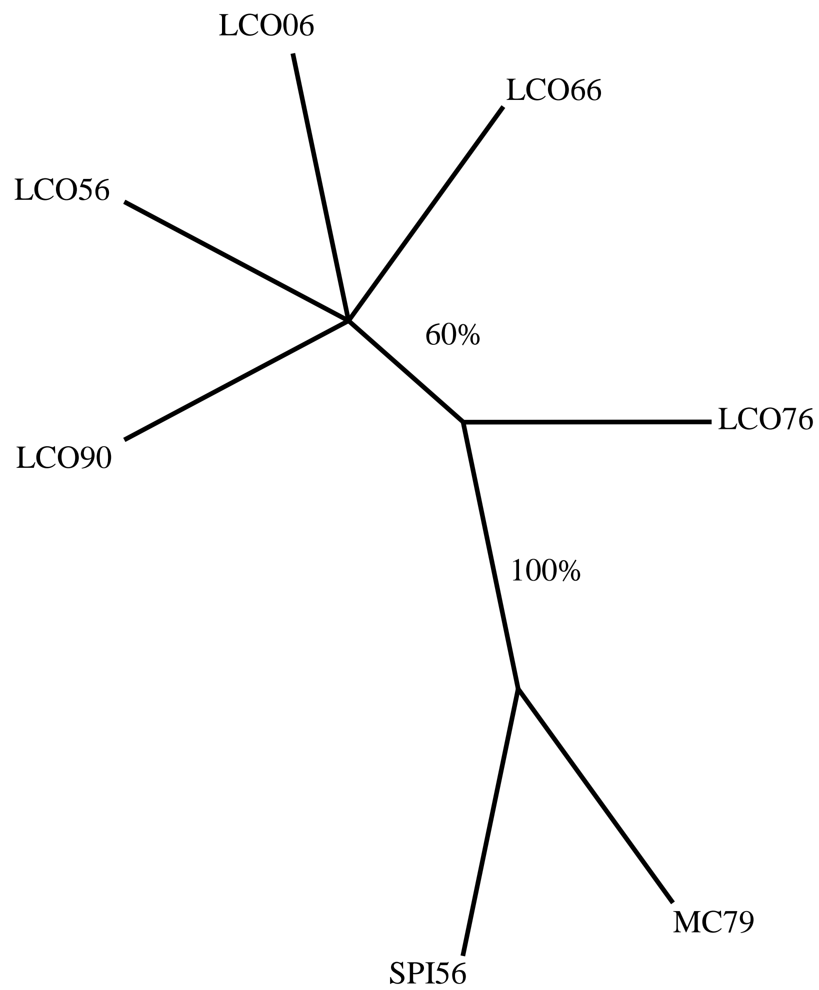

Figure 3. Unrooted NJ tree showing population clustering based on Cavalli-Sforza and Edwards (1967) genetic distance. Node support represents the percent resolution of that node in 1,000 bootstrap pseudoreplicates.

Appendix 1. Allele frequencies for each microsatellite locus used to assess the genetic integrity of LCO's muskellunge population.

Locus/Allele	**Population**						
D126	**LCO56**	**SPI56**	**LCO66**	**LCO76**	**MUD79**	**LCO90**	**LCO06**
128	0.2625	0.0741	0.2045	0.1224	0.2347	0.2551	0.3000
132	0.0125	0.2778	0.0909	0.3163	0.2653	0.1633	0.0857
136	0.7250	0.6481	0.7045	0.5612	0.5000	0.5816	0.6143
A104							
161	0.1250	0.0172	0.0714	0.0625	0.1000	0.0851	0.0694
163	0.5625	0.7414	0.5833	0.5625	0.7111	0.5319	0.5000
165	0.3125	0.1724	0.3333	0.3750	0.1778	0.3830	0.4306
167	0.0000	0.0690	0.0119	0.0000	0.0111	0.0000	0.0000
C1							
205	0.0000	0.0333	0.0128	0.0521	0.0227	0.0300	0.0286
209	0.0000	0.3667	0.0769	0.0000	0.3295	0.0600	0.0857
213	1.0000	0.6000	0.8974	0.8750	0.5909	0.8900	0.8429
217	0.0000	0.0000	0.0128	0.0729	0.0568	0.0200	0.0429
B120							
234	0.2353	0.4444	0.5000	0.4643	0.8621	0.5116	0.4722
236	0.7647	0.5556	0.5000	0.5357	0.1379	0.4884	0.5278
A102							
135	0.6310	0.3889	0.5652	0.5104	0.4783	0.5745	0.4306
137	0.3690	0.6111	0.4348	0.4896	0.5217	0.4255	0.5694
A10							
156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0143
158	0.8243	0.9464	0.8421	0.9082	0.9796	0.8830	0.8429
164	0.1757	0.0536	0.1579	0.0918	0.0204	0.1170	0.1429
D5							
201	0.0000	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000
209	0.0000	0.0000	0.0000	0.0417	0.0000	0.0125	0.0147
213	0.4583	0.0417	0.0469	0.2083	0.1034	0.1625	0.1176
221	0.0833	0.0417	0.1563	0.0208	0.0517	0.0000	0.0588
225	0.0000	0.0000	0.0781	0.0625	0.0345	0.0000	0.0588
229	0.0000	0.0000	0.0313	0.0208	0.0000	0.0500	0.0441
233	0.0833	0.1667	0.0000	0.1250	0.0517	0.0500	0.0147
237	0.0000	0.0417	0.0000	0.0208	0.3276	0.0000	0.0000
241	0.0000	0.1250	0.0313	0.0729	0.0690	0.0625	0.0735
245	0.0833	0.0000	0.0781	0.0938	0.0862	0.1250	0.1029
249	0.1250	0.2500	0.1250	0.0417	0.0345	0.1000	0.1324
253	0.0833	0.0833	0.1406	0.0833	0.0517	0.1750	0.1029
257	0.0000	0.1250	0.1250	0.0313	0.1724	0.1375	0.0882
261	0.0000	0.0417	0.0156	0.0000	0.0000	0.0125	0.0735

Appendix 1. (Continued).

Locus/Allele	Populations						
D5 (Cont.)	**LCO56**	**SPI56**	**LCO66**	**LCO76**	**MUD79**	**LCO90**	**LCO06**
265	0.0417	0.0417	0.0781	0.0938	0.0172	0.0500	0.1029
269	0.0417	0.0417	0.0469	0.0208	0.0000	0.0250	0.0000
273	0.0000	0.0000	0.0313	0.0521	0.0000	0.0250	0.0147
277	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000
281	0.0000	0.0000	0.0000	0.0000	0.0000	0.0125	0.0000
D12a							
181	0.0000	0.0000	0.0135	0.0111	0.0000	0.0000	0.0147
193	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0147
197	0.0000	0.0000	0.0000	0.0222	0.0000	0.0556	0.0294
201	0.1600	0.0625	0.1757	0.1778	0.1778	0.1111	0.1912
205	0.4400	0.1875	0.4459	0.3000	0.2000	0.3778	0.2353
209	0.1200	0.3542	0.2027	0.1667	0.2778	0.1222	0.2059
213	0.1200	0.2083	0.0811	0.2333	0.2222	0.1556	0.1765
217	0.1400	0.1667	0.0270	0.0556	0.0778	0.1333	0.1176
221	0.0200	0.0208	0.0270	0.0111	0.0444	0.0333	0.0147
225	0.0000	0.0000	0.0270	0.0000	0.0000	0.0111	0.0000
D116							
233	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000
239	0.0000	0.0000	0.0000	0.0213	0.0000	0.0244	0.0143
243	0.0000	0.0000	0.1471	0.0638	0.0333	0.0854	0.0571
247	0.4231	0.2000	0.2353	0.2553	0.2667	0.2561	0.2571
251	0.3077	0.1000	0.2794	0.1809	0.0500	0.2439	0.1286
255	0.1923	0.0500	0.0735	0.0213	0.0333	0.0854	0.1571
259	0.0000	0.0000	0.0147	0.0213	0.0000	0.0244	0.0286
263	0.0000	0.1500	0.0000	0.0213	0.0167	0.0000	0.0286
267	0.0000	0.2000	0.0735	0.1064	0.1667	0.0732	0.0429
271	0.0769	0.0500	0.0147	0.1277	0.2167	0.0610	0.0571
275	0.0000	0.0000	0.0294	0.0851	0.0167	0.0610	0.1000
279	0.0000	0.2500	0.1324	0.0745	0.1667	0.0488	0.0714
283	0.0000	0.0000	0.0000	0.0106	0.0333	0.0122	0.0000
287	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0429
291	0.0000	0.0000	0.0000	0.0000	0.0000	0.0244	0.0143

Chapter 2:

CONTEMPORARY GENETIC STOCK IDENTIFICATION OF MUSKELLUNGE IN NORTHERN WISCONSIN

Abstract –The Wisconsin Department of Natural Resources (WDNR) has managed muskellunge populations through a combination of regulations and stocking. Wisconsin's specific management goals include providing a range of angling opportunities, including trophy fisheries and protecting the genetic integrity of native populations. Defining existing stock structure, which is the result of natural processes and past introductions, is a prerequisite to selecting appropriate brood sources and defining operational stock boundaries to ensure adequate protection of genetic integrity in supplementally stocked fish. Presently, Wisconsin manages within zones representing a composite of genetic data and hydrology data that correlates with major watersheds. Nevertheless, the current management zones have been questioned and identification of stock boundaries within the state would allow more scientifically stringent management of the muskellunge in WI. The main objective of this study was to determine if genetic structure exists among naturally recruiting muskellunge populations in Wisconsin. Samples (~50/population) were collected by the WDNR during the spring of 2006. Genetic diversity at 14 microsatellite loci was used to delineate the genetic structure among 24 populations from throughout the native range of muskellunge in the state. Genetic stock identification, clustering approaches, and analysis of molecular variance (AMOVA) were used to delineate groupings of populations corresponding to genetic stocks and/or genetic management units. All populations were genetically distinct likely due to genetic drift. Despite the high level of population diversity, structure consistent

with major watershed boundaries (eastern and western Wisconsin) existed. These results show management of muskellunge on genetically defined population structure is feasible in Wisconsin.

INTRODUCTION

The WDNR's muskellunge propagation program recently underwent a series of strategic modifications aimed at meeting the goal of protecting the genetic integrity of local, naturally reproducing populations (Sloss 2004). The focus of these modifications was minimizing the risk to naturally recruiting, putatively native populations of muskellunge. To further these efforts and effectively manage Wisconsin's muskellunge populations into the future, a more resolved pattern of genetic stock structure is needed.

A key concept in managing the genetic integrity of fishery resources is the stock concept and how populations are distributed across the landscape (Kutkuhn 1981; STOCS 1981). A fishery stock is defined as an intraspecific group of randomly mating individuals that is reproductively isolated, shares a common gene pool, and has temporal and spatial stability (modified from Larkin 1972; Ihssen et al. 1981). The fishery stock concept is based on the idea that managing for the long-term sustainability of the component stocks will lead to long term stability of the entire resource (STOCS 1981). The use of the stock concept in fishery management programs allows multiple populations to be grouped for management purposes while still managing a biologically cohesive unit (Dizon et al. 1992). In recent years, a fishery stock has become somewhat synonymous with the management unit (MU). An MU is formally defined as the ecological component of larger evolutionary significant units that are diagnosed as a population(s) that exhibit significant allele frequency differences (Moritz 1999).

A critical assumption of any stock management approach is that the stocks being managed have been identified in a standardized, reliable, and biologically relevant manner (Laikre et al. 2005). In practice, fisheries managers often recognize stocks as a

group of organisms harvested in a particular area. This approach is dependent on what and how much information is available including data on age structure, life history, and other phenotypic and demographic estimates. Collecting these data requires extensive field-based efforts that are often not feasible (Carvalho and Hauser 1994), particularly with less abundant apex predators. Furthermore, meristic, morphological, and life history data can converge and/or diverge due to environmental effects thereby not measuring isolation/separation of gene pools and/or populations (Shaklee and Currens 2003). However, it is preferable to base stock identification on more definable measures. Genetic data has become a favored method for stock identification due to the large amount of data that can be quickly generated from small non-lethal amounts of tissue (Miller and Kapuscinski 1996) and the ability of genetic data to statistically assess the level of connectivity between populations, and thus, the degree of isolation between populations (Shaklee and Currens 2003).

The WDNR currently attempts to maintain the genetic integrity of their muskellunge resource using genetic management zones (GMZs) originally developed as part of a molecular genetic study conducted in the mid-1990s (Figure 1; Fields et al. 1997). These GMZs are used for delineating regions where stocking can occur within the zone with lower risk of outbreeding depression but is prohibited across zones due to the potential risk of outbreeding depression. Fields et al. (1997) sampled ten muskellunge populations (n = 26-30 individuals/population) from four major watersheds within northern Wisconsin (Upper Chippewa, St. Croix, Upper Wisconsin River, and Lake Superior; Figure 2) and analyzed allozyme and mitochondrial DNA (mtDNA) diversity to describe stock structure. The mtDNA data showed no variability among populations, so

no stock structure was resolved. The allozyme analysis showed only two polymorphic loci and resolved little to no genetic structure among Wisconsin muskellunge populations. Despite the overall lack of resolution, five stocks of Wisconsin muskellunge were conservatively suggested based on watershed boundaries within the state: Lake Superior Drainage, St. Croix River Drainage, Mississippi River Drainage, Wisconsin River Drainage, and Lake Michigan Drainage (Fields et al. 1997). These five stocks represent the current GMZs used to manage Wisconsin's muskellunge.

Despite the conservative resolution of five GMZs in Fields et al. (1997), confidently resolved genetic structure among muskellunge populations was not recovered for several reasons. The study was confounded by low genetic variability among sampled loci (both allozymes and mtDNA), relatively low sample sizes (both number of populations and number of individuals/population), and related low statistical power. Only two allozyme loci (out of 61 originally surveyed for muskellunge variation) showed any polymorphism within Wisconsin populations, and the levels of polymorphism were low, resulting in limited resolution of genetic structure. Secondly, sample sizes employed by Fields et al. were below most contemporary standards for confident resolution of genetic structure. Ryman et al. (2006) showed that the use of a small number of loci, such as in Fields et al. (1997), coupled with low variability (number of alleles = 2-3 in Fields et al. 1997) requires sample sizes of at least 50 individuals to achieve even moderate power ($1-\beta \approx 0.5$). Further, Ruzzante (1998) found similar sample size and polymorphism requirements in simulations examining the ability of highly polymorphic loci to discern population differentiation. The small number of Wisconsin muskellunge populations included in their study was of concern in delineating Wisconsin

genetic structure; Wisconsin has >700 muskellunge populations throughout the state. Although their study focused on muskellunge throughout the Midwest, only 10 populations were sampled in the northern third of Wisconsin, representing a small proportion (<2.0%) of the total muskellunge populations in this region. The GMZs as outlined by Fields et al. (1997) should be considered conservative management zones. Recent developments in methods for conservation genetic studies, such as highly polymorphic microsatellite loci and non-lethal sampling techniques, could provide valuable data and potential clarity to the resolution of muskellunge GMZs in Wisconsin.

The goal of this study was to determine an initial genetic stock structure prediction for naturally recruiting populations of muskellunge in northern Wisconsin. The specific objectives of this study were (1) to determine the utility of newly developed microsatellite DNA markers (Sloss et al. 2008) to estimate population differentiation and underlying genetic structure among muskellunge populations, (2) to determine if genetic structure occurs among muskellunge populations, and (3) to test whether any observed structure was consistent with contemporary management units.

METHODS

Study Area and Sampling Design

The study area was northern Wisconsin, defined for this study as all waters north of State Highway 29 (Figure 4). This area included the ceded territory, which contains the majority of Wisconsin's muskellunge waters (623/711; Simonson 2002), and includes the bulk of the muskellunge's native range in Wisconsin (Figure 5; Becker 1983). Since such a large area and number of populations were considered, populations were chosen in consultation with the WDNR Fisheries and Science Services personnel. To be included in the study, populations ideally met three criteria: 1) natural recruitment, 2) perceived native population, and 3) no recent stocking events (i.e., >10 years since last recorded stocking). The target number of populations was 25, distributed across the geographical area of this study. Criteria three was relaxed to allow for better geographical coverage and to meet the target goal of 25 populations. Fifty individuals per population were sampled based on the suggestions of Ruzzante (1998), a study that examined the effects of sample size on genetic structure among simulated populations. This sample size, coupled with the number and distribution of populations, provided a high probability of identifying stock structure if it existed across the muskellunge's contemporary distribution.

The majority of the muskellunge populations were sampled in conjunction with the WDNR's spring fyke net surveys. In addition to the target sample populations, tissue samples and phenotypic data were also collected from the lakes that the WDNR considers possible brood lakes for Wisconsin's propagation program (Sloss 2004). Phenotypic data collected included total length (cm), weight (g), and sex (when possible). Genetic

samples consisted of a fin clip preserved in individually labeled vials with 95% EtOH or fin clips, scales, and/or spines dried and stored in individually labeled scale envelopes. Scales and fin rays were collected with the standard WDNR protocol whereas fin clips were collected with the standard operating procedure of the Molecular Conservation Genetics Laboratory (MCGL; Appendix 1).

Genetic Analysis

DNA was extracted from the fin-clip samples using the Wizard® Promega Genomic DNA purification kit (Promega Corporation, Madison, WI) and from the archived scale and fin ray samples using the QIAGEN DNeasy® extraction kit (QIAGEN Inc., Valencia, CA). Both extraction methods followed the manufacturer's suggested protocol except the final elution of extracted DNA was in 200 µl of Tris-low-EDTA buffer. DNA quality was evaluated by electrophoresing the DNA in a 1% agarose gel in the presence of ethidium bromide, visualized with UV light, and compared to a molecular weight ladder BioLine Hyperladder™ I (Bioline USA Inc., Randolph, MA). DNA quantities were measured with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples were normalized to equal DNA concentrations (25 ng/µl) to ensure consistent results in subsequent genotype procedures.

Fourteen microsatellite loci developed (Sloss et al. 2008) for the genetic analysis of muskellunge (Table 1) were used to genotype all sampled individuals. Multiplex PCR reactions (3 loci/reaction; Table 1) were used to optimize time, effort, and cost while genotyping samples (Sloss et al. 2008). Loci were PCR amplified with fluorescently labeled primers and analyzed on an ABI Prism® 377XL automated DNA sequencer

(Applied Biosystems Inc., Foster City, CA). Allele sizes were determined by comparison to an internal size standard (GeneFlo™ 625, Chimerx Inc., Milwaukee, WI) using GeneScan® (Applied Biosystems, Inc.).

Statistical Analysis

Usefulness of markers.—Several basic diversity measures were calculated for evaluating the differences among populations and to estimate the overall usefulness of the microsatellite markers employed to estimate genetic structure. Three primary measures of genetic diversity were used to assess the utility of the markers based on recommendations of Ruzzante (1998) and direct comparison with the previous genetic study of Fields et al. (1997). Measures of allelic diversity, the number of alleles/locus in a population, were used to determine diversity differences between populations. Allelic richness (A_r) was estimated based on the rarefaction method of Kalinowski (2004) to account for unequal sample sizes using HP-Rare (Kalinowski 2005). Heterozygosity was also used to determine basic genetic differences between populations. Both expected heterozygosity (H_e) and observed heterozygosity (H_o) were used to estimate the level of genetic diversity within populations and can be used to determine genetic differences between populations (Allendorf and Luikart 2007). Expected heterozygosity (H_e) is the frequency of heterozygotes expected in a population with random mating (Frankham et al. 2002; Allendorf and Luikart 2007). Observed heterozygosity (H_o) was directly measured as the proportion of sampled individuals that were heterozygotes (Allendorf and Luikart 2007). The H_o and H_e of each population was calculated in GenAlEx v6 (Peakall and Smouse 2006).

Genetic stock identification.—Genetic stock identification uses a series of hierarchical tests of population structure to establish stable groups of populations that account for significant genetic similarities within the groups and significant genetic differences among groups (Shaklee and Currens 2003). The general approach of GSI is to 1) establish the ability of the allelic frequencies to describe the genotypic distributions observed within and between samples (similar to the basic diversity measures), 2) examine the distribution of genetic diversity across samples to identify potential patterns/structure in the data, and 3) test competing hypotheses that could explain the observed patterns of genetic diversity (Shaklee and Currens 2003).

The initial tests of GSI consist of testing the population/loci samples for conformance to Hardy-Weinberg equilibrium (HWE) expectations and independence of loci using a gametic disequilibrium test. Population samples were tested for conformance to HWE using the exact test of Guo and Thompson (1992) as implemented in GENEPOP 3.4 (Raymond and Rousset 1995) that uses a Markov Chain with 1,000 dememorization steps, 100 batches and 1,000 iterations. Due to known issues with low expected genotype frequencies and exact tests of HWE, locus/population tests showing significant deviation from HWE underwent pooling of rare genotypes (all genotypes with expected frequency <1% were pooled) and re-testing using a chi-square test (Crisp et al. 1978; Hedrick 2000). A gametic disequilibrium test for independence of each locus was conducted in GENEPOP 3.4 (Raymond and Rousset 1995) using 1,000 dememorization steps, 100 batches and 1,000 iterations. For both the HWE and the gametic disequilibrium tests, alpha (initial 0.05) was corrected for multiple pairwise tests using a sequential Bonferroni correction (Rice 1989).

The next phase of GSI consists of testing for the presence of significant genetic structure among the sampled populations. This phase tests a null hypothesis of panmixia across all sampled populations. A test of genic differentiation that assumes that divergent populations have different allele frequency distributions was performed (H_o= all distributions equal; Raymond and Rousset 1995). The test of global genic differentiation was conducted in GENEPOP 3.4 (Raymond and Rousset 1995) using default settings (1,000 dememorization steps, 100 batches and 1,000 iterations). An alternative estimate of differentiation, theta (θ; Weir and Cockerham 1984), was estimated for all pairwise population comparisons. Theta is an F_{ST} analog that estimates the degree of genetic subdivision between two populations such that values of θ close to zero are essentially no genetic difference and increasing values (to a maximum of one) are indicative of increasing differentiation. Estimation of θ was performed in Arlequin 3.11 (Excoffier et al. 2005) with 5,000 permutations of the data to test the null hypothesis that θ equals zero.

Following the confirmation of genetic structure among samples, estimates of genetic relations among populations was performed to develop a series of putative groups that are subsequently tested in a series of hierarchical tests (Shaklee and Currens 2003). An unrooted neighbor joining (NJ; Saitou and Nei 1987) tree based on Cavalli-Sforza and Edwards' (CSE; 1967) chord distance was used as a heuristic tool for identifying putative groups of populations. The confidence of the recovered NJ tree topologies was determined using 500 bootstrap pseudoreplicates. All genetic distance measures, NJ trees, and bootstrap confidence values were constructed using POWER MARKER (Liu and Muse 2005) and the CONSENSE program in PHYLIP v3.67 (Felsenstein 2007). The resulting unrooted trees were visualized in TREEVIEW (Page 1996).

The final phase of GSI consists of hierarchical tests assessing the genetic significance of groups estimated from the proceeding step (Shaklee and Currens 2003). An analysis of molecular variance (AMOVA) is essentially analogous to a nested ANOVA except that it tests the distribution of molecular variance components within and among groups as opposed to mean variance (Excoffier et al. 1992). AMOVA examines the total molecular variance (calculated as a series of Euclidean distances) and estimates the molecular variance attributable to various 'hierarchical levels' in the analysis. To test the putative groups derived from heuristically examining the unrooted NJ trees, the level of variance attributed to differences among groups (i.e., putative population groups from the NJ trees) and among populations within groups (i.e., the populations found inside a single putative group) was estimated in Arlequin v3.11 (Excoffier et al. 2005). The significance of the variance estimates (within populations, among groups, and among populations within groups) was calculated using 5,000 permutations of the original allelic data according to Arlequin v3.11 (Excoffier et al. 2005). Significant among group genetic variance was evidence of biologically relevant groups being tested. However, when significant within-group variance was also observed, additional AMOVAs were performed by testing less inclusive groups (i.e., more groups with less populations within each).

Alternatively, the presence of significant within-group variance was examined by conducting pairwise tests of population differentiation (θ; Weir and Cockerham 1984) to determine where specific differences occurred. Pairwise values of θ were estimated using FSTAT v2.9.3.2 (Goudet 1995) with significance estimated using a locus bootstrap

procedure and a combined locus p-value within FSTAT (Goudet 1995; Petit et al. 2001). For all pairwise tests, alpha was adjusted using the sequential Bonferroni (Rice 1989).

Potential influences of stocked populations.—Despite the desire to only include populations with no or limited stocking history, some populations were included in the original analysis that violated this prerequisite. Because these populations could result in conflicting signal if admixed fish were included in the analysis, two additional series of GSI tests were performed using a) only currently recognized Class I (i.e., naturally reproducing) muskellunge waters (Simonson 2002) and b) populations that have no recorded stocking events since 1990. These two criteria were chosen to provide the best possible resolution of genetic structure among Wisconsin's muskellunge populations and to allow a comparison with the GSI results with all sampled populations included.

Test of contemporary management units—Contemporary management units were tested to determine if they constituted significant biological (genetic) units. Groups of sampled populations were constructed based on their current management unit designation and analyzed using AMOVA. If the groups are biologically significant, the AMOVA should produce a significant among group variance. All tests were conducted in Arlequin v3.11 (Excoffier et al. 2005) as previously described.

RESULTS

Twenty-four muskellunge populations were sampled in conjunction with the WDNR's spring 2006 fyke netting surveys (Table 2; Figure 6). Fin clips were collected from lakes that met the first two inclusion criteria (natural recruitment and suspected native population). Sample sizes per population ranged from 21 (Lower Clam, Sawyer County) to 50 (several populations) fish (Table 4). Lake Chippewa, a flowage on the Chippewa River, is thought to have an east and a west spawning aggregate; as such, they were sampled and analyzed as two different samples. Three populations (Ghost Lake, Sawyer County; Spider Lake, Ashland County; Towanda Lake, Vilas County) were excluded from the study because they had fewer than 20 sampled individuals. One thousand thirteen individual muskellunge samples representing 23 populations were genotyped at 14 microsatellite loci to analyze genetic diversity and examine muskellunge genetic structure in northern Wisconsin.

Usefulness of the Markers

A total of 114 alleles across 14 loci were observed with a mean of 71.4 alleles per population (SD = 8.08; range 90 alleles in Caldron Falls, Marinette County and 55 alleles in Pine Lake, Iron County). The mean number of alleles/locus ranged widely among populations 3.93 (Pine Lake, Iron County) to 6.43 (Caldron Falls, Marinette County) (Table 3) and among loci (2 in Ema B110 to 25 in Ema D5; Appendix 2). Allele frequencies also showed high levels of variation (Appendix 2). For example, Ema D6 exhibited large differences in the most common allele between populations with allele 165 varying from a high frequency of 44.79% (Mud Lake/Callahan Lake, Sawyer

County) to a low frequency of 3.03% (Pine Lake, Iron County). A second allele at Ema D6 (229) was also present in all populations and ranged from a high frequency of 51.09% (North Nokomis Lake, Oneida County) to a low of 1.04% (Mud Lake/Callahan Lake, Sawyer County). Heterozygosity varied among populations with H_e ranging from 0.4914 (Pine Lake, Iron County) to 0.6150 (Big Crooked Lake, Vilas County) and H_o ranging from 0.4893 (Big Crooked Lake, Vilas County) to 0.6061 (Birch Lake, Vilas County) (Table 3). Private alleles were observed in five sampled populations (Butternut Lake, Price County; Tomahawk Lake, Oneida County; Kentuck Lake, Nicolet/Vilas County; Spider Lake, Sawyer County; Seven Island Lake, Lincoln County).

Genetic Stock Identification

All loci conformed to HWE and exhibited no significant gametic disequilibrium. Of the 336 HWE calculations, 29 tests were initially significant at the 0.05 alpha-level, and all comparisons were non-significant following rare allele pooling (Hedrick 2000) and sequential Bonferroni correction of alpha (Rice 1989). Initial tests of gametic disequilibrium showed 29 out of 336 comparisons were significant at the 0.05 level. Following sequential Bonferroni correction (Rice 1989), 10 comparisons remained significant. Given the low number (2.9% of all tests) that were significant and the lack of consistent loci combinations at disequilibrium, all loci combinations were considered to be in equilibrium; therefore, all loci were considered independent for subsequent analyses.

A global test of genic differentiation showed significant ($p = <0.00001$) genetic structure existed among the sampled populations, essentially rejecting the null hypothesis

of panmixia across all sampled populations. Pairwise tests of genic differentiation showed a high degree of uniqueness among muskellunge populations with only 17 of the 276 comparisons (6.16%) showing similar allele frequency distributions (Table 5) following sequential Bonferroni correction. Genetic subdivision among populations, as estimated by θ, showed 36 of 276 comparisons (13.04%) were not significantly different from zero following sequential Bonferroni correction.

A spatial representation of population relationships (NJ tree of CSE distance) showed a geographical east/west split among populations (Figure 6). Because of the inclusion of several recently stocked populations in the final data, an alternative NJ tree was constructed using only populations not stocked since 1990 (Figure 7). The east/west split observed in the initial tree (all populations; Figure 6) was more pronounced in the alternative tree, that included only populations that have not been stocked since before 1990 (Figure 7).

An abbreviated GSI approach was used in this study to estimate a preliminary genetic structure of muskellunge in Wisconsin. The GSI approach (described previously) was truncated at a maximum of five groups (as opposed to complete delineation of gene pools). Analysis of molecular variance tests showed significant genetic structure among the sampled populations consistent with genic differentiation results. Groups were chosen based on groupings observed in the CSE-based NJ trees. Five AMOVA tests were performed (Table 5). The first AMOVA (two groups: east/west split) had the largest between group variance (2.0%). The majority of variance came from differences between individuals within groups ($\approx$ 94%). Subsequent AMOVAs attempting to delineate additional genetic structure within each of these two groups, consistently

produced significant among group variances ($p = <0.001 – 0.015$) but did not find significant within-group patterns of variance.

Tests of Contemporary Units

Contemporary management units (per Fields et al. 1997) failed to explain the distribution of genetic variation observed among the sampled populations (Table 6). The AMOVA results showed that contemporary management units did not account for significant genetic variance among the groups (% variance = -0.06%; $p = 0.4781$). The observed within group variance (5.08% of total variance) was significant ($p < 0.0001$) suggesting the populations within these groups had a significant level of heterogeneity. These data showed contemporary management units do not represent genetically definable groupings.

DISCUSSION

Usefulness of Genetic Markers

Microsatellite markers have become a commonly used molecular marker when delineating stock boundaries because of their higher levels of polymorphism compared to other markers (e.g. allozymes and mtDNA), small tissue requirements, and ability to generate large amounts of data (Hallerman 2003). The microsatellite markers developed by Sloss et al. (2008) showed similar basic diversity values as microsatellite markers developed for other species (Miller and Kapuscinski 1996; Schable et al. 2002; Hauswaldt and Glenn 2003; Keeney and Heist 2003; Reading et al. 2003). Microsatellite variation was also higher than that seen in studies looking at population structuring of another esocid, northern pike (*Esox lucius*; Miller and Kapuscinski 1996; Laikre et al. 2005).

The microsatellite markers used in this study showed enough variation to delineate genetic structure, if it exists, based on the combination of number of usable loci (14), number of alleles (114) at all loci, and the sample sizes per population (N = 21-50). Kalinowski (2002) concluded that genetic markers that show high levels of polymorphism are better suited when calculating genetic distances. His recommendation for detecting slight genetic difference between populations was a minimum of 33 independent alleles for 100 diploid individuals. The loci in the present study showed more than double this number in most populations, suggesting adequate sampling of allelic diversity. Kalinowski (2005) further showed polymorphic loci do not necessarily require a large sample size when calculating genetic distances (including Cavalli-Sforza and Edwards chord distance and F_{ST}). In fact, large sample sizes can disrupt the

coefficient of variation by revealing too much variability between study groups (Kalinowski 2005). Ruzzante (1998) further supports the use of a moderate number of samples (20-50) given expected F_{ST} values > 0.01-0.02. Therefore, the sample sizes and levels of diversity observed among the suite of 14 microsatellite markers used in the present study are sufficient to predict genetic structure among Wisconsin's muskellunge population.

The predicted level of resolution for the present study was higher than that of the previous genetic structure predictions by Fields et al. (1997) because of the aforementioned higher polymorphism of the microsatellite markers and increased sample sizes compared to Fields et al. (1997). Fields et al (1997) found only two polymorphic allozyme loci (out of 61 originally surveyed for muskellunge variation) within Wisconsin populations with low levels of polymorphism (A = 2-3) resulting in limited resolution of genetic structure (Kalinowski 2002; Kalinowski 2005). In their study, these markers yielded enough information to delineate conservative management zones; however, with the development of multiple, polymorphic microsatellite markers more defined stock structure is achievable. Sample coverage of the present study was superior to the previous study of Fields et al. (1997) in terms of coverage of the native muskellunge range in Wisconsin and number of samples per population. Sample sizes in the present study were chosen to maximize statistical power during analysis (Ruzzante 1998; Ryman et al. 2006). Sample sizes employed by Fields et al. (1997) were below most contemporary standards for confident resolution of genetic structure (N =18-30). Therefore, based on larger population sampling, larger individuals/population, and a

larger number of genetic loci, this study has identified underlying differences among populations within or between current watershed boundaries.

Genetic Structure of Muskellunge in Wisconsin

Significant structure exists among sampled populations of muskellunge in Wisconsin based on both genic differentiation tests and AMOVA. The degree of difference among populations was striking given 93.84% of all pairwise genic differentiation comparisons and 86.96% of θ comparisons were significant. This level of divergence among populations was somewhat expected given the natural history and distribution of muskellunge in this region such as small population size and relative isolation of populations on the landscape.

Small effective population sizes result in high levels of genetic drift and, subsequently, high levels of divergence among even close geographic populations. Muskellunge population density in Wisconsin is typically <1 fish/acre (Simonson 2002); most systems in this study were <1,000 acres in size (however, Chippewa Flowage ≈ 15,000 acres). The effective size of a population is often ~10-11% of the census size in wildlife populations (Frankham 1995). Applying this reduction to the populations of muskellunge in this study suggests the effective population size of most populations in Wisconsin are <100 fish/population. This level of divergence should be detectable by polymorphic genetic markers such as microsatellites (Miller and Senanan 2003). A similar pattern of divergence and differentiation was observed in northern pike that, like muskellunge, exhibit low genetic variation and small populations (Senanan and Kapuscinski 2000; Miller and Senanan 2003; Laikre et al. 2005). Senanan and

Kapuscinski (2000) were able to distinguish between pike populations across the northern hemisphere based mainly on F_{ST} values. In addition, Small et al. (2007) observed significant differences between rainbow trout (*Oncorhynchus mykiss*) populations in the Spokane River drainage based on F_{ST} comparisons. The differences observed among muskellunge populations are likely because the sampled populations are small, isolated muskellunge populations with relatively rapid allele frequency changes due to genetic drift.

Despite high levels of diversity between populations, the NJ tree showed hierarchical contemporary structure present in northern Wisconsin. The study populations were grouped into an eastern and a western group, which loosely relates to the Upper Chippewa River and the Upper Wisconsin River genetic management zones (*sensu* Fields et al. 1997). Hierarchical AMOVAs supported this basic split of the populations into two major groupings. The initial east/west AMOVA (Table 5a) showed the highest proportion of variance explained among groups (2%; $p < 0.00001$) of all AMOVA analyses conducted. Subsequent AMOVA analyses failed to yield higher proportions of among group variance. Therefore, the initial baseline prediction of genetic structure among muskellunge population in Wisconsin consists of two primary genetic units, a western unit mostly consistent with the Upper Chippewa River watershed minus the headwater regions and western Lake Superior drainage, and the eastern unit consistent with the Upper Wisconsin River watershed, including the Upper Chippewa River headwater populations, eastern Lake Superior drainage and Lake Michigan drainage.

Genetic structure consistent with river drainages is well documented in a number of aquatic species (O'Connell et al 1996; Brunner et al. 1998; Wenburg et al. 1998; Beacham et al. 1999; Carlsson et el 1999; Beacham et al. 2000; King et al. 2001; Wilson et al. 2004; Beacham et al. 2005). Senanan and Kapuscinski (2000) found differences among northern pike populations mostly consistent with drainage patterns across the northern hemisphere. The observed muskellunge genetic structure in this study was mostly consistent with the *a priori* prediction that we would see genetic differences between major drainages with one major exception: samples in the Lac du Flambeau region of the Upper Chippewa River drainage were consistently resolved as part of the Upper Wisconsin River system. This finding of differences consistent with watersheds was also consistent with Fields et al. (1997) who observed differences among the Midwest watersheds in their study.

Contemporary management units in Wisconsin are based on the watershed designations of Fields et al. (1997). The AMOVA of contemporary management zones showed no significant variance explained by the current scenario ($p = 0.1437$). Therefore, the contemporary management units fail to account for a significant proportion of genetic variance and should be adjusted based on the current genetic data.

The major disjunction between the genetic structure resolved in the present study and the contemporary management units was in the resolution of Upper Chippewa River headwater populations and the Lake Superior drainage being split into east and west components. Disruptions in the resolution of genetic structure compared to geographical distribution are generally due to either geological events or anthropogenic causes such as stocking.

The major geological impact on fish species in the upper Midwest was the Wisconsin glaciation (~9-10,000 ybp; Crossman 1986). Following colonization of Wisconsin's waters, the muskellunge likely had undergone a period of relatively isolated divergence resulting in the standing genetic diversity observed in this study. Poissant et al. (2005) found that stream-dwelling brook charr (*Salvelinus fontinalis*) of Gros Morne National Park (Newfoundland, Canada) exhibited genetic structure mostly consistent with genetic drift acting on populations isolated by the historic geological features and historic colonization of the area. Historical muskellunge colonization of upper Midwest rivers and subsequent lake systems could explain the east-west genetic split if contemporary watershed boundaries are not consistent with historical boundaries. If this were the case, consistent patterns of resolution would be expected in other fish species. A similar study on walleye (*Sander vitreus*) in Wisconsin found a disjunction in the contemporary watershed boundaries and the genetic structure similar to that observed in the present study (Hammen 2009). However, stocking of muskellunge and walleye could also explain such disjunction.

The 100+ year stocking history in the state of Wisconsin could be driving the results observed in the present study. The observed east-west grouping occurs around the logistical range of stocking for the Governor Tommy G. Thompson State Fish Hatchery (Spooner, WI) and the Art Oehmcke State Fish Hatchery (Woodruff, WI). The Oehmcke hatchery traditionally uses brood sources from the Upper Wisconsin watershed and the Thompson hatchery uses Upper Chippewa watershed sources. The populations in the Upper Chippewa River headwater regions are in much closer proximity to the Oehmcke hatchery than the Thompson hatchery. Continued stocking in the immediate proximity of

these two hatcheries over time would predictably result in similarities among populations stocked from a single hatchery, regardless of their contemporary management unit designation. The probability of such cross-management unit stockings is high, especially prior to the completion of Fields et al. (1997).

The inclusion of stocked populations in this study introduced a potentially confounding factor. Since stocked fish are generally not physically marked for easy detection, any population that had been stocked in recent years could have had stocked fish sampled as representative of the native fishery. Furthermore, the current study may show populations in various states of transition (Poissant et al. 2005) because stocked fish could be successfully reproducing with the native populations creating introgressed populations. Over time, these populations could become a representative of artificial genetic structure caused in part by stocking. However, survival rates of supplementary stocked fish are generally poor (Hanson and Margenau 1992; Jennings et al. 2005). Predation, starvation, and stress are major factors in survival of stocked fish (Hanson and Margenau 1992). In addition, Margenau (1992) found overwinter survival rates to be quite low in muskellunge. A second factor in the reproductive success of stocked fish is homing, or a fish's tendency to return to its natal spawning grounds. Crossman (1990) found that muskellunge home during spring spawning during a mark-recapture study in Stony Lake in Ontario, Canada. Other studies have observed lack of homing ability in stocked and transplanted fish, which led to low survival of stocked fish (Bams 1976; Gharrett and Smoker 1991; Gilk 2004). Low reproductive success of stocked fish, the distinctiveness of each population observed (F_{ST} values and genic differentiation), and the

genetic distances observed between populations in the NJ trees essentially eliminates genetic structure based solely on stocking regimes.

Whether geology or human-based means are responsible for the disjunction in contemporary genetic structure from watershed boundaries, significant genetic structure still appears to exist among muskellunge in Wisconsin. This structure is likely based on natural patterns of genetic divergence, population founding, and general population dynamics. The failure of contemporary genetic management units to significantly explain the observed genetic variance strongly suggests adjustments be made to the muskellunge management units.

Management Implications

This study provides an initial estimate of genetic diversity within and among Wisconsin's naturally recruiting muskellunge populations and an initial estimate of genetic structure within Wisconsin's muskellunge resources. However, more information can and will be gathered during the second portion of this four-year study. The stock concept is based on the theory that managing the component stocks of the entire population in an area will maximize genetic diversity and health of the entire population. In this study, two groups of muskellunge were found based on the Upper Chippewa and Upper Wisconsin River drainages, suggesting these two groups can be managed and monitored as two different stocks to improve management effectiveness. Currently, the WDNR monitors muskellunge populations based on "abundance, size structure, and relative abundance of the associated fish community," (Simonson 2002). Since a minimum of two stocks have been identified, the WDNR can tailor their monitoring

efforts to these two separate groups to better understand the biological and ecological functions of these populations.

Future Research

This project was the first half of a four-year project, and more work is necessary to fully understand the genetic structure of muskellunge in northern Wisconsin. The first and foremost need is more population samples representing the Lake Superior and Lake Michigan Basins. The Lake Superior Basin is especially important because the sampled populations from that area are under-represented compared to the number of suspected naturally recruiting populations. This area is also managed as a genetic management zone; however, currently this zone does not appear to be a viable genetic management zone. By adding more populations to the current dataset, it may be possible to identify a viable Superior management zone with confidence. More samples are also needed in areas outside of the main muskellunge areas (Sawyer and Vilas counties). This will also allow a better test of the management boundaries and, ultimately, allow a refined genetic stock model of Wisconsin muskellunge.

In addition to more samples, the impact of stocking on the genetic diversity and integrity of muskellunge populations needs to be assessed; especially in terms of impacts on the resolved boundaries of this study. Since improved resolution using only populations that had not been stock since 1990, analysis of historical brood sources and their potential admixture with populations in the study could allow a better idea of the impact of stocking across the study region. This can be done by viewing the sampled populations as potential admixtures and seeing how the brood stocks potentially

introgressed into the native populations using maximum likelihood and Bayesian assignment tests.

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Table 1. Microsatellite loci used in the current study and description of primer sequence, size range, and number of alleles for each locus (Sloss et al. 2008).

Locus	Primer Sequence (5'-3')	Number of Alleles	Allele Size (bp)
Ema A5	TGGGACATTTGCCTCAAG	4	216-230
	CCATTGGTTCCATTTATTGC		
Ema A10	GCCAGATGTTCCTCTTCG	6	152-164
	TGGTCCAGAAAGCGTTATG		
Ema A11	TACCGTCACACACAGATGC	5	136-146
	TGGTTCTCAAACTTTTTACACC		
Ema A102	GGAACAGGTAGTGGGCAGAG	4	131-139
	CTTGGTGTGGGGTTTTGTG		
Ema A104	TGCAGTCTGGAACGACATC	4	161-167
	TGCTCACAGCAATCTCATG		
Ema B110	TGCCCCGTATCTCTCAAC	4	183-191
	GGGTCTGTGTGGAAATAAATG		
Ema B120	TGTTCCTGAAAGAGTTTTGTTG	2	234-236
	CGAGGGAGATGGAGACTG		
Ema C1	CATTGTCTGCCTGAGGTATCT	4	205-221
	AAATCCAGTGTGACAGAAGTTG		
Ema D4	TCCCTATCGTAAATTACACACG	5	196-212
	CAGAATGTGGCATTTTTAACAG		
Ema D5	CCGTAGACGCACAAAAAC	25	201-285
	TGGTTATCTGGCATCATTG		
Ema D6	TCACTCTCGCAATTTCTATCTG	20	165-269
	GGGGACAGGTAATTTGTAACTG		
Ema D12a	CGTATGAACAGTAGGTTTTGTCTG	11	181-229
	GATGGTGGATTGTGCCTATC		
Ema D114	TGATCCACAAACACCTGAGTAG	9	270-302
	CAAATCCTTCCTCAACAGATTC		
Ema D116	GCAAAAGGACACAACACTG	14	239-295
	CGAGCAGAGGGAAACTAAG		
Ema D126a	CCAATCAGAATGTGGCATTT	3	128-136
	AAAGGAACCCTGAAGTCAG		

Table 2. Class 1 and Class 2 populations listed with abbreviation, county, class and last stocking date according to the WDNR fish stocking database. Classes are based on reproductive status of the population: 0 = unknown recruitment, 1 = natural recruitment, 2 = natural recruitment with additional stocking, 3 = no natural recruitment.

Population	Class	County	Last Stocked
Amnicon Lake (AM)	2	Douglas	1997
Big Arbor Vitae Lake (BA)	2	Vilas	2004
Big Crooked Lake (BI)	2	Vilas	No Record
Birch Lake (BC)	1	Vilas	1980
Butternut Lake (BN)	2	Price	1999
Day Lake (DAY)	1	Ashland	1990
Ghost Lake (GH)	2	Sawyer	1993
Harris Lake (HA)	1	Vilas	1978
Horsehead Lake (HH)	1	Vilas	No Record
Kentuck Lake (KT)	1	Nicolet/Vilas	No Record
Lake Chippewa (WCF/EC)	2	Sawyer	2005
Lake Tomahawk (TH)	2	Oneida	2000
Lower Calm Lake (LC)	2	Sawyer	No Record
Mineral Lake (MN)	1	Ashland	1976
Moose Lake (MO)	1	Sawyer	No Record
Mud Lake/Callahan Lake (MC)	1	Sawyer	1982
North/South Twin Lake (NT)	2	Vilas	1995
North Nokomis Lake (NN)	2	Oneida	1996
Pine Lake (PI)	1	Iron	1975
Plum Lake (PM)	2	Vilas	1999
Seven Island Lake (SI)	2	Lincoln	1976
Spider Lake (SA)	2	Ashland	1992
Spider Lake (SS)	1	Sawyer	1984
Towanda Lake (TO)	1	Vilas	1984
Wolf Lake (WO)	2	Vilas	No Record

Table 3. Population statistics for all 24 sampled populations including sample size (N), number of loci genotyped (Loci), unbiased heterozygosity (H_e) and standard deviation (H_e SD), observed heterozygosity (H_o) and standard deviation (H_o SD), and allelic richness (A_r).

Population	N	Loci	H_e	H_e SD	H_o	H_o SD	A_r
BN	45	14	0.5694	0.0584	0.5408	0.0206	5.57
AM	50	14	0.5719	0.0609	0.5649	0.0188	5.86
MC	49	14	0.5357	0.0637	0.5266	0.0192	5.79
WCF	40	14	0.5870	0.0595	0.5902	0.0209	5.57
TH	49	14	0.5671	0.0641	0.5715	0.0189	5.57
BC	40	14	0.4989	0.0771	0.4893	0.0211	4.93
DAY	50	14	0.5567	0.0612	0.5471	0.0188	5.64
PM	34	14	0.5911	0.0566	0.5897	0.0226	5.57
BA	50	14	0.5745	0.0633	0.5471	0.0188	5.86
KT	41	14	0.5517	0.0677	0.5363	0.0208	5.21
EC	49	14	0.5794	0.0597	0.5795	0.0189	6.21
SS	43	14	0.5288	0.0704	0.5280	0.0207	5.64
LC	21	14	0.5725	0.0594	0.5578	0.0290	5.07
NN	46	14	0.5547	0.0511	0.5661	0.0199	5.00
HA	37	14	0.5382	0.0662	0.4901	0.0223	4.71
HH	23	14	0.5353	0.0582	0.5589	0.0277	4.21
SI	48	14	0.5544	0.0665	0.5939	0.0190	4.86
NT	34	14	0.5742	0.0532	0.5905	0.0226	5.50
MN	51	14	0.5336	0.0603	0.5444	0.0188	5.07
BI	33	14	0.6129	0.0536	0.6061	0.0227	4.93
MO	40	14	0.5183	0.0698	0.4994	0.0211	5.29
CF	48	14	0.5837	0.0617	0.5956	0.0190	6.43
PI	38	14	0.4871	0.0675	0.4960	0.0229	3.93
WO	50	14	0.5367	0.0627	0.5504	0.0189	5.07

Table 4. Population pairwise comparison of allele frequency distributions across all loci. The genic differentiation p-value is below the diagonal and the F_{ST} values are above the diagonal. Bold values are values that were significant after sequential Bonferroni correction (Rice 1989).

	BN	AM	MC	WCF	TH	BC	DAY	PM	BA	KT	EC	SS
BN	*	0.026	0.053	0.013	0.023	0.114	0.014	0.028	0.017	0.084	0.009	0.029
AM	<0.001	*	0.086	0.028	0.029	0.151	0.015	0.043	0.041	0.105	0.020	0.046
MC	<0.001	<0.001	*	0.050	0.069	0.078	0.065	0.068	0.065	0.091	0.036	0.049
WCF	<0.001	<0.001	<0.001	*	**0.009**	0.077	**0.010**	**0.016**	0.005	0.053	-0.001	0.046
TH	<0.001	<0.001	<0.001	<0.001	*	0.063	0.015	0.009	**0.010**	0.059	0.012	0.061
BC	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.113	0.063	0.066	0.067	0.069	0.158
DAY	**0.002**	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.023	0.023	0.069	0.011	0.043
PM	<0.001	<0.001	<0.001	<0.001	**0.023**	<0.001	<0.001	*	-0.002	0.029	**0.009**	0.086
BA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.408**	*	0.033	0.006	0.069
KT	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.047	0.135
EC	<0.001	<0.001	<0.001	**0.004**	<0.001	<0.001	<0.001	**0.003**	<0.001	<0.001	*	0.036
SS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*
LC	**0.006**	**0.027**	<0.001	<0.001	<0.001	<0.001	**0.063**	<0.001	<0.001	<0.001	**0.001**	<0.001
NN	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
HA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
HH	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NT	<0.001	<0.001	<0.001	**0.002**	**0.002**	<0.001	<0.001	**0.052**	**0.041**	<0.001	**0.046**	<0.001
MN	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MO	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CF	**0.004**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.023**	**0.011**	<0.001	<0.001	<0.001
PI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
WO	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4. Continued.

	LC	NN	HA	HH	SI	NT	MN	BI	MO	CF	PI	WO
BN	0.009	0.075	0.074	0.139	0.063	0.008	0.035	0.081	0.061	**0.008**	0.123	0.072
AM	0.007	0.077	0.068	0.134	0.077	**0.013**	0.029	0.078	0.097	0.009	0.150	0.093
MC	0.046	0.115	0.098	0.140	0.098	0.038	0.108	0.112	**0.012**	0.072	0.153	0.082
WCF	0.011	0.042	0.044	0.097	0.036	0.006	0.050	0.038	0.056	**0.008**	0.075	0.044
TH	**0.016**	0.044	0.033	0.095	0.048	0.013	0.035	0.040	0.077	**0.008**	0.066	0.044
BC	0.118	0.098	0.073	0.139	0.081	0.086	0.138	0.083	0.086	0.105	0.069	0.049
DAY	0.000	0.060	0.052	0.104	0.074	0.018	0.042	0.073	0.072	0.004	0.105	0.073
PM	**0.020**	0.028	0.036	0.056	0.025	**0.012**	0.027	0.035	0.079	**0.014**	0.038	0.014
BA	**0.016**	0.027	0.044	0.091	**0.016**	0.008	0.034	0.041	0.065	**0.012**	0.049	0.018
KT	0.063	0.020	0.054	0.077	0.035	0.059	0.098	0.050	0.082	0.069	0.054	0.030
EC	0.007	0.044	0.034	0.087	0.036	-0.003	0.030	0.051	0.044	0.006	0.075	0.032
SS	0.023	0.137	0.133	0.183	0.131	0.026	0.080	0.127	0.057	0.039	0.215	0.119
LC	*	0.050	0.071	0.109	0.062	0.000	0.030	0.067	0.051	0.002	0.126	0.058
NN	<0.001	*	0.043	0.076	0.027	0.053	0.073	0.051	0.104	0.056	0.054	0.027
HA	<0.001	<0.001	*	0.097	0.051	0.059	0.060	0.073	0.107	0.054	0.080	0.061
HH	<0.001	<0.001	<0.001	*	0.099	0.097	0.109	0.084	0.160	0.100	0.083	0.061
SI	<0.001	<0.001	<0.001	<0.001	*	0.039	0.064	0.049	0.090	0.058	0.056	0.027
NT	**0.016**	<0.001	<0.001	<0.001	<0.001	*	0.022	0.048	0.046	0.005	0.101	0.037
MN	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.098	0.122	0.024	0.124	0.066
BI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.108	0.050	0.058	0.057
MO	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.080	0.153	0.079
CF	**0.005**	<0.001	<0.001	<0.001	<0.001	**0.137**	<0.001	<0.001	<0.001	*	0.103	0.057
PI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*	0.032
WO	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*

Table 5. Analysis of molecular variance groupings, sum of squares, percent variation, and p-values. GIS approach used was based on the topography of the phylogenetic tree. The first two group AMOVA (a) showed the highest among-group variation. Subsequent AMOVA tests (b), (c), and (d) failed to yield higher among-group variation.

2 Group AMOVA		Source of variation	Sum of Squares	% of Variation	p-value
Group 1		Among Groups	97.58	2.00	<0.00001
	BN				
	AM	Among Populations within Groups	364.28	3.82	<0.00001
	MC				
	WCF				
	DAY	Within Populations	7501.9	94.18	<0.00001
	EC				
	SS				
	LC				
	NT				
	MN				
	MO				
	CF				
Group 2					
	TH				
	BC				
	PM				
	BA				
	KT				
	NN				
	HA				
	HH				
	SI				
	WO				
	PI				
	BI				

Table 5 (b). Continued

3 Group AMOVA		Source of variation	Sum of Squares	% of Variation	p-value
Group 1		Among Groups	107.61	1.53	0.00020
	BN				
	AM	Among Populations within Groups	354.28	3.93	<0.00001
	MC				
	DAY				
	SS	Within Populations	7501.92	94.54	<0.00001
	LC				
	MN				
	MO				
	CF				
Group 2					
	TH				
	BC				
	PM				
	BA				
	KT				
	NN				
	HA				
	HH				
	SI				
	WO				
	PI				
	BI				
Group 3					
	WCF				
	EC				
	NT				

Table 5 (c). Continued

4 Group AMOVA		Source of variation	Sum of Squares	% of Variation	p-value
Group 1		Among Groups	133.28	1.48	0.00020
	BN				
	AM	Among Populations within Groups	328.58	3.81	<0.00001
	MC				
	DAY				
	SS	Within Populations	7501.92	94.71	<0.00001
	LC				
	MN				
	MO				
	CF				
Group 2					
	BC				
	KT				
	NN				
	HA				
	HH				
	SI				
	WO				
	PI				
Group 3					
	WCF				
	EC				
	NT				
Group 4					
	TH				
	PM				
	BA				
	BI				

Table 5 (d). Continued

5 Group AMOVA		Source of variation	Sum of Squares	% of Variation	p-value
Group 1		Among Groups	167.74	1.66	<0.00001
	BN				
	MC				
	DAY	Among Populations within Groups	294.12	3.56	<0.00001
	SS				
	LC	Within Populations	7501.92	94.78	<0.00001
	MO				
Group 2					
	BC				
	KT				
	NN				
	HA				
	HH				
	SI				
	WO				
	PI				
Group 3					
	WCF				
	EC				
	NT				
Group 4					
	TH				
	PM				
	BA				
	BI				
Group 5					
	CF				
	MN				
	AM				

Table 6. AMOVA groupings, sum of squares, percent variation, and p-values of the current management units (Group 1 = Upper Chippewa; Group 2 = Lake Superior; Group 3 = Upper Wisconsin; Group 4 = Green Bay).

4 Group AMOVA		Source of variation	Sum of Squares	% of Variation	p-value
Group 1		Among Groups	72.74	0.33	0.14370
	BC				
	BI	Among Populations within Groups	361.43	4.82	<0.00001
	BN				
	DAY				
	EC	Within Populations	3371.59	-1.02	0.93842
	MC				
	WCF				
	SS				
	LC				
	MO				
	WO				
Group 2					
	AM				
	HA				
	HH				
	MN				
	PI				
Group 3					
	TH				
	PM				
	BA				
	NN				
	SI				
	NT				
Group 4					
	KT				
	CF				

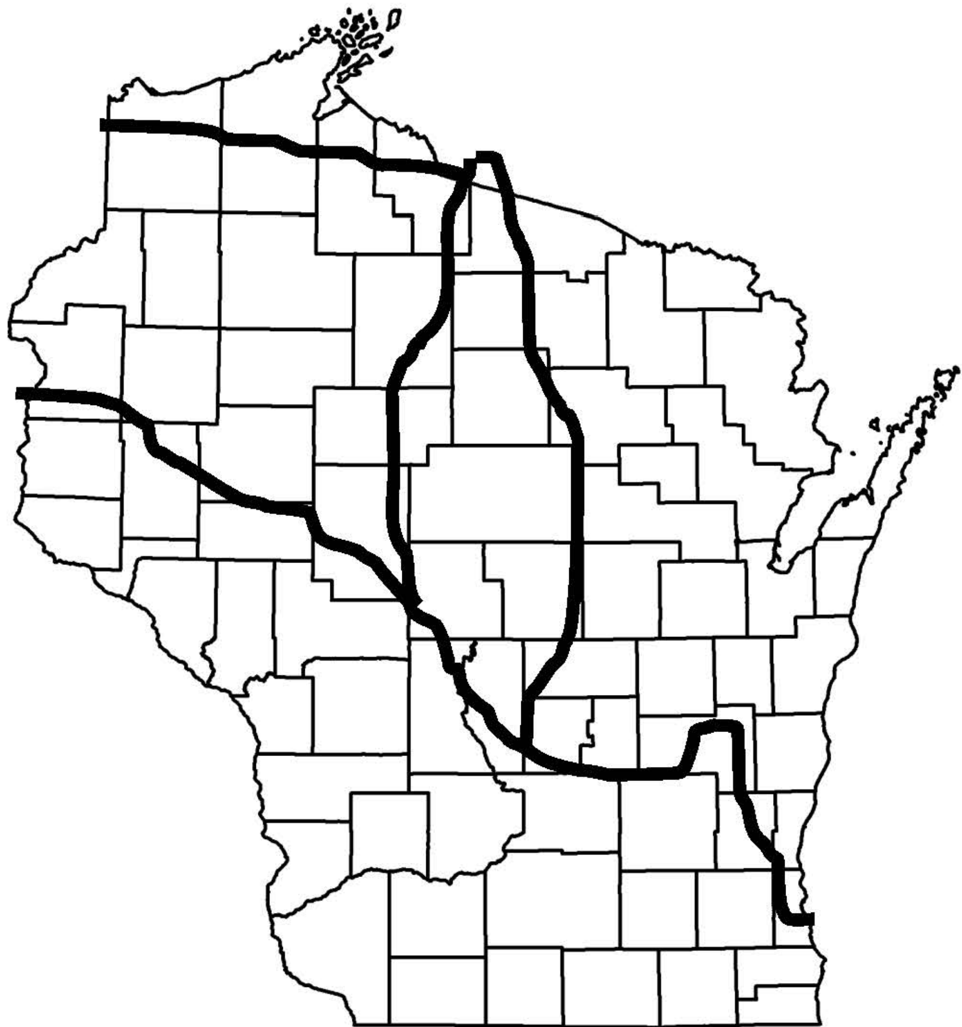

Figure 1. Current genetic management zones suggested by Fields et al. (1997) based partially on allozyme and mtDNA data.

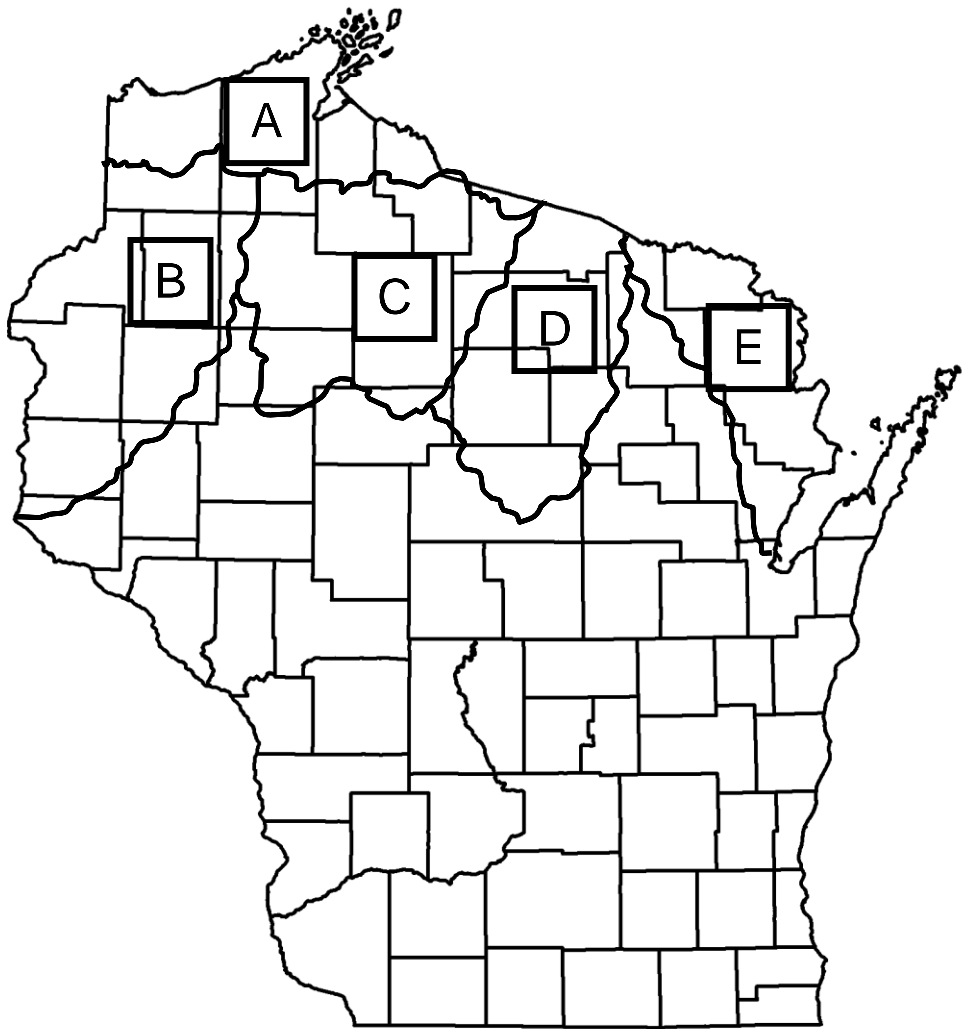

Figure 2. Major watersheds in northern Wisconsin: (A) Lake Superior, (B) Upper St. Croix, (C) Upper Chippewa, (D) Upper Wisconsin, and (E) Green Bay (Lake Michigan).

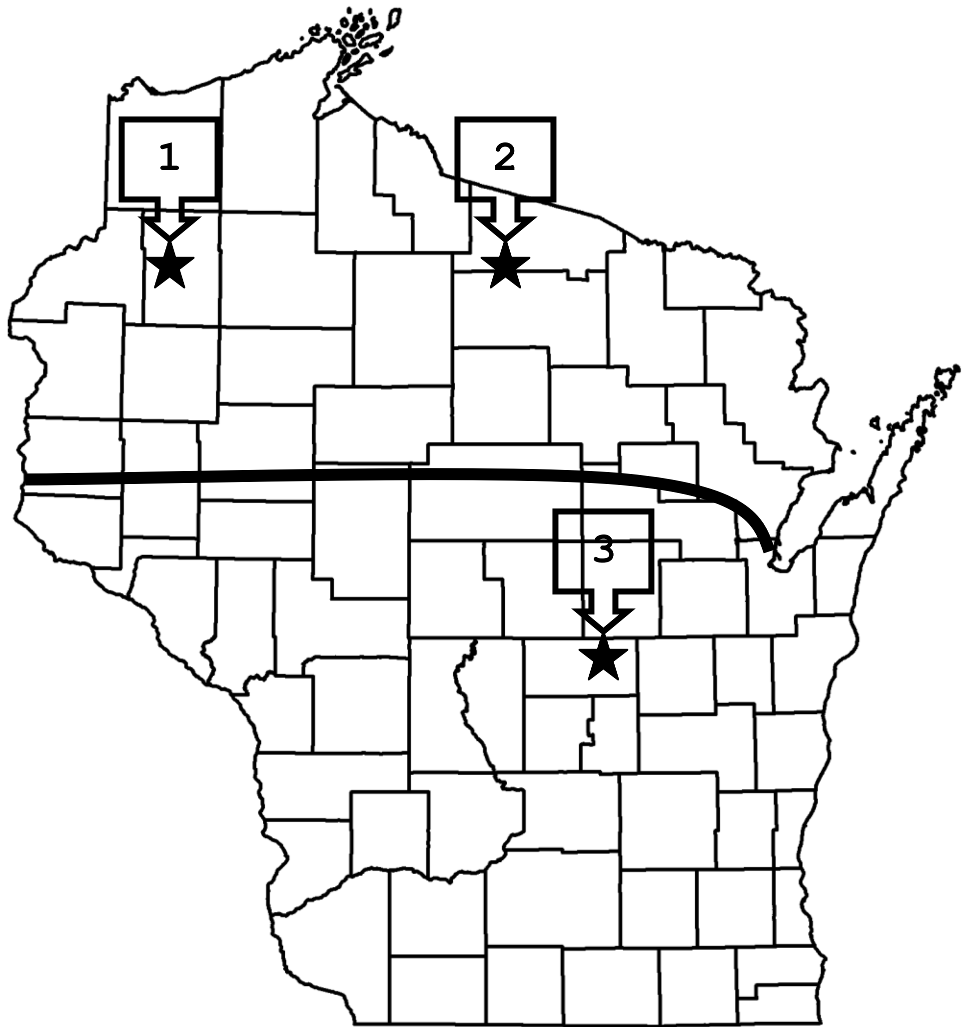

Figure 3. Three hatcheries involved with muskellunge propagation in Wisconsin and their relative location in the state. (1) Governor Tommy G. Thompson State Fish Hatchery, (2) Art Oehmcke State Fish Hatchery, and (3) Wild Rose State Fish Hatchery. The black line represents State Highway 29, which was chosen as the southern boundary of the study.

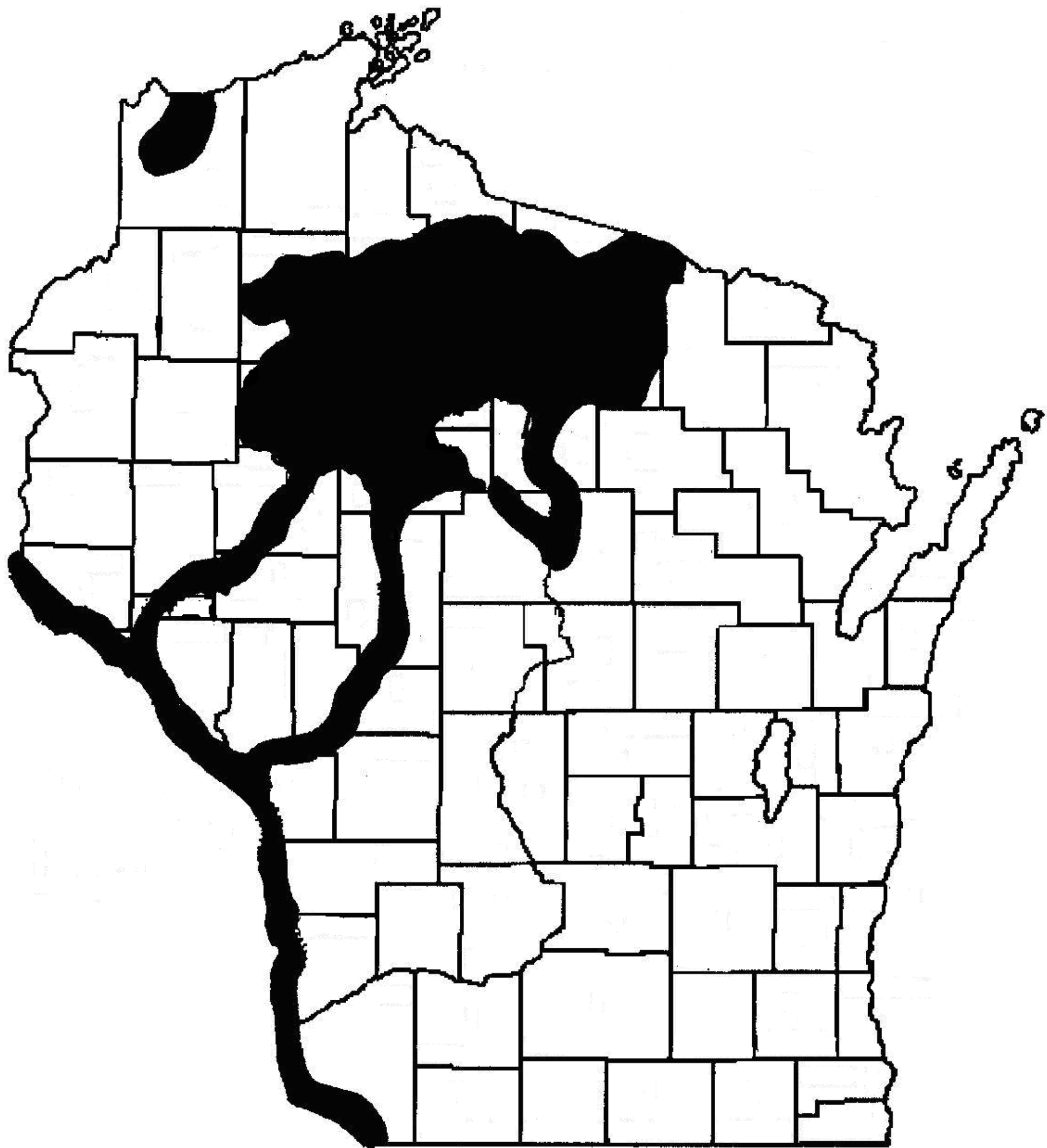

Figure 4. Native range of muskellunge in Wisconsin according to Becker (1983).

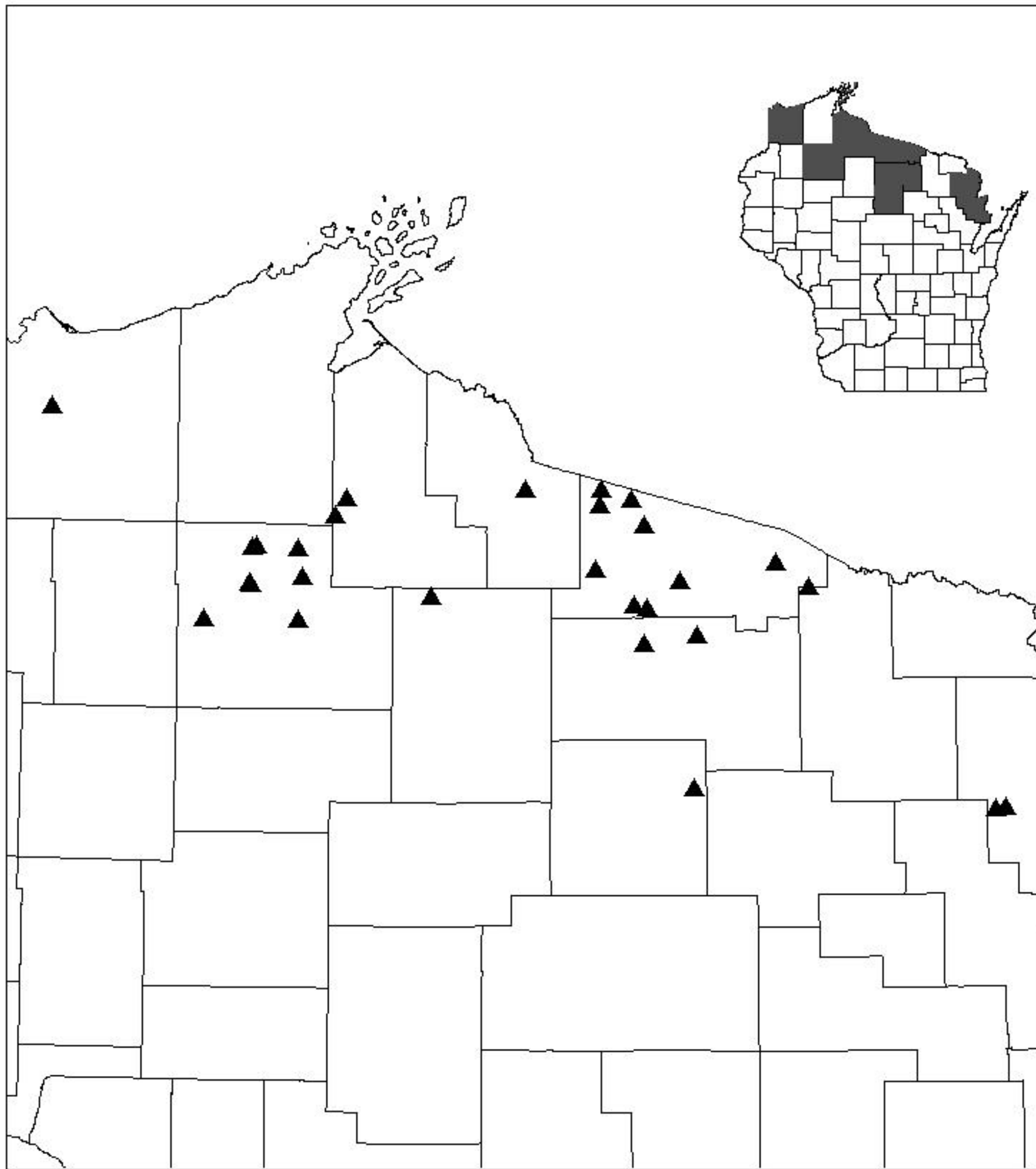

Figure 5. Relative distribution and location of sampled populations in this study. The inset map shows the counties containing sampled populations.

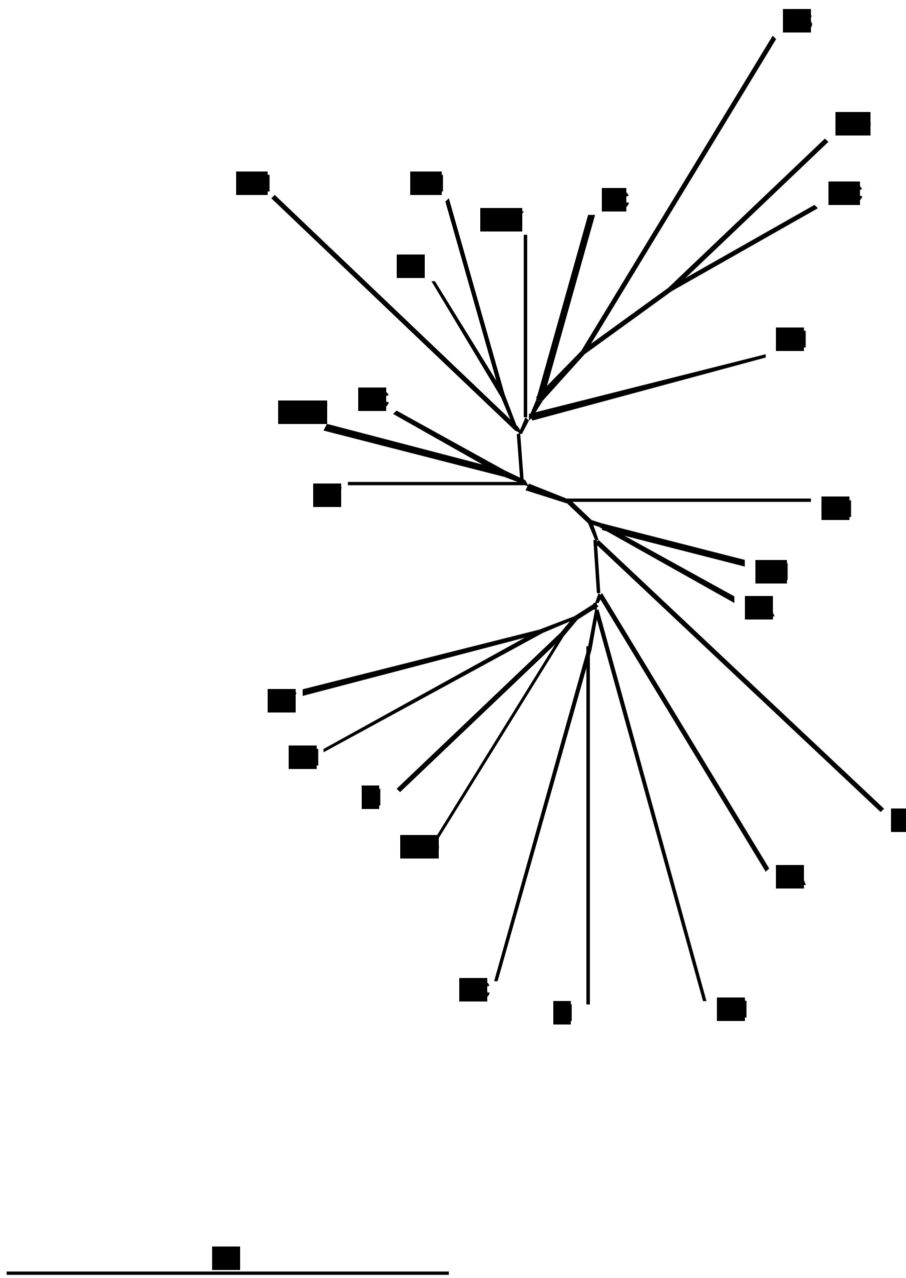

Figure 6. Unrooted NJ tree of all sampled populations created using Cavalli-Sforza and Edwards (1967) chord distance.

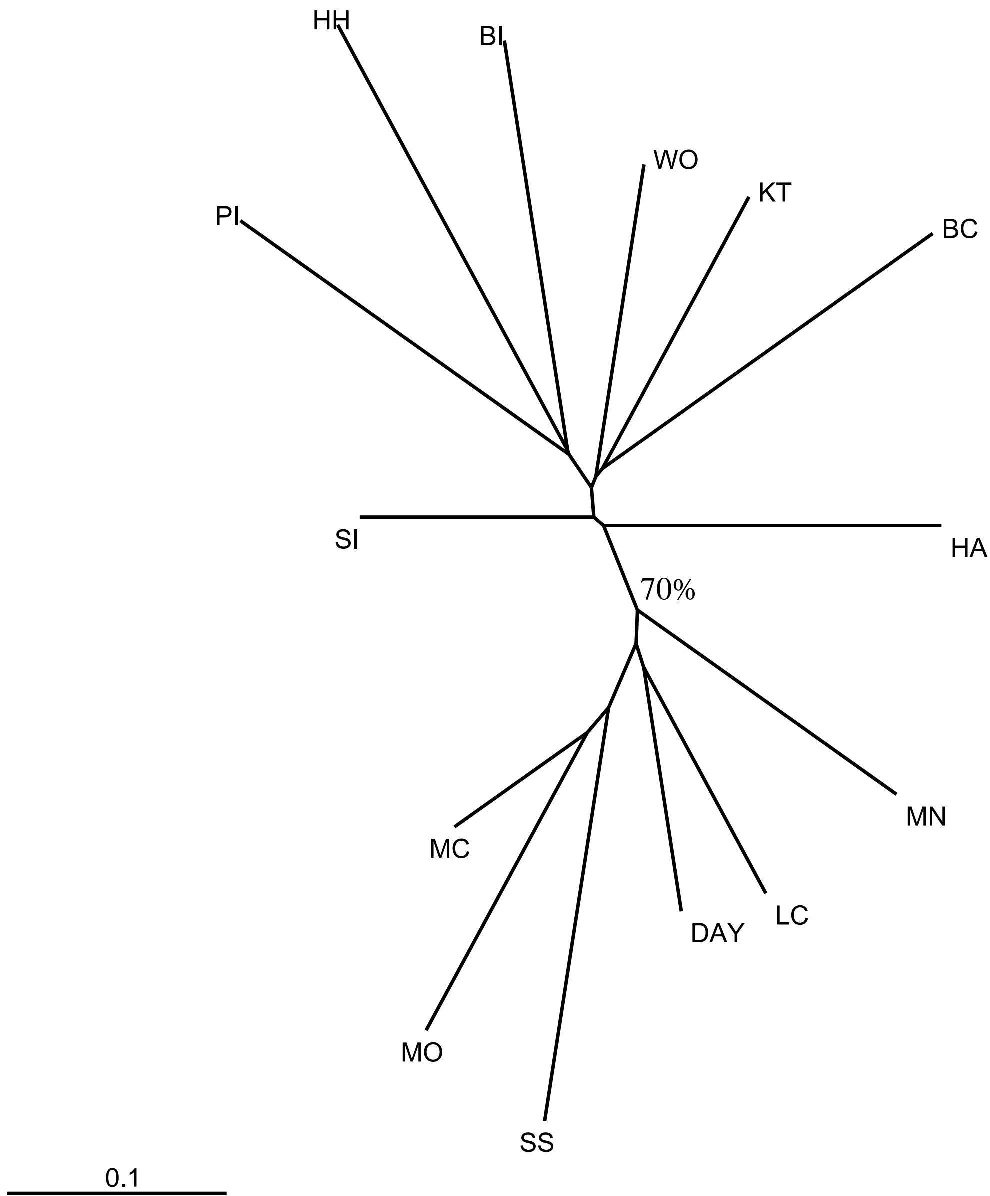

Figure 7. Unrooted NJ tree of all populations that have not been stocked since 1990 created using Cavalli-Sforza and Edwards (1967) chord distance. Node support based on 500 bootstrap pseudoreplicates. Nodes with support <50% are not labeled.

Appendix 1. Standard operating protocol for field collection of fin-clips.

Items Needed for this Procedure:

1) Labeled screw cap tubes in tube rack
2) Forceps and tissue scissors
3) Clipboard with datasheet and writing utensil
4) Squeeze bottle filled with 95% ethanol

Procedure:

1) Organize work space to maximize fish handling efficiency.
2) Collect morphological data (i.e., length, weight, etc.).
3) Before releasing fish, collect tissue sample.
4) Cut a "nickel" size piece of fin tissue usually from the caudal or pelvic fin using scissors.
5) Using forceps, place tissue in the labeled screw cap tube (Note: place tissue in tubes in consecutive order beginning with the smallest number to minimize confusion).
6) Fill tubes with ethanol and screw on cap securely to prevent the ethanol from evaporating (Note: if handling a large number of fish, you can wait for a pause in sampling to add ethanol; just make sure the lids are put back on the tubes to prevent mixing tubes and lids).
7) Place tubes in tube rack in sequential order.
8) Record tube number on the data sheet so tissue samples can be matched up with morphological data collected for each individual.
9) Rinse scissors and forceps in water between samples to minimize contamination risk (Note: lake/river water is sufficient; no visible blood, 'slime', or tissue should be present between samples).
10) Label tube box with site specific information (location, date, range of sample numbers, and name of individuals collecting sample).

Appendix 2. Allele frequency data for all sampled muskellunge populations.

Locus/Alleles	Populations											
D5	**BN**	**AM**	**MC**	**WCF**	**TH**	**BC**	**DAY**	**PM**	**BA**	**KT**	**EC**	**SS**
201	0.012	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
205	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.000	0.000	0.013	0.000	0.000
209	0.000	0.000	0.000	0.025	0.041	0.050	0.000	0.059	0.050	0.100	0.000	0.012
213	0.047	0.100	0.063	0.088	0.143	0.025	0.190	0.147	0.120	0.050	0.071	0.061
217	0.012	0.040	0.021	0.013	0.020	0.075	0.010	0.029	0.020	0.000	0.020	0.000
221	0.012	0.090	0.042	0.088	0.071	0.100	0.040	0.044	0.060	0.063	0.071	0.012
225	0.035	0.050	0.052	0.013	0.031	0.125	0.050	0.044	0.010	0.013	0.051	0.000
229	0.023	0.020	0.042	0.050	0.000	0.000	0.010	0.015	0.010	0.050	0.020	0.000
233	0.058	0.050	0.021	0.025	0.010	0.000	0.030	0.015	0.020	0.025	0.041	0.073
237	0.023	0.010	0.115	0.075	0.020	0.025	0.000	0.059	0.040	0.100	0.102	0.110
241	0.047	0.020	0.188	0.125	0.061	0.038	0.010	0.029	0.090	0.050	0.051	0.037
245	0.198	0.090	0.135	0.075	0.153	0.075	0.180	0.132	0.190	0.088	0.133	0.073
249	0.128	0.110	0.010	0.100	0.102	0.050	0.190	0.132	0.120	0.100	0.092	0.134
253	0.093	0.090	0.083	0.088	0.061	0.100	0.100	0.132	0.110	0.150	0.102	0.146
257	0.128	0.060	0.188	0.050	0.071	0.025	0.050	0.074	0.040	0.113	0.010	0.085
261	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.015	0.020	0.000	0.051	0.024
265	0.058	0.080	0.010	0.038	0.153	0.000	0.060	0.044	0.050	0.000	0.082	0.073
269	0.012	0.030	0.000	0.063	0.020	0.000	0.030	0.000	0.010	0.050	0.010	0.061
273	0.081	0.110	0.021	0.088	0.041	0.000	0.050	0.029	0.000	0.013	0.031	0.024
277	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.010	0.025	0.051	0.000
281	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
285	0.035	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.010	0.037
289	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024
291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012
D6												
165	0.267	0.200	0.448	0.238	0.235	0.375	0.170	0.206	0.190	0.317	0.255	0.295
213	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
217	0.000	0.000	0.000	0.013	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000
221	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.044	0.000	0.000	0.041	0.013
225	0.023	0.020	0.052	0.025	0.000	0.000	0.080	0.000	0.020	0.000	0.112	0.000
229	0.256	0.200	0.010	0.200	0.214	0.025	0.380	0.250	0.280	0.134	0.184	0.115
233	0.093	0.080	0.115	0.163	0.102	0.113	0.050	0.074	0.070	0.110	0.092	0.064
237	0.116	0.180	0.146	0.113	0.194	0.038	0.140	0.162	0.200	0.049	0.122	0.269
241	0.093	0.090	0.073	0.200	0.092	0.088	0.060	0.103	0.090	0.293	0.041	0.064
245	0.047	0.020	0.010	0.000	0.051	0.113	0.030	0.044	0.070	0.012	0.061	0.064
249	0.023	0.110	0.052	0.000	0.031	0.063	0.050	0.088	0.030	0.049	0.010	0.064
253	0.035	0.030	0.021	0.000	0.031	0.038	0.000	0.029	0.050	0.024	0.031	0.013
257	0.047	0.040	0.042	0.013	0.020	0.000	0.020	0.000	0.000	0.000	0.031	0.026
261	0.000	0.010	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
265	0.000	0.000	0.031	0.025	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.013
269	0.000	0.000	0.000	0.000	0.000	0.138	0.010	0.000	0.000	0.000	0.000	0.000
277	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000

Appendix 2. Continued.

Locus/Alleles	Populations										
D116	**BN**	**AM**	**MC**	**WCF**	**TH**	**BC**	**DAY**	**PM**	**BA**	**KT**	**EC**	**SS**
239	0.023	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.020	0.037	0.010	0.000
243	0.035	0.120	0.010	0.113	0.010	0.000	0.060	0.015	0.000	0.000	0.051	0.013
247	0.279	0.200	0.219	0.188	0.337	0.238	0.260	0.294	0.230	0.342	0.367	0.150
251	0.233	0.270	0.031	0.113	0.184	0.063	0.290	0.132	0.260	0.110	0.082	0.100
255	0.035	0.120	0.010	0.088	0.102	0.050	0.040	0.074	0.120	0.110	0.133	0.125
259	0.047	0.010	0.000	0.100	0.031	0.063	0.060	0.088	0.150	0.134	0.020	0.038
263	0.000	0.000	0.010	0.000	0.010	0.413	0.000	0.044	0.030	0.024	0.031	0.038
267	0.070	0.070	0.271	0.113	0.061	0.063	0.070	0.044	0.060	0.122	0.020	0.125
271	0.093	0.070	0.260	0.150	0.031	0.088	0.050	0.059	0.030	0.000	0.153	0.113
275	0.035	0.070	0.073	0.075	0.082	0.000	0.050	0.147	0.040	0.061	0.051	0.200
279	0.151	0.060	0.094	0.013	0.092	0.025	0.080	0.029	0.040	0.037	0.071	0.063
283	0.000	0.010	0.010	0.013	0.000	0.000	0.020	0.029	0.010	0.024	0.000	0.013
287	0.000	0.000	0.000	0.013	0.051	0.000	0.010	0.044	0.010	0.000	0.000	0.013
291	0.000	0.000	0.010	0.025	0.010	0.000	0.000	0.000	0.000	0.000	0.010	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
A10												
154	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
156	0.080	0.010	0.051	0.025	0.000	0.013	0.040	0.015	0.010	0.000	0.010	0.023
158	0.761	0.810	0.908	0.838	0.837	0.963	0.850	0.809	0.800	0.878	0.898	0.814
160	0.000	0.010	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.012	0.000	0.000
164	0.159	0.160	0.041	0.138	0.133	0.025	0.110	0.177	0.180	0.110	0.092	0.163
D12												
181	0.036	0.000	0.000	0.013	0.061	0.000	0.030	0.059	0.010	0.000	0.000	0.024
185	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
193	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.029	0.010	0.037	0.010	0.000
197	0.060	0.031	0.000	0.000	0.000	0.063	0.020	0.015	0.060	0.024	0.031	0.000
201	0.131	0.174	0.160	0.290	0.174	0.100	0.250	0.147	0.150	0.073	0.184	0.083
205	0.214	0.469	0.213	0.171	0.255	0.050	0.330	0.250	0.190	0.122	0.194	0.238
209	0.274	0.112	0.234	0.132	0.122	0.088	0.180	0.088	0.140	0.281	0.174	0.333
213	0.202	0.163	0.266	0.237	0.296	0.488	0.130	0.235	0.270	0.244	0.265	0.274
217	0.071	0.010	0.075	0.132	0.092	0.213	0.030	0.162	0.150	0.171	0.082	0.048
221	0.000	0.010	0.043	0.026	0.000	0.000	0.030	0.015	0.020	0.049	0.061	0.000
225	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
229	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A5												
216	0.000	0.010	0.010	0.066	0.184	0.175	0.030	0.103	0.070	0.024	0.041	0.000
226	0.830	0.800	0.847	0.776	0.714	0.675	0.820	0.765	0.820	0.646	0.796	0.893
228	0.171	0.190	0.143	0.158	0.102	0.150	0.150	0.132	0.110	0.329	0.163	0.107
A102												
131	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
133	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
135	0.478	0.620	0.408	0.400	0.469	0.175	0.450	0.265	0.300	0.134	0.388	0.640
137	0.511	0.380	0.592	0.600	0.521	0.825	0.550	0.735	0.700	0.866	0.612	0.361

Appendix 2. Continued.

Locus/Alleles	Populations											
A11	**BN**	**AM**	**MC**	**WCF**	**TH**	**BC**	**DAY**	**PM**	**BA**	**KT**	**EC**	**SS**
132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000
136	0.182	0.230	0.092	0.125	0.112	0.038	0.060	0.212	0.190	0.061	0.184	0.049
138	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
140	0.046	0.020	0.010	0.000	0.020	0.013	0.010	0.030	0.000	0.000	0.041	0.000
144	0.739	0.750	0.878	0.838	0.847	0.950	0.920	0.758	0.810	0.927	0.745	0.939
146	0.034	0.000	0.010	0.038	0.020	0.000	0.010	0.000	0.000	0.000	0.020	0.012
B110												
165	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
183	0.607	0.663	0.592	0.564	0.388	0.275	0.540	0.409	0.510	0.476	0.602	0.854
185	0.143	0.112	0.327	0.244	0.255	0.400	0.200	0.106	0.110	0.146	0.163	0.110
191	0.238	0.225	0.082	0.192	0.357	0.325	0.260	0.485	0.380	0.378	0.235	0.037
D114												
270	0.000	0.052	0.021	0.026	0.020	0.000	0.010	0.000	0.020	0.012	0.010	0.013
274	0.553	0.438	0.362	0.577	0.582	0.388	0.520	0.470	0.370	0.390	0.520	0.218
278	0.105	0.188	0.106	0.103	0.122	0.100	0.140	0.121	0.240	0.342	0.102	0.269
282	0.316	0.260	0.362	0.244	0.194	0.113	0.230	0.318	0.290	0.207	0.265	0.359
286	0.026	0.042	0.043	0.013	0.020	0.238	0.000	0.000	0.040	0.049	0.051	0.051
290	0.000	0.010	0.075	0.039	0.051	0.050	0.050	0.030	0.030	0.000	0.031	0.051
294	0.000	0.010	0.021	0.000	0.010	0.113	0.050	0.061	0.010	0.000	0.020	0.000
298	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039
A104												
161	0.022	0.200	0.112	0.250	0.122	0.238	0.060	0.103	0.140	0.122	0.194	0.058
163	0.689	0.410	0.755	0.438	0.449	0.550	0.490	0.441	0.460	0.317	0.490	0.616
165	0.267	0.380	0.122	0.313	0.429	0.213	0.430	0.427	0.380	0.561	0.306	0.267
167	0.022	0.010	0.010	0.000	0.000	0.000	0.020	0.029	0.020	0.000	0.010	0.058
169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C1												
205	0.047	0.020	0.031	0.050	0.000	0.025	0.120	0.029	0.000	0.000	0.020	0.037
209	0.012	0.060	0.388	0.063	0.031	0.063	0.030	0.118	0.050	0.110	0.092	0.195
213	0.895	0.910	0.551	0.813	0.949	0.838	0.820	0.794	0.890	0.683	0.806	0.768
217	0.047	0.000	0.031	0.063	0.020	0.075	0.030	0.059	0.060	0.207	0.082	0.000
221	0.000	0.010	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D126												
128	0.211	0.300	0.214	0.225	0.194	0.138	0.250	0.235	0.280	0.402	0.214	0.035
132	0.356	0.160	0.204	0.375	0.286	0.200	0.220	0.235	0.340	0.171	0.235	0.372
136	0.433	0.540	0.582	0.400	0.520	0.663	0.530	0.529	0.380	0.427	0.551	0.593
B120												
234	0.583	0.540	0.827	0.600	0.677	0.975	0.500	0.662	0.690	0.829	0.635	0.631
236	0.417	0.460	0.174	0.400	0.323	0.025	0.500	0.338	0.310	0.171	0.365	0.369

Appendix 2. Continued.

Locus/Alleles	Populations											
D5	LC	NN	HA	HH	SI	NT	MN	BI	MO	CF	PI	WO
201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
205	0.000	0.000	0.015	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.010
209	0.000	0.063	0.000	0.000	0.104	0.029	0.020	0.000	0.000	0.021	0.000	0.051
213	0.119	0.113	0.044	0.000	0.115	0.074	0.080	0.000	0.075	0.135	0.000	0.092
217	0.000	0.000	0.177	0.000	0.031	0.044	0.000	0.091	0.050	0.021	0.000	0.061
221	0.071	0.063	0.044	0.023	0.031	0.059	0.030	0.091	0.225	0.073	0.133	0.041
225	0.024	0.025	0.000	0.000	0.000	0.015	0.040	0.015	0.075	0.021	0.017	0.031
229	0.000	0.025	0.088	0.000	0.083	0.015	0.010	0.000	0.000	0.031	0.000	0.031
233	0.071	0.038	0.044	0.000	0.000	0.074	0.010	0.015	0.025	0.042	0.000	0.010
237	0.024	0.025	0.015	0.182	0.031	0.015	0.030	0.015	0.013	0.021	0.067	0.051
241	0.000	0.013	0.265	0.182	0.177	0.044	0.030	0.121	0.113	0.042	0.100	0.112
245	0.095	0.263	0.088	0.114	0.073	0.221	0.200	0.197	0.088	0.094	0.000	0.082
249	0.286	0.125	0.118	0.068	0.125	0.177	0.150	0.046	0.025	0.115	0.067	0.143
253	0.071	0.138	0.044	0.136	0.115	0.074	0.120	0.182	0.025	0.115	0.367	0.153
257	0.000	0.038	0.015	0.205	0.031	0.074	0.160	0.000	0.050	0.031	0.150	0.020
261	0.024	0.000	0.015	0.046	0.010	0.000	0.010	0.030	0.075	0.042	0.050	0.031
265	0.071	0.000	0.000	0.046	0.031	0.029	0.070	0.061	0.050	0.073	0.033	0.051
269	0.024	0.025	0.015	0.000	0.000	0.015	0.000	0.136	0.063	0.010	0.017	0.000
273	0.119	0.038	0.015	0.000	0.000	0.029	0.030	0.000	0.000	0.073	0.000	0.031
277	0.000	0.013	0.000	0.000	0.000	0.000	0.010	0.000	0.050	0.021	0.000	0.000
281	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.010	0.000	0.000
285	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.010	0.000	0.000
289	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
291	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D6												
165	0.143	0.141	0.333	0.283	0.208	0.364	0.367	0.273	0.263	0.344	0.030	0.230
213	0.071	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
217	0.000	0.000	0.014	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
221	0.000	0.033	0.014	0.000	0.010	0.015	0.000	0.046	0.000	0.000	0.000	0.000
225	0.000	0.000	0.014	0.000	0.000	0.015	0.000	0.015	0.000	0.010	0.000	0.000
229	0.310	0.511	0.125	0.065	0.292	0.227	0.235	0.121	0.025	0.240	0.121	0.090
233	0.048	0.054	0.056	0.000	0.125	0.030	0.031	0.121	0.225	0.083	0.015	0.040
237	0.095	0.065	0.111	0.022	0.115	0.197	0.143	0.212	0.100	0.073	0.091	0.370
241	0.048	0.087	0.125	0.152	0.198	0.091	0.102	0.136	0.025	0.146	0.303	0.260
245	0.024	0.098	0.153	0.196	0.052	0.015	0.020	0.030	0.000	0.073	0.000	0.000
249	0.143	0.011	0.000	0.261	0.000	0.030	0.051	0.046	0.025	0.021	0.152	0.000
253	0.024	0.000	0.042	0.000	0.000	0.015	0.041	0.000	0.138	0.000	0.136	0.000
257	0.024	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.150	0.010	0.000	0.000
261	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
265	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.136	0.010
269	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000
277	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

Locus/Alleles	Populations											
D116	**LC**	**NN**	**HA**	**HH**	**SI**	**NT**	**MN**	**BI**	**MO**	**CF**	**PI**	**WO**
239	0.000	0.035	0.000	0.022	0.000	0.000	0.020	0.000	0.000	0.031	0.000	0.000
243	0.095	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.025	0.031	0.000	0.061
247	0.167	0.221	0.216	0.239	0.219	0.324	0.140	0.242	0.338	0.281	0.547	0.429
251	0.381	0.419	0.216	0.152	0.229	0.177	0.410	0.106	0.038	0.188	0.250	0.041
255	0.095	0.058	0.027	0.130	0.115	0.162	0.060	0.076	0.025	0.125	0.078	0.194
259	0.000	0.151	0.108	0.174	0.240	0.074	0.050	0.091	0.050	0.104	0.000	0.071
263	0.000	0.000	0.054	0.283	0.010	0.029	0.100	0.106	0.113	0.031	0.000	0.051
267	0.048	0.081	0.311	0.000	0.146	0.044	0.130	0.030	0.013	0.094	0.000	0.020
271	0.071	0.000	0.068	0.000	0.000	0.000	0.070	0.015	0.288	0.031	0.078	0.061
275	0.048	0.000	0.000	0.000	0.042	0.044	0.020	0.076	0.025	0.042	0.000	0.000
279	0.048	0.023	0.000	0.000	0.000	0.059	0.000	0.167	0.013	0.031	0.047	0.061
283	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.091	0.013	0.010	0.000	0.010
287	0.000	0.012	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000
291	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
A10												
154	0.024	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.031
156	0.119	0.022	0.000	0.022	0.000	0.044	0.177	0.000	0.063	0.031	0.066	0.000
158	0.762	0.880	0.944	0.957	0.854	0.809	0.745	0.636	0.863	0.750	0.934	0.908
160	0.000	0.022	0.000	0.000	0.021	0.015	0.000	0.000	0.000	0.010	0.000	0.000
164	0.095	0.076	0.056	0.022	0.104	0.132	0.078	0.364	0.075	0.208	0.000	0.061
D12												
181	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
185	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.031	0.000	0.000
197	0.000	0.174	0.043	0.044	0.010	0.015	0.010	0.000	0.000	0.094	0.000	0.122
201	0.119	0.198	0.271	0.522	0.219	0.132	0.088	0.303	0.231	0.156	0.378	0.163
205	0.405	0.233	0.171	0.130	0.177	0.279	0.373	0.091	0.103	0.208	0.068	0.143
209	0.119	0.070	0.114	0.022	0.073	0.235	0.177	0.106	0.256	0.125	0.041	0.061
213	0.214	0.279	0.343	0.109	0.219	0.206	0.226	0.333	0.385	0.188	0.500	0.388
217	0.071	0.047	0.057	0.109	0.229	0.132	0.128	0.136	0.000	0.156	0.014	0.102
221	0.048	0.000	0.000	0.065	0.052	0.000	0.000	0.015	0.026	0.021	0.000	0.020
225	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
229	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A5												
216	0.000	0.067	0.029	0.022	0.053	0.076	0.010	0.258	0.013	0.031	0.176	0.031
226	0.881	0.678	0.729	0.630	0.681	0.833	0.902	0.424	0.850	0.813	0.595	0.837
228	0.119	0.256	0.243	0.348	0.266	0.091	0.088	0.318	0.138	0.156	0.230	0.133
A102												
131	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.010	0.000	0.000
133	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
135	0.524	0.283	0.278	0.239	0.219	0.485	0.461	0.379	0.400	0.510	0.053	0.190
137	0.476	0.717	0.722	0.761	0.781	0.515	0.539	0.606	0.600	0.479	0.947	0.810

Appendix 2. Continued.

Locus/Alleles	Populations											
A11	**LC**	**NN**	**HA**	**HH**	**SI**	**NT**	**MN**	**BI**	**MO**	**CF**	**PI**	**WO**
132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030
136	0.071	0.148	0.292	0.239	0.333	0.191	0.430	0.121	0.013	0.128	0.136	0.150
138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
140	0.024	0.102	0.000	0.000	0.000	0.044	0.000	0.000	0.038	0.032	0.000	0.090
144	0.905	0.750	0.708	0.761	0.667	0.735	0.570	0.879	0.950	0.840	0.864	0.730
146	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
B110												
165	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.011	0.000	0.000
183	0.643	0.466	0.375	0.348	0.575	0.632	0.560	0.394	0.738	0.553	0.183	0.470
185	0.095	0.159	0.472	0.044	0.075	0.074	0.060	0.121	0.188	0.106	0.067	0.050
191	0.262	0.375	0.153	0.609	0.351	0.294	0.380	0.470	0.075	0.330	0.750	0.480
D114												
270	0.000	0.024	0.028	0.065	0.032	0.015	0.022	0.030	0.100	0.053	0.000	0.000
274	0.476	0.619	0.111	0.174	0.426	0.559	0.711	0.258	0.513	0.426	0.283	0.460
278	0.143	0.143	0.403	0.239	0.372	0.177	0.133	0.167	0.163	0.170	0.133	0.280
282	0.238	0.131	0.319	0.500	0.085	0.221	0.089	0.515	0.175	0.255	0.517	0.240
286	0.048	0.012	0.000	0.000	0.000	0.029	0.000	0.000	0.050	0.021	0.000	0.000
290	0.095	0.024	0.139	0.000	0.085	0.000	0.044	0.000	0.000	0.011	0.067	0.010
294	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000	0.010
298	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000
A104												
161	0.071	0.120	0.095	0.065	0.219	0.235	0.049	0.394	0.088	0.128	0.189	0.210
163	0.500	0.217	0.324	0.217	0.385	0.471	0.392	0.242	0.713	0.447	0.392	0.350
165	0.429	0.663	0.581	0.717	0.396	0.294	0.529	0.333	0.200	0.404	0.419	0.440
167	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.030	0.000	0.011	0.000	0.000
169	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.011	0.000	0.000
C1												
205	0.024	0.033	0.000	0.022	0.000	0.015	0.000	0.000	0.175	0.021	0.000	0.000
209	0.143	0.000	0.000	0.391	0.031	0.147	0.029	0.121	0.213	0.042	0.000	0.130
213	0.738	0.728	0.946	0.522	0.927	0.794	0.961	0.773	0.588	0.896	0.924	0.740
217	0.095	0.239	0.054	0.065	0.042	0.044	0.010	0.106	0.025	0.031	0.076	0.130
221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
D126												
128	0.286	0.424	0.324	0.022	0.406	0.206	0.137	0.318	0.325	0.208	0.265	0.170
132	0.262	0.294	0.054	0.239	0.302	0.265	0.235	0.212	0.175	0.240	0.309	0.330
136	0.452	0.283	0.622	0.739	0.292	0.529	0.628	0.470	0.500	0.552	0.427	0.500
B120												
234	0.619	0.796	0.722	0.761	0.883	0.706	0.633	0.742	0.925	0.542	0.736	0.888
236	0.381	0.205	0.278	0.239	0.117	0.294	0.367	0.258	0.075	0.458	0.264	0.112