GENETIC, MORPHOLOGICAL AND ISOTOPIC POPULATION STRUCTURE OF LAKE WHITEFISH (*Coregonus clupeaformis*) IN NORTHERN LAKE WINNIPEG AND PLAYGREEN LAKE

BY

WILLIAM V. MAVROS

A Thesis

Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

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Department of Zoology
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ABSTRACT

Lake whitefish (*Coregonus clupeaformis*) populations from various sites on northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake were characterized by biochemical, morphological and stable isotope analyses. Genetic composition of the fish was determined by the use of starch gel electrophoresis based on 36 genetic loci for six spawning aggregations collected in 1989, and based on 14 genetic loci for four spawning aggregations collected in 1975. Phenotypic characteristics were obtained from 23 morphometric measurements and from 9 meristic counts. Carbon, nitrogen and sulphur stable isotope tracers were utilised to delineate among stocks of lake whitefish. A secondary aim of this project was to test the null hypothesis that the construction of the Lake Winnipeg Regulation Project (LWR) has not caused significant changes in the genetic and morphological relationships of lake whitefish stocks in the LWR development area.

Allelic frequencies differed significantly among stocks at the MDH-B1,2 loci, indicating that lake whitefish in northern Lake Winnipeg and Playgreen Lake can be differentiated into at least two distinct genetic subpopulations. These two differentiated stocks were present before LWR and they have remained genetically distinct after the construction of LWR. Morphological analysis of the 1989 lake whitefish samples revealed that all six samples were significantly different from each other, and were in

agreement with previous morphological studies conducted in 1975. Inter-year comparison of Little Playgreen Lake, Big Black River and Grand Rapids populations indicated that stock integrity did not change over time but morphological characteristics of the stocks did change over time. The various stocks differed in their C, N and S stable isotope composition, indicating that adult lake whitefish seem to feed in specific locations in Lake Winnipeg but exact locations and range of feeding areas could not be distinguished without knowing particular source isotope signals.

This multiple approach study confirms the presence of multiple stocks of lake whitefish and fails to reject the null hypothesis that LWR has not altered the stock structure of lake whitefish in the north basin of Lake Winnipeg, Playgreen Lake and Little Playgreen Lake.

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GENERAL INTRODUCTION

The main emphasis of this study dealt with the identification of stocks of lake whitefish in northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake utilizing biochemical techniques for the analysis of genetically determined characteristics, morphological methodologies for phenotypic analyses, and stable isotope techniques for the analysis of stock separation due to dietary differences. The secondary objective of this study was to determine whether the genetic, phenotypic and/or isotopic structuring of the populations of lake whitefish in northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake have changed temporally as a result of the construction and subsequent operation of the Lake Winnipeg Regulation Project (LWR). The null hypothesis that there has been no change was tested by comparing the stock structure of lake whitefish populations in 1989 to the stock structure present in 1975 (Kristofferson 1978; Kristofferson and Clayton 1990). Previous tagging studies (Kennedy 1954; Pollard 1973) and morphological research (Kristofferson 1978; Kristofferson & Clayton 1990) have suggested the existence of at least three phenotypically distinct forms of lake whitefish from Little Playgreen Lake and the north basin of Lake Winnipeg.

Genetic (biochemical) characteristics, as measured by electrophoresis, are not environmentally modifiable and are not known to be sensitive to changes due to short term environmental modifications (Allendorf & Utter

1979). Genetic analysis is a more definitive tool in identifying the long-term temporal and spatial stability of lake whitefish stocks, whereas morphometric analysis is extremely useful in identifying short term differentiation in stocks and in delineating environmental effects on stocks (Imhof et al. 1980; Casselman et al. 1981; Savvaitova et al. 1989). Carbon (C), sulphur (S) and nitrogen (N) stable isotopes can be utilized as markers to delineate the spatial feeding pattern of lake whitefish stocks. Differences in the feeding locations and/or diets among stocks can lead to isotopic differences in fish tissue that can indicate different stocks of lake whitefish. Since stable isotopic compositions of tissues can be considered indicative of the assimilated diet, both long-term and short-term environmental trends can be identified (Peterson & Fry 1987).

There is evidence that there are movements of lake whitefish between Playgreen Lake and the north end of Lake Winnipeg (Pollard 1973). Some stocks migrate downstream from their feeding grounds in Lake Winnipeg to utilize Playgreen Lake and Little Playgreen Lake for spawning during late fall and then reverse their migration upstream into Lake Winnipeg to overwinter and feed. Pollard (1973) tagged 2934 lake whitefish at Warren Landing, at the outlet of Lake Winnipeg, between September 25th and October 7th 1970 and noted that 16 fish were recaptured downstream in the river channels (mouth of Gunisao River) by Norway House and in Little Playgreen Lake (Fig. 1). This interbasin movement of whitefish is critical to

fishermen since 90% of the pre-LWR commercial whitefish catch in Playgreen Lake was dependent on this movement between the lakes (Kuiper & Booy 1968). Changes in the geographic and/or temporal distribution of flows between Lake Winnipeg and Playgreen Lake might be expected to have disrupted lake whitefish migration patterns between the two lakes and possibly the structure of lake whitefish stocks in the area. Of major concern are the effects that LWR hydroelectric development may have on lake whitefish that utilize the affected basins.

Hydroelectric development has been a contentious issue with Manitoba fishermen during the last two decades with controversy arising from the construction and operation of LWR, in particular Two Mile Channel (2MC). The LWR project was constructed by Manitoba Hydro over the period 1971 to 1976 with the primary aim of the project being to regulate the level of Lake Winnipeg in order to provide larger assured winter flows into the Nelson River for hydroelectric power production on the lower Nelson. The secondary aim of the LWR project was to increase the outflow capacity from Lake Winnipeg. Warren Landing was the only outlet of Lake Winnipeg but since the construction of the 2MC outlet and the operation of the LWR, the geographic and seasonal distribution of flows through the Playgreen Lake area have been altered (Fig. 1). The gradient through Warren Landing has been reduced by 75% by the construction of 2MC thus greatly reducing flows through the natural channel (LWCNR

Study Board tech. report, 1971 - 75). During normal and high water levels in Lake Winnipeg, Warren Landing carries most of the outflow of Lake Winnipeg (about 60 - 70%; MacLaren Plansearch 1985). Natural seasonal variation has been changed and water levels on Playgreen Lake and Lake Winnipeg are now lower at the end of winter (March) and higher at the commencement of winter (October) than they were before LWR, although the lake is regulated within its historic range of water levels (regulated between 711 - 715 feet above sea level).

Local fishermen have claimed that separate stocks of lake whitefish occur and that the seasonal movements of these stocks between Lake Winnipeg and Playgreen Lake have changed since the construction of LWR. These fishermen have also reported decreased catches of lake whitefish and have attributed this decline in catch to deleterious effects caused by the construction of LWR (Flett, pers. comm.). Many of these fishermen believe that lake whitefish behaviour is strongly moderated by lake currents and they have attributed the decrease in the catch of lake whitefish to the change in lake currents caused by 2MC.

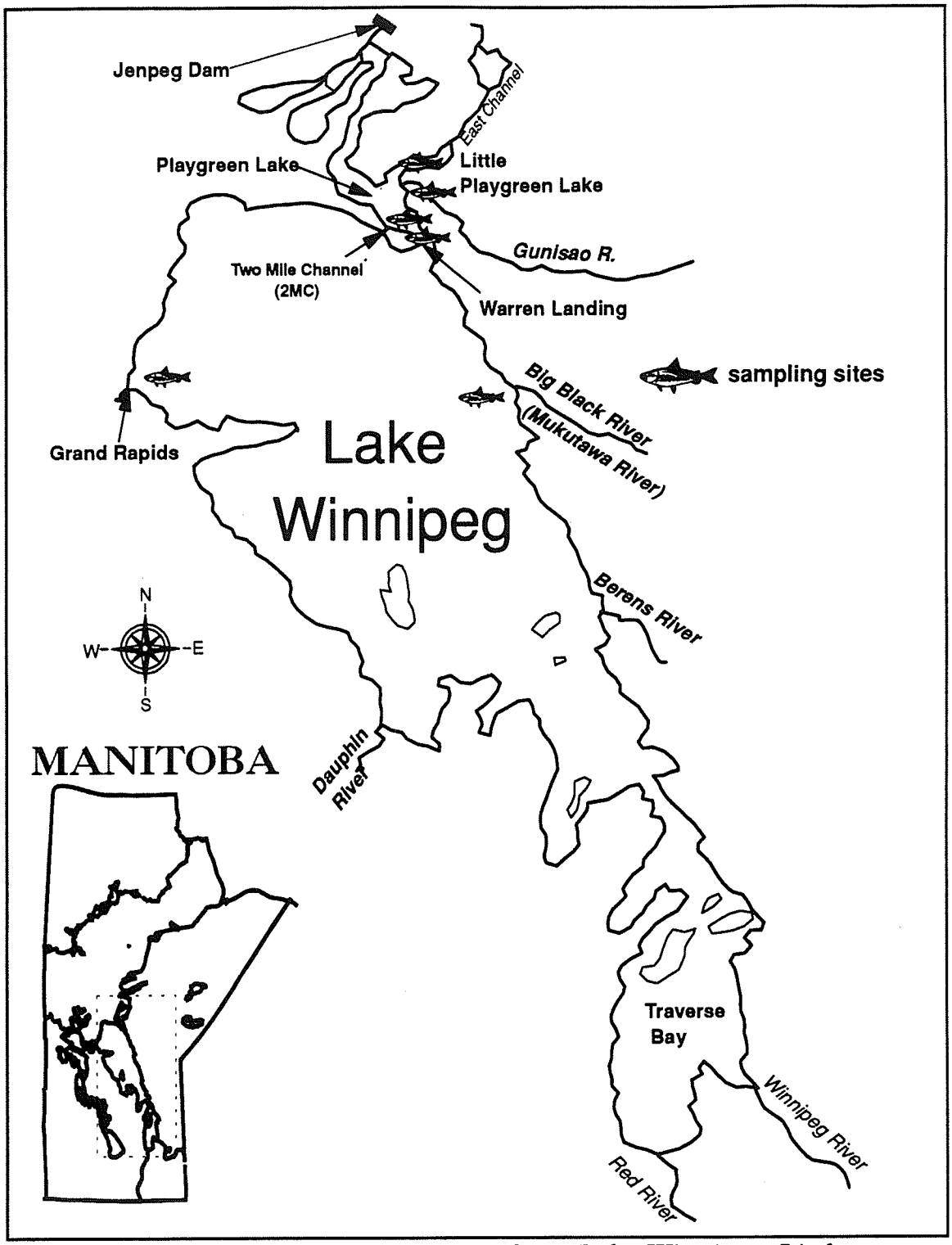

Fig. 1. Location of sampling sites in northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake.

PART I:

GENETIC & MORPHOLOGICAL ANALYSIS OF LAKE WHITEFISH

INTRODUCTION

The main emphasis of this part of the investigation was to discriminate between spawning aggregations of lake whitefish in northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake (pre-LWR and post-LWR) by utilizing biochemical genetic analysis for genetically determined differences and morphological analysis for phenotypic differences among populations sampled. This study also attempted to determine whether the genetic and/or phenotypic structuring of the populations of lake whitefish in northern Lake Winnipeg and in Playgreen Lake has changed as a result of the construction and subsequent operation of LWR. The null hypothesis that there has been no change was tested by comparing the genetic and phenotypic stock structure of lake whitefish populations in 1989 to the genetic and phenotypic stock structure present in 1975 (Kristofferson 1978). The study design for this inter-year comparison used three different sites: a) two Lake Winnipeg sites, Grand Rapids and Big Black River (also known as Mukutawa River), as reference sites which have been less affected by LWR and b) the Little Playgreen Lake site as an experimental site which may have been affected to a greater degree by LWR.

Larkin (1972) has defined a stock of fish as a population which shares a common environment and gene pool. Ricker's (1972) criteria for a stock involve fish spawning in specific locations that are temporally and spatially reproductively segregated. These definitions imply a degree of reproductive isolation between various stocks of a particular fish species in a specific area. For certain freshwater fishes, reproductive isolation of stocks often results from stock isolating mechanisms such as site imprinting and homing behaviour of individual fish to their natal spawning grounds (Horrall 1981). Reproductive isolation will generally be expected to result in genetic differences between stocks that will tend to accumulate due to (random) genetic drift and/or selection.

The genetic differences expected among various stocks of fish and the environmental differences present in their habitats have allowed the development of population genetic procedures to identify and differentiate stocks (Casselman et al. 1981; Ihssen et al. 1981; Todd 1981; Kristofferson 1978). Stock characteristics are usually measured in samples of fish taken from spawning areas at the time of spawning, when stocks would be expected to have segregated themselves into reproductively isolated units. Direct assessment of genetic differences is possible by a number of means, the most common of which is the electrophoretic analysis of allelic variation.

Horizontal starch gel electrophoresis is the separation of a mixture of electrically charged molecules in an electric field through a starch gel and is

one of the most useful techniques devised to date for studying genetic variability within and among populations of organisms (Abersold et al. 1987). Starch gel electrophoresis has been used extensively to study intraspecific genetic variation of coregonids (Clayton and Franzin 1970; Clayton et al. 1973; Franzin and Clayton 1977; Kristofferson 1978; Kirkpatrick and Selander 1979; Imhof et al. 1980; Casselman et al. 1981; Vuorinen 1984; Kristofferson and Clayton 1990). Genetic characteristics, as measured by electrophoresis, are not modifiable by short-term environmental conditions and are therefore powerful attributes for identifying and characterizing different stocks of fish. The spawning aggregates of whitefish were tested, by horizontal starch gel electrophoresis, for differences in protein migration in order to obtain insights into both within-group and among-group genetic variation (Winans 1980; Richardson 1983; Shaklee 1984).

Stock genetics is also an increasingly utilized tool in the measurement and study of environmental impacts on fish populations and fisheries. For example, Bodaly et al. (1984) showed that a significant change in the stock genetics of lake whitefish had taken place in Southern Indian Lake, Manitoba and adjacent water bodies concurrent with disruptions in fish populations caused by the impoundment of the lake and the blockage of fish migrations via the natural outlet of the lake.

Populations of coregonid fishes in large lake systems have been shown in many instances to be divided into discrete stocks (e.g., Imhof et al. 1980; Casselman et al. 1981; Ihssen et al. 1981; Todd et al. 1981). Kristofferson (1978, Kristofferson and Clayton 1990) was able to discern two distinct genotypic stocks of lake whitefish in Lake Winnipeg and connecting water bodies. Six genetic loci were found to be polymorphic in these Lake Winnipeg populations, but there were no significant differences in allelic frequencies between populations of lake whitefish located in northern Lake Winnipeg and Little Playgreen Lake (Kristofferson 1978; Kristofferson and Clayton 1990). These results form part of the pre-development (pre-LWR) baseline for the present study.

Phenotypic characteristics can be of practical importance in delineating stocks (Kristofferson 1978; Ihssen et al. 1981; Casselman et al. 1981; Todd et al. 1981; Beacham 1985; MacCrimmon and Claytor 1985; Kristofferson & Clayton 1990; Karakousis et al. 1991) and in determining the influence of anthropogenic perturbations (indicated by increased phenotypic diversity, as demonstrated by Savvaitova et al. 1989). The use of morphological characteristics has some limitations in that they are polygenically inherited, have low heritability and are prone to be influenced by short term environmental variation (Casselman et al. 1981; Karakousis et al. 1991). Although these characters are modifiable by environmental variation, they can be as valuable in indicating stock discreteness as genetic

characters (Casselman et al. 1981; Kristofferson and Clayton 1990). In a study of lake whitefish populations in Lake Winnipeg and connecting water bodies, which was based on samples collected in 1975, Kristofferson (1978, Kristofferson and Clayton 1990) utilized morphological comparisons to identify three different stocks of lake whitefish in the north end of Lake Winnipeg and Little Playgreen Lake. Distinct stocks were noted for Grand Rapids on the northwest shore of Lake Winnipeg, for the Big Black, Poplar and Berens Rivers complex on the northeast shore of Lake Winnipeg, and for Little Playgreen Lake, on the Nelson River outlet of Lake Winnipeg.

Larkin's (1972) definition of fish stocks implies that different fish stocks of the same species utilize different environments for spawning, egg incubation and early life stages. These are periods when environmental influences can alter morphological traits. Therefore, differing environmental conditions among areas can lead to morphological differences among stocks which are not based solely on genetic differences, i.e. they are at least in part environmentally induced. This is especially the case for coregonid fishes in which extreme morphological plasticity is present (Lindsey 1981). Morphological differences between stocks result from genetic and/or environmental differences and these can be measured directly. The identification of discrete stocks of lake whitefish is important for the maintenance of genetic diversity of lake whitefish, but the possibility exitst that lake whitefish phenotypic characteristics have changed due to habitat

perturbations caused by LWR. Different environmental conditions (modified flow, ice cover, temperature fluctuations, food availability, etc.) arising from the operation of LWR may have changed the characteristics of the lake whitefish even before embryogenesis, causing larvae to develop differently from the parental stock (Tåning 1952). If different genetic and phenotypic stocks were present prior to LWR development, these stocks of lake whitefish would have either persisted or else have altered genetic relationships or phenotypic characteristics due to perturbation. Several researchers have found that meristic variation could be influenced by environmental effects such as temperature (Svärdson 1952; Tåning 1952; Blouw et al. 1988). Since phenotypic variation is likely linked to divergent environmental conditions, whitefish exposed to different environmental conditions during development may exhibit phenotypic variation over time and space.

METHODS

SPECIMEN COLLECTION

Samples of fifty mature lake whitefish (*Coregonus clupeaformis*) were collected by gill netting from six different spawning sites in northern Lake Winnipeg and Playgreen Lake. Gang nets with mesh size of 108, 113 and 133 mm. stretch mesh were used in order to catch mature whitefish. Sampling was conducted in late October 1989 to coincide with the probable lake whitefish spawning run through Warren Landing (Pollard 1973; Kristofferson 1978). The fish were frozen and shipped to the Freshwater Institute (FWI) in Winnipeg where they were processed.

I) GENETICS

For genetic (biochemical) analyses of 1989 fish, a sample of red and white muscle and a sample of liver were removed from each fish. These tissue samples were then frozen (-30 C) for later use in electrophoretic analyses.

Frozen muscle samples (-30 C) of lake whitefish collected from Little Playgreen Lake, Warren Landing, Grand Rapids, and Big Black River (Fig. 1) by Kristofferson in 1975 were obtained to determine pre-LWR stock structure. Biochemical analyses for these four samples were conducted using only white muscle tissue extracts. Since only white muscle tissue

extracts were available for the 1975 analysis, the number of enzymes that could be assayed by starch gel electrophoresis was reduced. The possibility of finding temporal differences between lake whitefish samples was therefore restricted to only polymorphic loci expressed in white muscle tissue. IDHP-4 allele frequencies were scored from gels run by Kristofferson (1978) because the allelic resolution obtained from the frozen white muscle tissue was poor.

Horizontal starch gel electrophoresis was performed following the methodology outlined by Vuorinen (1984) and under the electrophoretic conditions described in Bodaly et al. (1991). Thirty-six loci were screened in samples collected in 1989 whereas fourteen loci were screened in samples collected in 1975. Previous studies have shown that the following enzyme loci are likely to be polymorphic in these populations of lake whitefish and therefore useful in genetically comparing the various spawning aggregations: MDH-B1,2 (treated as two loci with equal allelic frequencies), IDDH-1,2 (SDH), G3PDH-1, G3PDH-3, LDH-B2 (liver), IDHP-3, IDHP-4, and MEP-3,4 (Imhof et al. 1980; Casselman et al. 1981; Ihssen et al. 1981; Kristofferson 1978; Kristofferson and Clayton 1990; Bodaly et al. 1991). Table 1 gives the genetic loci examined, their abbreviations, and the tissue of primary expression (muscle, eye or liver). Table 2 lists alleles observed at polymorphic loci with their relative mobilities on electrophoretic gels.

Table 1. Enzymes screened with number of loci and tissues (M=muscle; L=liver). All loci were examined for the 1989 samples; G3PDH-1, IDHP-3, IDHP-4, LDH-B2, MDH-B1,2, MEP-1,2, MEP-3, MEP-4 were examined for the 1975 samples.

Enzyme name	Enzyme number	Abbreviation	No. loci screened	Tissue
Aspartate aminotransferase	2.6.1.1	mAAT	1	M
		sAAT	2	M
Alcohol dehydrogenase	1.1.1.1	ADH	1	L
Creatine kinase	2.7.3.2	CK-A	2	M
Esterase	3.1.1.1	EST	1	L
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	3	M
Glucose-6-phosphate isomerase	5.3.1.9	GPI-A	2	L
		GPI-B	2	M
L-Iditol dehydrogenase [Sorbitol dehydrogenase]	1.1.1.14	IDDH	2	L
Isocitrate dehydrogenase	1.1.1.42	mIDHP	2	M
		sIDHP	2	L
Lactate dehydrogenase	1.1.1.27	LDH-A	2	M
		LDH-B	2	L
Malate dehydrogenase	1.1.1.37	sMDH-A	2	L
		sMDH-B	2	M
$NADP^+$-dependent malic enzyme	1.1.1.40	mMEP	2	M
		sMEP	2	L
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	1	M,L
Phosphoglucomutase	5.4.2.2	PGM	2	M,L
Superoxide dismutase	1.15.1.1	sSOD	1	L

Table 2. Relative mobilities of alleles at polymorphic loci under electrophoretic conditions explained in the text. Alleles and locus nomenclature follow Bodaly et al. (1991) which should be consulted for allele designations in previous studies.

Locus	Allele	Mobility
G3PDH-1	a	0
	b	-85
	d	210
G3PDH-3	a	100
	e	65
IDHP-3	a	100
	d	190
IDHP-4	a	100
	c	118
LDH-B2	a	100
	b	90
MDH-B1,2	a	120
	b	100
MEP-1,2	a	100
	b	40
MEP-3,4	a	100
	b	135

Statistical analyses were done with BIOSYS-1 (Swofford and Selander 1981) except were noted. Allele frequencies at polymorphic loci were calculated for each location. Phenotypic distributions were tested for Castle-Hardy-Weinberg (C-H-W) equilibrium by a chi-square test to determine homogeneity within samples (Swofford and Selander 1981). Mean heterozygosity (both direct-count and Hardy-Weinberg unbiased estimate) was computed for each population sample analyzed. Direct-count is the proportion of individuals sampled that are actually heterozygous and the Hardy-Weinberg unbiased estimate is an unbiased estimate (Nei 1978) based on conditional expectations. Inter-stock homogeneity was evaluated by using a G-test based on maximum likelihood ratios (Sokal and Rohlf 1981). Chi-square contingency analyses of heterogeneity among populations at all loci were calculated to analyze inter-stock heterogeneity between the lake whitefish spawning stocks. Phylogenetic trees based on Nei's unbiased genetic distance (1978) were constructed using the unweighted pair group method of arithmetic averages (UPGMA) cluster method. The UPGMA method was chosen because it was the preferred clustering method for constructing dendrograms for electrophoretic data from lake whitefish studies (Imhof et al. 1980; Casselman et al. 1981; Ihssen et al. 1981).

Waples' maximum likelihood method (Waples 1988) was used to estimate genotypic frequencies from the MDH-B1,2 isoloci. This method uses the distribution of phenotypic scores to estimate the population allele

frequencies for the individual gene loci (P and Q) with the highest probability of producing the observed phenotypic distribution.

II) MORPHOLOGY

Twenty-one morphometric measurements and nine meristic counts (Fig. 2) were taken from each fish caught in 1989 following the methodology outlined by Reist (1985). The fish were partially thawed in order to facilitate accurate counts and measurements. Straight line measurements (to the nearest 1.0 mm) parallel to the long axis of the body on the left side of the fish were taken using a measuring board. All other measurements were taken with digital calipers graduated to 0.1 mm. Gill raker counts and gill arch measurements were taken from the first right gill arch (Reist 1985). Morphometric variables measured were as follows: standard length (STL), fork length (FRL), preorbital length (POL), orbital length (OOL), postorbital length (PSL), trunk length (TTL), dorsal length (DOL), lumbar length (LUL), anal length (ANL), caudal peduncle length (CPL), head depth (HDD), body depth (BDD), caudal peduncle depth (CPD), interorbital width (IOW), maxillary length (MXL), maxillary width (MXW), pectoral fin length (PCL), pelvic fin length (PVL), adipose fin length (ADL), gill raker length (GRL), and lower arch length (LAL) (Lindsey 1962; Bodaly 1979) (Fig. 2). Two other variables which were deemed useful for discrimination of stocks (Reist, pers. comm.; Kliewer 1970), gill raker space (GRS) and fork size

(FRS), were not measured directly but were calculated from available measurements. Fork size (FRS) was calculated by subtracting FRL from STL. Meristic counts were made with the naked eye, on the left side of the fish where possible. Meristic counts were as follows: lateral line scales (LLS), supra pelvic scales (SPS), scales above lateral line (ULS), dorsal fin ray count (DRC), anal fin ray count (ARC), pectoral fin ray count (PRC), pelvic fin ray count (VRC), upper gill raker count (UGR) and lower gill raker count (LGR) (Hubbs & Lagler 1974; Lindsey 1962) (Fig. 2). Gill raker space (GRS) was obtained by dividing LAL by LGR. The biological variables sex (SEX), maturity of fish (MAT) and gonad weight (GWT) were also recorded. Kristofferson (1978) found no evidence of sexual dimorphism in Lake Winnipeg whitefish, so no attempt was made to distinguish between sexes during morphological analyses.

A temporal comparison was conducted between 1989 fish and 1975 fish (Kristofferson 1978) from the Little Playgreen Lake, Big Black River and Grand Rapids sites, the only sites for which there were morphological data sets for both sampling periods. This resulted in using two sampling sites not directly affected by LWR (Big Black River and Grand Rapids) and an experimental site which was directly affected by LWR (Little Playgreen Lake). There were no 1975 fish extant, so the 1989 data set was adjusted with extra measurements and counts used by Kristofferson (1978) in the 1975 data set. This was done because Kristofferson (1978) used

measurements that were not taken on the 1989 fish. Eight extra measures and counts were taken on the 1989 fish, as follows: pectoral fin origin (PCO), dorsal to adipose distance (DLAD), pelvic fin origin (VLO), ventral to adipose distance (VLAL), head depth (HD), body width (BDWD), anal depth (AND), caudal peduncle scale count (PNRS) (Kristofferson 1978). Adipose fin length (ADL) was omitted because the subjective nature of the measurement and of the high probability of researcher bias in the measurement technique. To facilitate a a direct comparison, some of the 1989 measurements were combined to conform to the twenty-seven measurements and counts used in the 1975 data set. Since this part of this study is a comparison over time, there is the possibility that there might be a bias in the data due to researcher effect, i.e. due to differences in techniques between researchers when more than one researcher is involved in morphological data collection. However, this could not be assessed as the 1975 samples and/or the researcher who conducted the measurements on these fish were unavailable. To decrease potential technique bias, the methods used for the 1975 measurements and counts were applied to the 1989 fish samples.

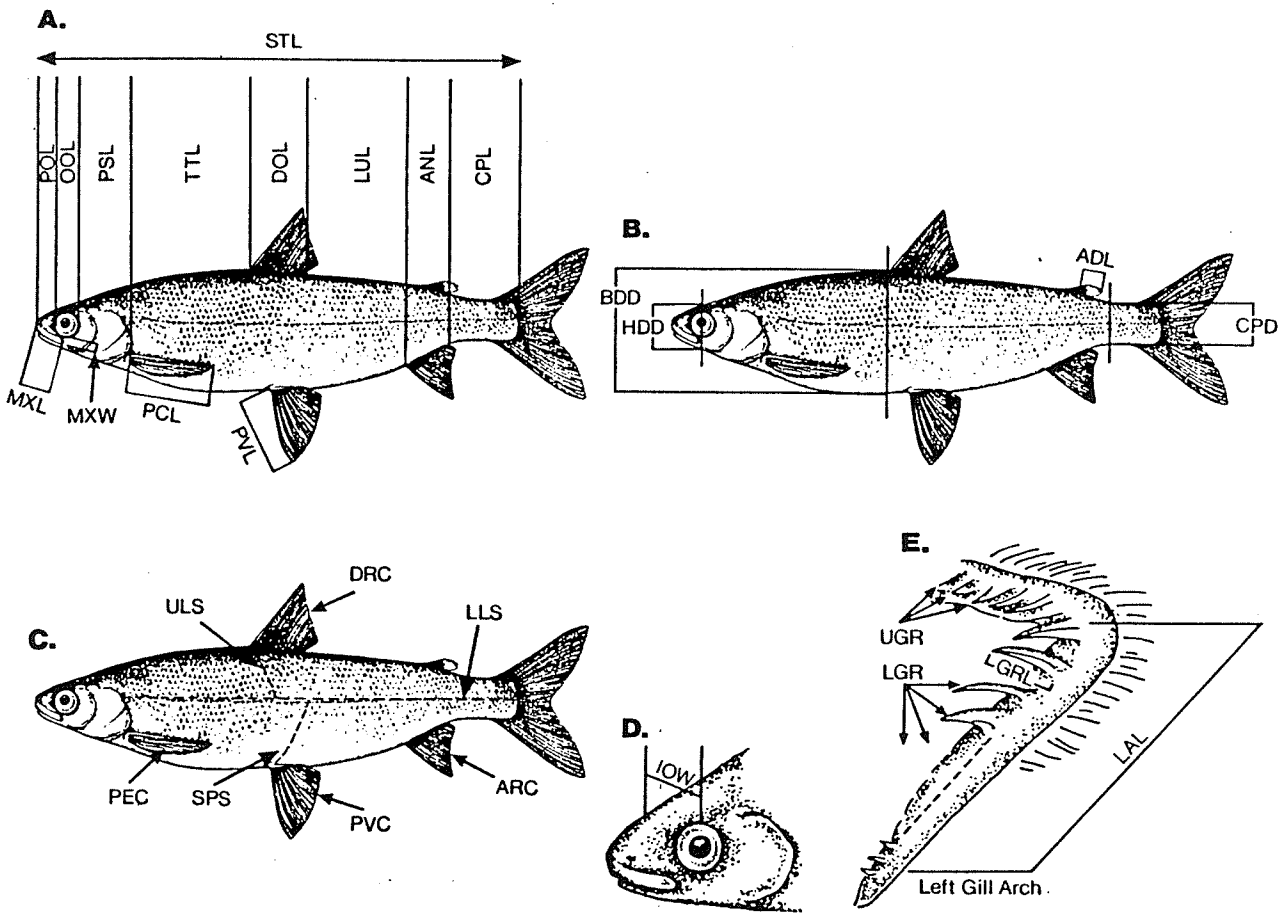

Fig. 2 Morphometric measurements and meristic counts taken on lake whitefish sampled in 1989 (taken from Reist pers. comm.)

Morphological Analyses

Morphological inter-stock distinctiveness of the 1989 samples was examined by applying canonical discriminant function analysis (CDFA) and discriminant function analysis (DFA) separately to the morphological data sets (residual, ratio, meristic and combined meristic/residual data sets). Canonical discriminant function analysis yields a coefficient for each variable that ranks its importance in discriminating the groups. Given that the means do differ, CDFA attempts to determine the degree of difference (Barnes 1990). Discriminant function analysis compares groups by creating artificial variables which are a linear function of each pair of original variables for each group (Barnes 1990). Each individual fish is then classified to the group it most closely resembles morphologically.

Whitefish exhibit allometric growth (Gould 1966; Thorpe 1976) which can confound comparisons of morphometric measurements between fish of different size. Since body shape rather than actual body size was being analyzed, the potential allometric size variation between and within groups had to be corrected. This was accomplished by using a regression technique that computed residuals by adjusting for the size covariate using a pooled within group regression slope (Thorpe 1975; Reist 1985; Barnes 1990). Calculations were computed using the Statistical Analysis System Version 6 (SAS Inc. 1985). Reist (1985) stated that ratios, while not as highly recommended as residuals, could be used to suppress potential allometric

variation. Casselman et al. (1981) used ratios of body measurements of lake whitefish on fork lengths to remove variations of fish size within each sample. Barnes (1990) used both techniques in a morphological study on lake whitefish and concluded there was not a very notable difference between the two techniques. Meristic data were not normally distributed because meristics are discrete variables (Blouw et al. 1988). Meristic counts were left unadjusted and were analyzed using non-parametric univariate tests since they are discrete variables. Canonical discriminant function analysis (parametric test) was also used on the meristic data because multivariate tests have been found to be extremely robust for even non-normal data (Mardia 1971; Sneath & Sokal 1973). Morphometric data were normally distributed and were tested for normality using parametric univariate techniques (Bartlett's test). Analyses were conducted separately on meristic, residual morphometric and combined residual morphometric and meristic data sets. Ratio adjusted morphometric data results were visually compared to the residual adjusted morphometric data results to examine congruence of values.

Univariate analysis of variance (ANOVA) was used to compare the variation among samples for individual characters. Discriminant function analysis (DFA) was performed *a posteriori* on the six groups to reclassify individual fish into their respective spawning stocks. Meristic, residual morphometric, ratio morphometric and combined residual morphometric and

meristic data sets were analyzed by DFA. There were differences in sample sizes among spawning groups in the morphological DFA analyses because only individual fish for which there were complete sets of observations were used (Pimental 1979; Brown 1989). Discriminant function analyses are presented in summary of reclassification tables (Table 9) and canonical discriminant function analyses of the 1989 sample group means are presented in 3-dimensional graphical comparisons (Figs. 5a-d).

The same analyses (CDFA and DFA) that were performed for the 1989 samples were conducted on the temporal comparison of 1975 fish to 1989 fish, with the exception that residuals were not calculated for morphometric variables for the temporal comparison. Duncan's multiple range test (alpha= 0.05) was used to determine statistically significant groupings for canonical discriminant function analyses for both 1989 and 1975 samples. Ratio morphometric values were used because they were not significantly different from residual morphometric values calculated for the 1989 whitefish data set and ratio values were easier to work with when the 1989 data set was being standardized to the 1975 measurements. An ANOVA between 1989 and 1975 results was conducted separately on 8 meristic counts and 18 morphometric measurements for each of the Grand Rapids, Big Black River and Little Playgreen Lake samples in order to determine if morphological change had occurred since the construction of LWR. Grand Rapids and Big Black River were used as control sites since

they were the sites farthest away from the direct influence of Two Mile Channel, and Little Playgreen Lake was used as an experimental site since it was downstream of Two Mile Channel.

RESULTS

I) GENETICS

Genetic variation in the 1989 samples was found at sixteen of the thirty-six loci screened. Eight of these were not used for subsequent analysis: three loci had unreliable genetic models (IDHP-3 and MEP-3,4); and five loci (AAT, EST, GPI-1, PGM-2, SOD) expressed rare alleles (average frequencies over all populations <0.01). Variation found at the remaining eight loci fit existing genetic models (Vuorinen 1984). Genetic variation in the 1975 sample was found at six of the loci screened. Observed phenotypic frequency distributions of MDH-B1,2, G3PDH-1, IDHP-4 and LDH-B2 were in compliance with expected Castle-Hardy-Weinberg distributions for both pre-LWR and post-LWR samples, with the exception of the pre-LWR Warren Landing sample G3PDH-1 whose allele frequencies violated equilibrium frequencies. Waple's (1988) maximum likelihood (ML) approach was used to analyze the genetic variation for the duplicated isoloci MDH-B1,2 and it revealed the presence of at least three distinct genetic

stocks. However, this quantitative approach based on phenotypic distribution patterns could not be used since inter-group allelic frequency comparisons of the individual gene loci (p and q) revealed too much ambiguity in the selection of either the p or q loci. MDH-B1,2 were therefore treated as duplicated loci (isoloci) with identical allelic frequencies at both loci. These isoloci have gene products with identical electrophoretic mobilities and are assumed to be inherited disomically (independent assortment) (Waples 1988). If both loci are heterozygous, then five different patterns of three bands of symmetric density will be observed (Leary & Booke 1990). Genotype identification is difficult but the frequency of alleles can be inferred from the banding pattern if the enzyme is treated as one encoded by a single locus represented by four copies (tetrasomic locus) (Leary & Booke 1990). MDH-B1 and MDH-B2 loci were separated and treated as two separate dimeric loci for calculations using BIOSYS-1 (Swafford & Selander 1981).

Phenotypic proportions observed for MDH-B1,2 differed significantly ($G= 9.92$, d.f.= 3, $P= 0.019$) among the subpopulations being compared in 1975. Samples were pooled *a posteriori* into similar groupings as suggested by Kristofferson and Clayton (1990) in order to determine which stocks differed. Little Playgreen Lake and Warren Landing samples were pooled together and the Grand Rapids and Big Black River samples were pooled together based on similarity of allele frequencies and on geographical

proximity of the samples (Fig. 3). Among-group phenotypic differences at the MDH-B1,2 loci for the 1975 pooled samples were significant (G= 6.68, d.f.= 1, P= 0.009).

Phenotypic proportions observed for MDH-B1,2 differed significantly (G= 14.24, d.f.= 5, P= 0.014) among the subpopulations being compared in 1989 (Table 3). Samples were pooled *a posteriori* on the basis of the 1975 samples in order to determine if any stock groupings differed. These "pooled" groupings were similar to results obtained for the pre-development (1975) samples. The samples taken in 1989 indicated that lake whitefish from Warren Landing and Little Playgreen Lake had similar MDH-B1,2 phenotypic proportions (G= 0.32, d.f.= 1, P= 0.948) and comprised one grouping (Fig. 3). Two additional 1989 samples, Gunisao River and Two Mile Channel were grouped with samples with similar allele frequencies from Big Black River and Grand Rapids. Inter-group differences were significant (G= 12.29, d.f.= 1, P= 0.001).

Allele frequencies at the MDH-B1,2 loci for the grouped samples were not significantly different between 1975 and 1989 (Warren Landing and Little Playgreen group, 1975 vs. 1989: G = 0.82, d.f.= 1, P= 0.381; Grand Rapids and Big Black River group, 1975 vs. 1989: G = 2.68, d.f.= 1, P= 0.116). IDHP-4, G3PDH-1 and LDH-4 allele frequencies were not significantly different among samples (for both pre-LWR and post-LWR sampling periods) and between samples (1975 and 1989) at the 5% level .

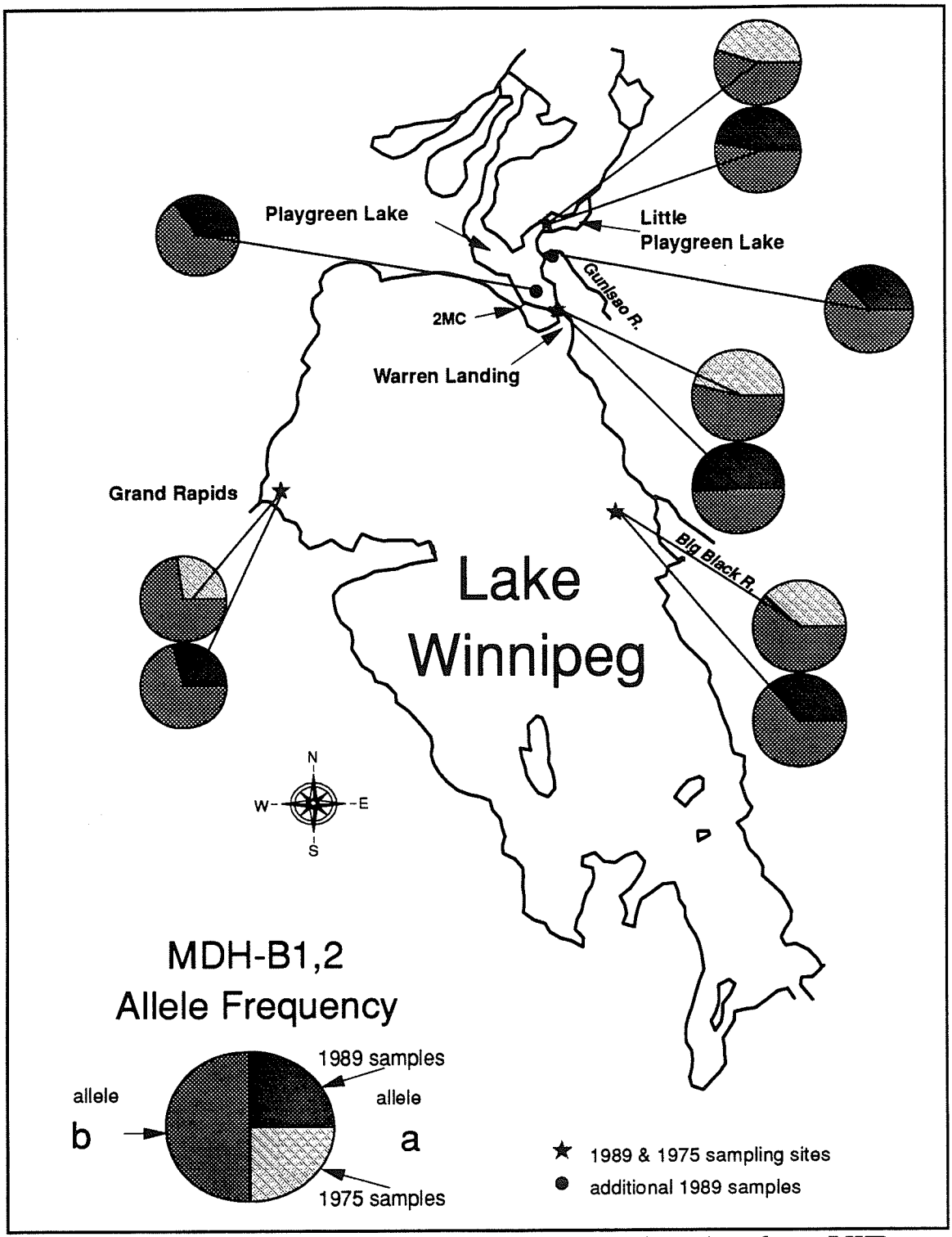

Fig. 3. MDH-B1,2 allelic frequencies for post-LWR (1989) and pre-LWR (1975) samples of lake whitefish caught in Lake Winnipeg and the outlet lakes.

Table 3. Sample sizes and allele frequencies at five polymorphic loci examined in six lake whitefish samples caught in 1989 from northern Lake Winnipeg, Playgreen Lake and Little Playgreen Lake.

	Population					
Locus and alleles	Little Playgreen L. n=50	Warren Landing n=48	Grand Rapids n=50	Big Black River n=50	Two Mile Channel n=50	Gunisao River n=50
G3PDH-1						
N	50	49	50	50	50	50
a	.220	.214	.200	.250	.210	.230
b	.320	.184	.220	.290	.300	.280
d	.460	.602	.580	.460	.490	.490
IDHP-4						
N	50	48	50	50	47	50
a	.850	.865	.790	.880	.851	.740
c	.150	.135	.210	.120	.149	.260
LDH-B2						
N	50	48	50	50	50	50
a	.040	.042	.090	.090	.100	.050
b	.960	.958	.910	.910	.900	.950
MDH-B1,2						
N	50	48	50	50	50	50
a	.470	.510	.290	.350	.350	.370
b	.530	.490	.710	.650	.650	.630

Table 4. Sample sizes and allele frequencies at five polymorphic loci examined in four lake whitefish samples caught in 1975 from northern Lake Winnipeg and Little Playgreen Lake (IDHP-4 allele frequencies obtained from gels run by Kristofferson (1975) because only muscle tissues were available for analysis).

	Population			
Locus and alleles	Little Playgreen L. n=52	Warren Landing n=51	Grand Rapids n=54	Big Black River n=55
G3PDH-1				
N	50	51	53	55
a	.250	.157	.226	.309
b	.270	.216	.245	.245
d	.480	.627	.528	.445
IDHP-4				
N	24	24	36	22
a	.896	.854	.944	.864
c	.104	.146	.056	.136
LDH-B2				
N	51	51	54	55
a	.020	.020	.028	.036
b	.980	.980	.972	.964
MDH-B1,2				
N	50	51	53	54
a	.450	.461	.274	.389
b	.550	.539	.726	.611

G3PDH-3 allele frequencies were not significantly different among samples at the 5% level for the post-LWR sampling period.

Genetic distance relationships are shown diagrammatically in Figures 4a-b, where samples are clustered in a UPGMA dendrogram on the basis of Nei's (1978) unbiased genetic distance. The grouping pattern observed was similar to that found by MDH-B1,2 allele frequency analysis. These genetic distances are similar to those found between sympatric stocks of lake whitefish by Kristofferson (1978), Casselman et al. (1981) and Ihssen et al. (1981).

Mean heterozygosity (H) ranged from 0.106 to 0.114 for the 1989 samples (Table 5) and from 0.114 to 0.148 for the 1975 samples (Table 6).

Chi-square contingency table analyses between spawning groups indicated that there were significant inter-group differences in heterogeneity for both 1989 (X^2= 110.31; df= 85, P= 0.033) and 1975 (X^2= 49.43; df= 21, P= 0.001) samples when all polymorphic loci were analyzed as a whole. Inter-group differences were significant at the MDH-B1,2 loci for both 1989 (X^2= 14.46; df= 5, P= 0.013) and 1975 (X^2= 9.68; df= 3, P= 0.022) samples (Tables 7-8).

Table 5. Genetic variability present at 36 loci for all six populations of lake whitefish sampled in 1989 (standard errors in parentheses).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	Hardy-Weinberg estimated
Little Playgreen L.	50.0	1.4	33.3	.106	.106
	(.0)	(.1)		(.033)	(.032)
Warren Ldn	47.6	1.4	36.1	.114	.114
	(.5)	(.1)		(.035)	(.032)
Gunisao R.	49.6	1.4	36.1	.112	.111
	(.3)	(.1)		(.033)	(.032)
Two Mile Channel	50.0	1.3	30.6	.111	.105
	(.0)	(.1)		(.035)	(.032)
Grand Rapids	50.0	1.4	38.9	.114	.109
	(.0)	(.1)		(.034)	(.031)
Big Black R.	50.0	1.4	36.1	.112	.106
	(.0)	(.1)		(.034)	(.032)

* A locus was considered polymorphic if the frequency of the most common allele does not exceed .99

Table 6. Genetic variability present at 14 loci for lake whitefish populations sampled in 1975 (standard errors in parentheses).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	Hardy-Weinberg estimated
Little Playgreen L.	51.5	1.5	49.2	.127	.124
	(.2)	(.2)		(.063)	(.061)
Warren Ldn.	51.0	1.5	49.2	.118	.123
	(.0)	(.2)		(.054)	(.057)
Grand Rapids	53.8	1.5	49.2	.112	.109
	(.1)	(.2)		(.055)	(.054)
Big Black R.	53.4	1.5	49.2	.167	.163
	(1.0)	(.2)		(.064)	(.062)

* A locus was considered polymorphic if the frequency of the most common allele does not exceed .99

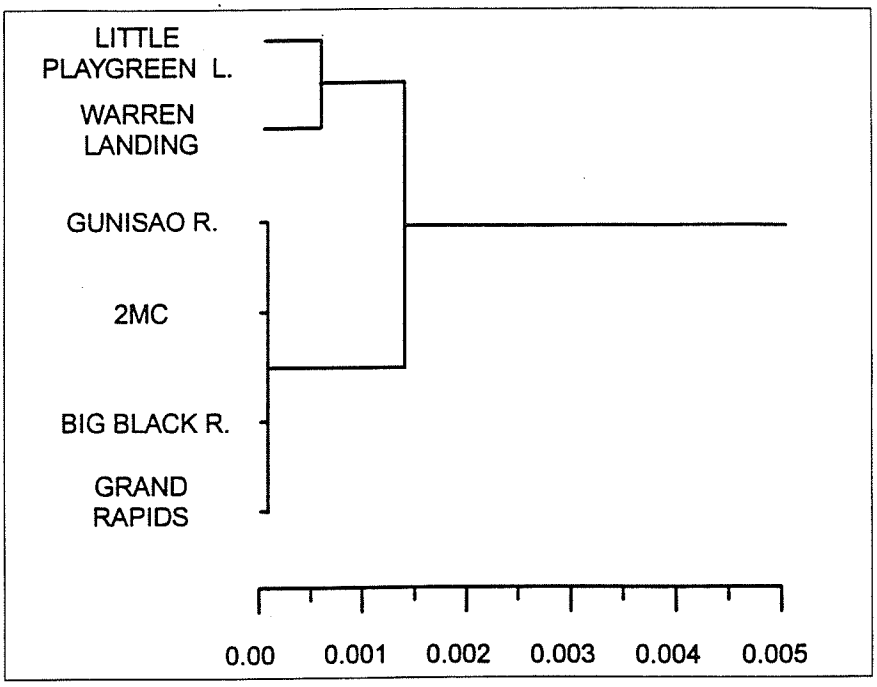

Fig. 4a. Dendrogram, constructed by UPGMA method, showing the genetic relatedness among samples. Nei's genetic (Nei 1978) distances were based on allele frequencies at 36 loci in lake whitefish sampled during 1989.

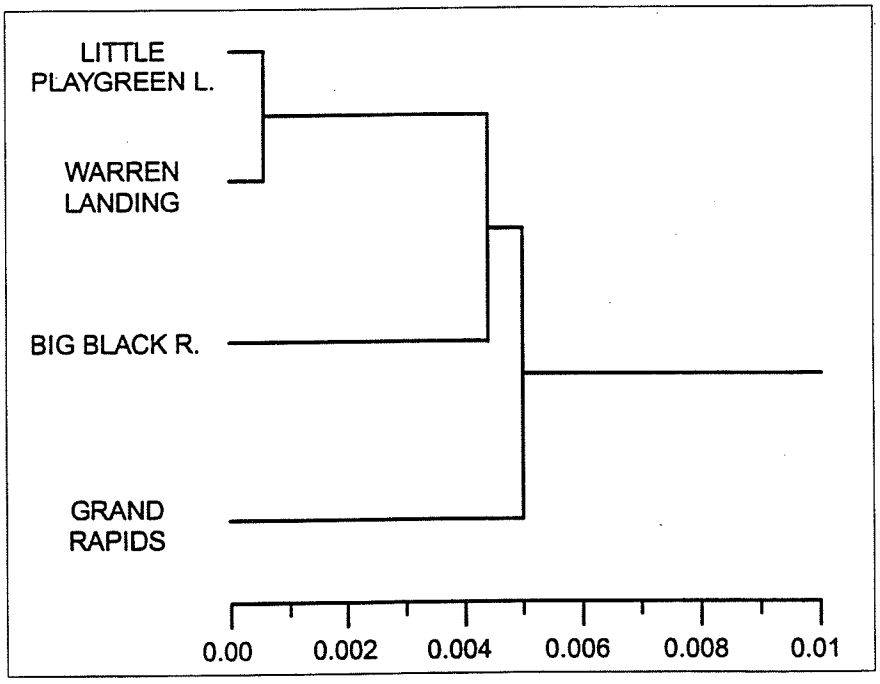

Fig. 4b. Dendrogram, constructed by UPGMA method, showing the genetic relatedness among samples. Nei's genetic distances (Nei 1978) were based on allele frequencies at 14 loci in lake whitefish sampled during 1975.

Table 7. Chi-square contingency table analysis of heterogeneity among populations of lake whitefish sampled in 1989.

Locus	No. of alleles	Chi-square	D.F.	P
G3PDH-1	3	9.49	10	.485
G3PDH-3	2	6.60	5	.251
IDHP-4	2	9.01	5	.108
LDH-B2	2	8.45	5	.132
MDH-B1	2	14.46	5	.012
MDH-B2	2	14.46	5	.012
MEP-1	2	4.06	5	.539
MEP-2	2	4.06	5	.539
AAT-1	2	5.21	5	.389
EST	2	10.58	5	.060
GPIB1	2	3.97	5	.553
PGM-2	2	4.14	5	.529
SOD-1	2	5.07	5	.407
(Totals)		99.63	70	.033

Table 8. Chi-square contingency table analysis of heterogeneity among populations of lake whitefish sampled in 1975.

Locus	No. of alleles	Chi-square	D.F.	P
G3PDH-1	3	9.68	6	.138
LDH-B2	2	0.80	3	.847
MDH-B1	2	9.67	3	.021
MDH-B2	2	9.67	3	.021
(Totals)		29.86	15	.020

II) MORPHOLOGY

Residual Adjusted Morphometric Data Set (1989)

Univariate analysis of variance (ANOVA) indicated that all characters, except DOL, differed significantly among the six samples. The first four canonical discriminant functions based on morphometric data were significant (P=0.0001) and respectively accounted for 66, 13, 12 and 7% of the variability (Appendix IIa). Measurements that provided the best discrimination were: head depth (HDD), body depth (BDD), preorbital length (POL), postorbital length (PSL) and trunk length (TTL) (Fig. 5a). Little Playgreen fish were distinguished by large body proportions. The Grand Rapids and Big Black River fish had the smallest body proportions.

The first canonical function separated Little Playgreen Lake, Gunisao River, Warren Landing - Two Mile Channel (2MC) and Big Black River - Grand Rapids fish on the basis of contrasts between high loadings of BDD and POL and low loadings of HDD, PSL and TTL. The second function separated Big Black River from Grand Rapids and Warren Landing from 2MC on the basis of contrasts between high loadings of HDD and low loadings for OOL. Little Playgreen Lake, 2MC, Warren Landing and Gunisao R. fish had large body proportions and small heads whereas Grand Rapids and Big Black River fish had small body proportions and large heads. An *a posteriori* discriminant function analysis, using all discriminant

functions, categorized 89.8% to 95.7% of the fish into their appropriate groupings (Table 9). A dendrogram based on Mahalanobis distance (D^2), using all variables, illustrated that three clusters were present: Big Black River and Grand Rapids formed one cluster, Warren Landing and Gunisao River formed another cluster and 2MC and Little Playgreen Lake formed the third cluster (Fig. 6). Also, this dendrogram indicated a closer relationship among the 2MC-Little Playgreen Lake-Warren Landing-Gunisao River samples than with two Lake Winnipeg samples (Grand Rapids-Big Black River).

Ratio Morphometric Data Set

Univariate analysis of variance (ANOVA) indicated that POL, OOL, PSL, TTL, LUL, ANL, CPL, HDD, BDD, CPD, IOW, MXL, MXW, ADL, LAL, GRL and GRS differed significantly among the six samples. The first three morphometric canonical discriminant functions were highly significant and respectively accounted for 61% (P=0.0001), 19% (P=0.0001), and 13% (P=0.0012) of the variability (Appendix IIb). The first canonical function separated the Little Playgreen Lake, Warren Landing - Gunisao River, 2MC, Grand Rapids and Big Black River samples on the basis of high POL and BDD loadings and low HDD loadings. The second canonical function separated Warren Landing from Gunisao River on the basis of high OOL

loadings and low HDD and POL loadings. The findings were similar to those observed for residual morphometrics.

An *a posteriori* discriminant function analysis correctly assigned between 83.3 - 94% of the fish into their original groupings (Table 9). A dendrogram of Mahalanobis distances (D^2), using all variables, illustrated that Warren Landing and Gunisao River were very similar and that the Big Black River-Grand Rapids cluster was very different from the other four samples (Fig. 6).

Meristic Data Set (1989)

Univariate analysis of variance (ANOVA) indicated that only ULS, DRC and UGR differed significantly. No significant differences were found among all the other meristic counts.

The first two canonical discriminant functions were significant for meristics and these two functions respectively accounted for 51% (P=0.0001) and 34% (P=0.016) of the variability (Appendix IIb). The best discriminators were lateral line scales (LLS), pectoral ray counts (PRC), upper gill raker counts (UGR), anal ray counts (ARC) and dorsal ray counts (DRC). The first canonical function separated the Warren Lnd., Big Black-Grand Rapids grouping from the Little Playgreen-2MC-Gunisao River grouping on the basis of high UGR and LLS loadings. The second canonical function separated Gunisao River from the Little Playgreen-2MC cluster on the basis

of high LLS and DRC loadings (Fig. 5c). Big Black River, Grand Rapids and Warren Landing fish had more lateral line scales and upper gill rakers than Gunisao River, 2MC and Little Playgreen Lake fish.

An *a posteriori* discriminant function analysis, using all discriminant functions correctly assigned 67% of the Big Black fish, 66% of the Gunisao fish, 61% of the Warren Landing fish and 46% of the Little Playgreen fish into their original groupings. The 2MC fish were reassigned as either Warren Landing, Gunisao or Big Black River fish. The Grand Rapids fish were mostly reclassified as either Big Black River fish or Gunisao River fish (Table 9). A dendrogram of Mahalanobis distance (D^2), using all meristic variables, indicated a similar clustering pattern in meristics as seen in the residual morphometric data except that the distances were an order of magnitude smaller for meristics (Fig. 6).

Combined Residual Morphometric and Meristic Data Set (1989)

The combined data set was influenced more by the morphometric results than the meristic results (Appendix IIc). Univariate analyses indicated that POL, OOL, PSL, TTL, LUL, ANL, CPL, HDD, BDD, CPD, IOW, MXL, MXW, PVL, ADL, GRL, LAL, LLS, PRC and UGR differed significantly (P=0.001) among the 1989 groups. Differences in morphology, as described by the first four canonical discriminant axes, accounted for 96% of the variability. The first canonical function accounted for 61% of the

variance and indicated significant differences (P=0.0001) among all groups except between Warren Landing and 2MC fish and between Big Black River and Grand Rapids fish on the basis of contrasts between high loadings for LAL and low loadings for GRS and LGR. Function 2, accounting for 14% of the variation, discriminated between Warren Landing fish and 2MC fish (P=0.0001) on the basis of high loadings for LAL and low loadings for GRS, LGR, CPD and LAL. The third canonical function was needed to reveal significant differences (P=0.0001) between Big Black River fish from Grand Rapids fish on the basis of high GRS and LGR loadings and low LAL loadings (Fig. 5d).

An *a posteriori* reclassification correctly assigned 97.8 - 100% of the fish into their original groupings (Table 9). A dendrogram based on Mahalanobis distance (D^2), using all variables, illustrated that the Big Black River and Grand Rapids cluster differed from the rest of the groupings, Warren Landing and Gunisao River samples clustered together, and Little Playgreen Lake and 2MC samples were grouped together (Fig. 6).

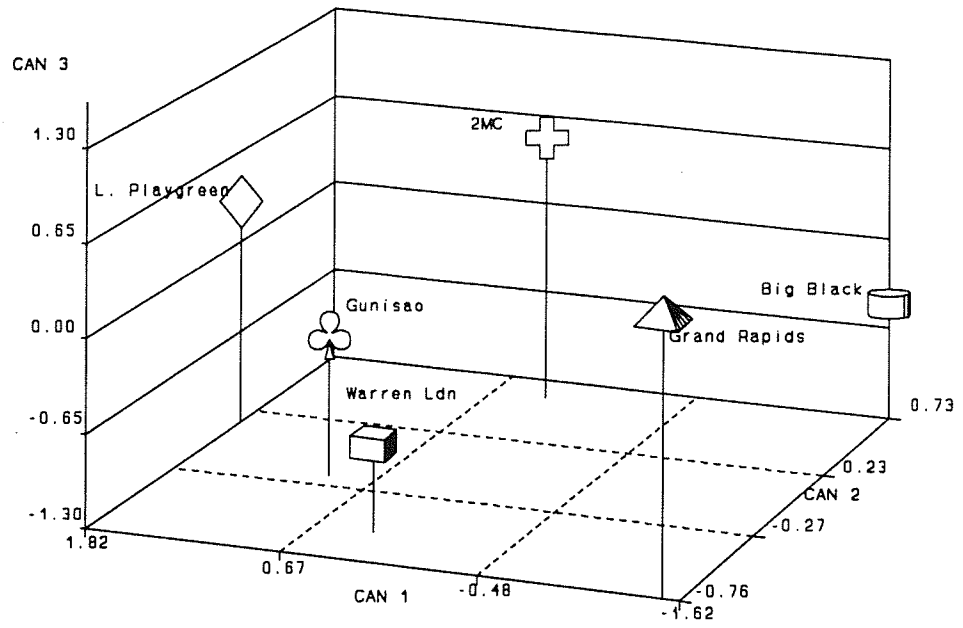

Fig. 5a. Canonical discriminant analysis centroid plot of residual morphometric values for 1989 samples.

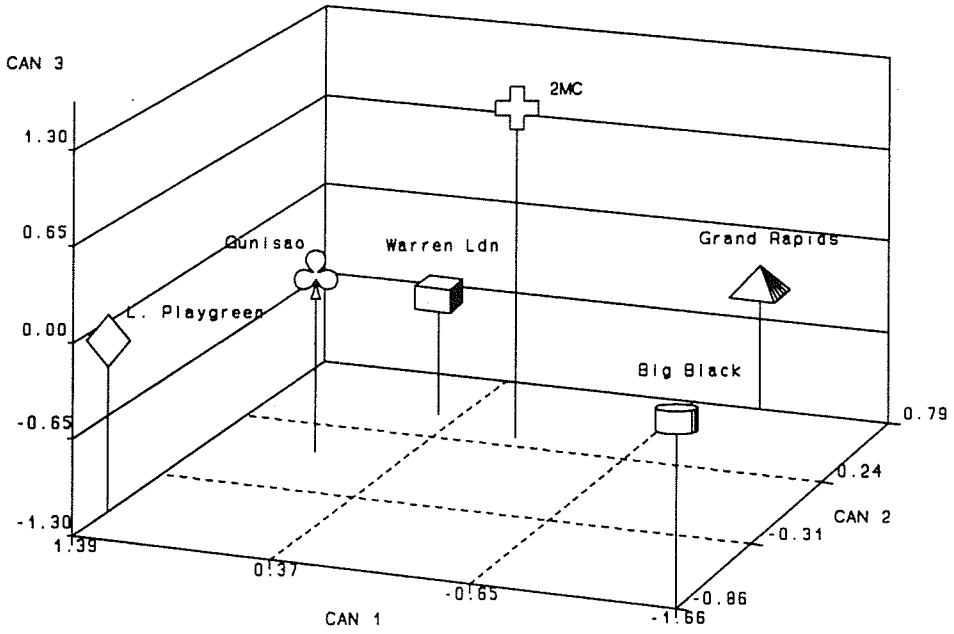

Fig. 5b. Canonical discriminant function analysis centroid plot of ratio morphometric values for 1989 samples.

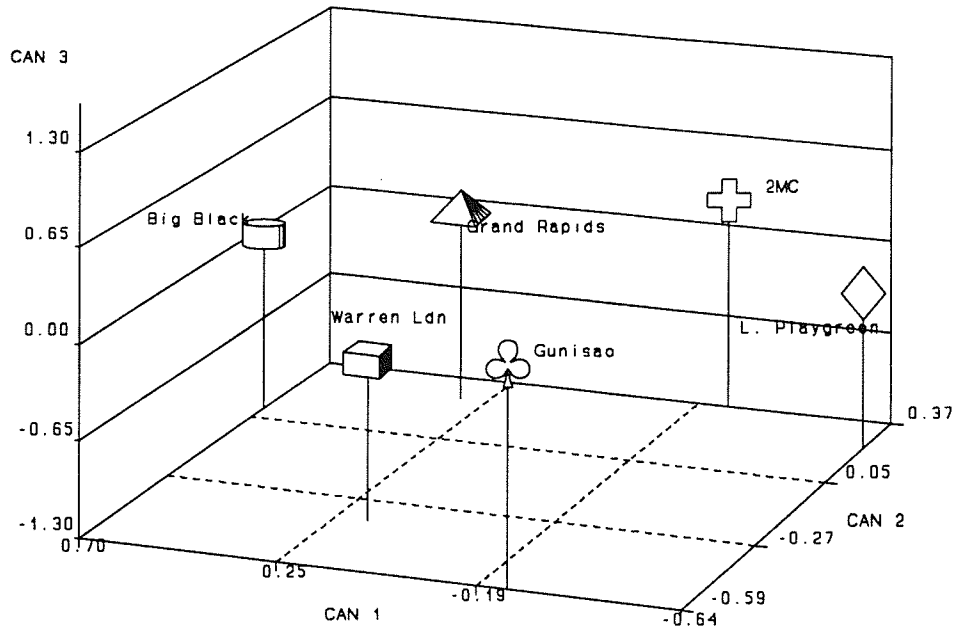

Fig. 5c. Canonical discriminant function analysis centroid plot of meristic values for 1989 samples.

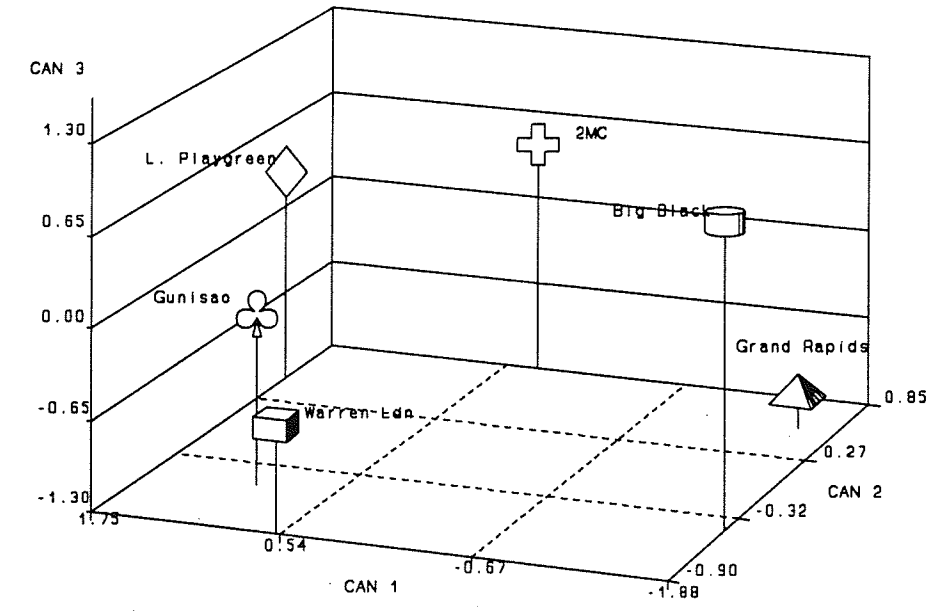

Fig. 5d. Canonical discriminant analysis centroid plot of combined residual morphometric values and meristic values for 1989 samples.

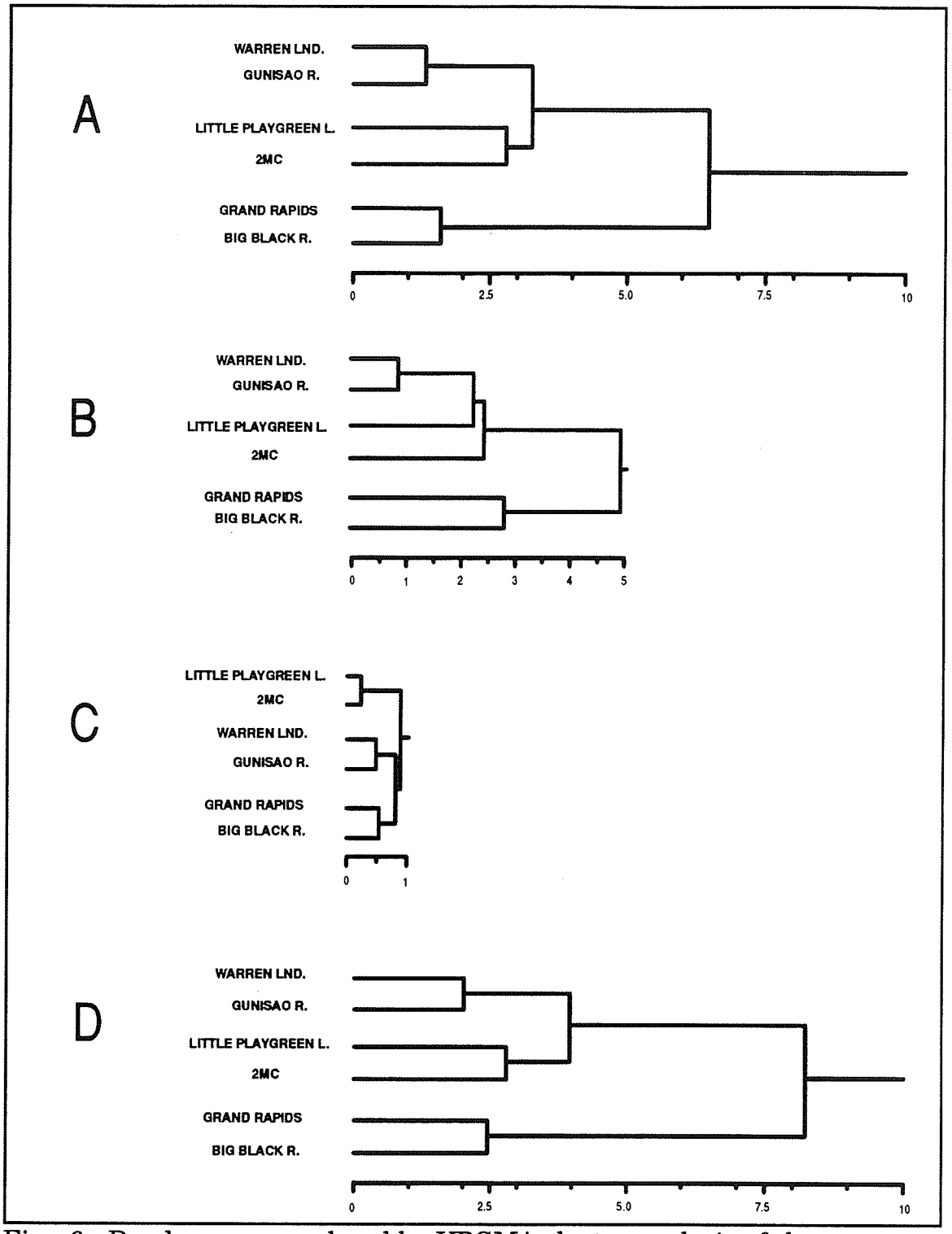

Fig. 6. Dendrograms produced by UPGMA cluster analysis of the Mahalanobis distances for 1989 lake whitefish samples for: A) residual morphometrics; B) ratio morphometrics; C) meristics and; D) combined residual morphometrics and meristics.

Table 9. Discriminant function reclassification of 1989 lake whitefish phenotypic samples.

	STOCK	% CORRECTLY CLASSIFIED	NUMBER OF OBSERVATIONS CLASSIFIED INTO GROUP						
			Warren Landing	Gunisao River	Little Playgreen	Two Mile Channel	Big Black River	Grand Rapids	Total
Residuals									
	Warren Landing	95.56	43	1	0	1	0	0	45
	Gunisao River	93.75	0	45	3	0	0	0	48
	Little Playgreen L.	95.12	0	1	39	1	0	0	41
	Two Mile Channel	93.88	0	2	1	46	0	0	49
	Big Black River	89.80	2	1	0	0	44	2	49
	Grand Rapids	95.74	0	0	0	1	1	45	47
Ratios									
	Warren Landing	91.67	44	1	1	0	1	1	48
	Gunisao River	90.00	2	45	1	1	0	1	50
	Little Playgreen L.	85.71	1	2	42	2	0	2	49
	Two Mile Channel	94.00	1	2	0	47	0	0	50
	Big Black River	92.00	0	1	0	1	46	2	50
	Grand Rapids	83.33	1	1	1	1	4	40	48
Meristics									
	Warren Landing	60.87	28	9	2	0	7	0	46
	Gunisao River	66.00	6	33	2	0	9	0	50
	Little Playgreen L.	46.00	10	12	23	0	5	0	50
	Two Mile Channel	10.42	12	10	6	5	14	1	48
	Big Black River	67.35	11	4	1	0	33	0	49
	Grand Rapids	4.17	5	12	9	1	19	2	48
Combined									
	Warren Landing	100.00	43	0	0	0	0	0	43
	Gunisao River	97.92	0	47	1	0	0	0	48
	Little Playgreen L.	100.00	0	0	41	0	0	0	41
	Two Mile Channel	97.83	0	0	0	45	0	1	46
	Big Black River	97.92	0	0	0	0	47	1	48
	Grand Rapids	100.00	0	0	0	0	0	44	44

1975 - 1989 MORPHOLOGICAL COMPARISON

The study conducted by Kristofferson (1978) and the present study indicate that there were significant inter-stock differences among lake whitefish groups during 1975 and during 1989. Canonical discriminant function analysis conducted on both 1975 and 1989 data sets (Duncan's Multiple Range Test) differentiated among all 1989 and 1975 samples for both morphometric (ratios) and meristic data sets (alpha= 0.05) (Figs. 7a-b). Scatter plots of the first and second canonicals for meristics and

morphometrics indicated that there was no change in variation between years for Little Playgreen Lake, Big Black River and Grand Rapids populations. Kristofferson (1975) and Kristofferson & Clayton (1990) indicated that the Big Black River fish were distinguished by relatively small body measurements such as pectoral fin length and head depth. Little Playgreen Lake fish were distinguished by large body measurements such as pectoral fin length, prepostorbital distance and head depth while Grand Rapids fish had the highest mean numbers of lateral line scales and caudal peduncle scales. Comparison of 1989 and 1975 samples indicated a significant (Duncan's Multiple Range Test) difference between years with narrow inter-stock clustering in the 1975 samples and wider inter-stock clustering in 1989 samples (Figs. 7a-b). Canonical discriminant function analysis (CDFA) of meristics indicated that the 1975 Grand Rapids sample overlapped with both the 1975 Little Playgreen Lake and the 1975 Big Black River samples. Whitefish sampled during 1975 had more lateral line scales, higher gill raker counts and fewer supra pelvic scales than whitefish sampled during 1989. Analysis of morphometrics indicated that the first canonical function accounted for 85% of the variation and was able to discriminate between all samples except the 1975 samples from Little Playgreen Lake and Grand Rapids. Fish collected during 1975 had smaller head depth measurements and larger body measurements than 1989 fish based on the first canonical axis. Whitefish collected during 1989 had

longer prepostorbital lengths (PPO), longer maxillary lengths (MXL), longer gill raker lengths (GRL), greater anal depths (AND) and shorter postorbital lengths (PSL). The second canonical axis accounted for 7% of the variation and indicated that Little Playgreen Lake and Grand Rapids fish (1989 & 1975) had smaller anal depths (AND), shorter maxillary lengths (MXL) and shorter postorbital lengths (PSL) than Big Black River fish.

The ANOVA conducted on 8 meristic counts and 18 morphometric measurements between 1989 and 1975 Little Playgreen Lake, Big Black River and Grand Rapids samples indicated that 8 morphometric variables and 4 meristic counts were significantly different for Little Playgreen Lake, 15 morphometric variables and 1 meristic count were significantly different for Big Black River and, 14 morphometric variables and 4 meristic counts were significantly different for Grand Rapids samples (Appendix IIIa-c). Variables that differed very significantly between years were, anal depth (AND) and caudal peduncle depth (CPD) for both the Big Black River and Grand Rapids samples (P=0.001), body width (BDWD) and postorbital length (PSL) for the Big Black River samples (P=0.001) and, scales above the lateral line (ULS) for the Little Playgreen Lake samples (P=0.001) (Appendix IIIa-c).

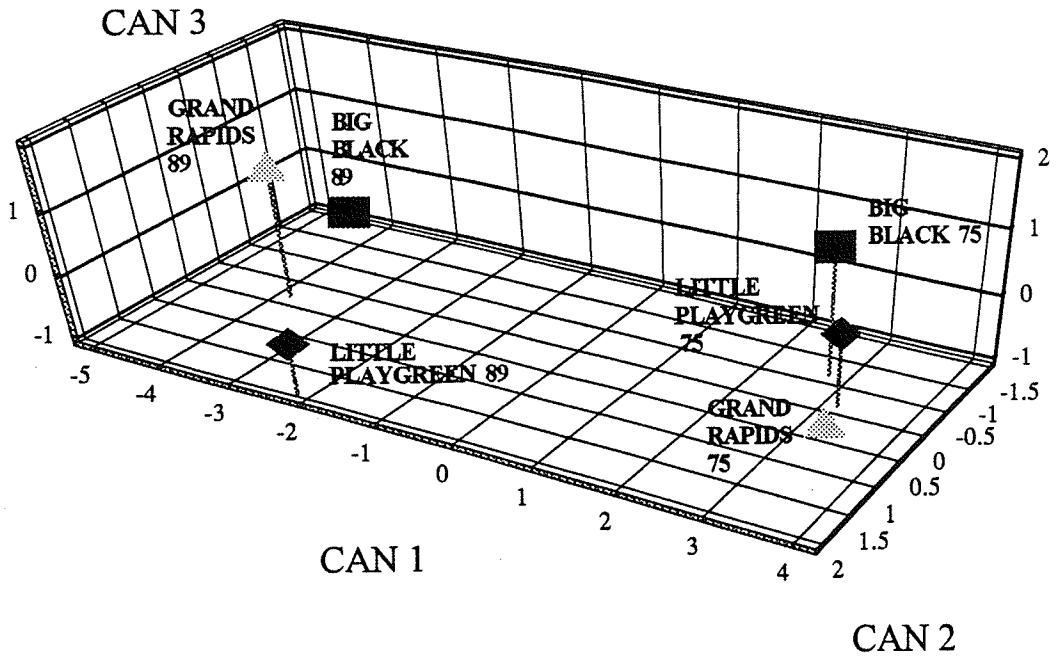

Fig. 7a. Canonical discriminant analysis centroid plot of three ratio morphometric canonical vectors for Little Playgreen Lake, Big Black River and Grand Rapids samples taken during 1975 and 1989.

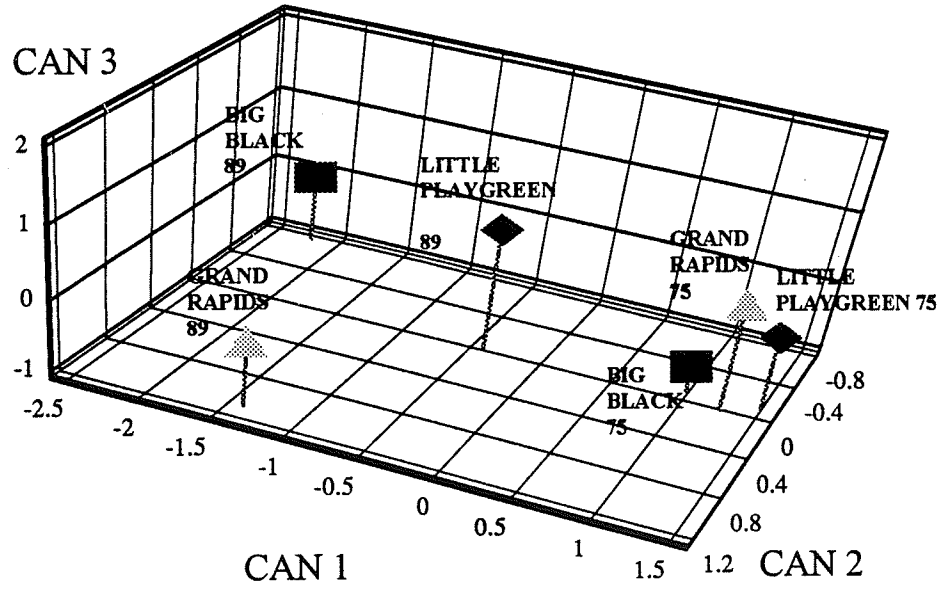

Fig. 7b. Canonical discriminant analysis centroid plot of three meristic canonical vectors for Little Playgreen Lake, Big Black River and Grand Rapids samples taken during 1975 and 1989.

DISCUSSION

I) GENETICS

A single locus is a very small portion of a genome but inferences about the amount of genetic differentiation among populations should be fairly reliable if they are based on at least 25 loci (Nei 1978) and in this study 36 loci were screened. LDH-B2 and G3PDH-1 loci were polymorphic for the populations of lake whitefish in this study, but only MDH-B1,2 differed among populations. Imhof et al. (1980) were able to differentiate among at least four stocks of lake whitefish in northern Lake Michigan using the enzymes MDH-B1,2 and LDH-B2. Casselman et al. (1981) were able to differentiate among at least two stocks of lake whitefish in Lake Huron using MDH-B1,2 and LDH-B2. G3PDH-1 was polymorphic in this study as well as in the studies conducted by Kristofferson (1978), Imhof et al. (1980) and Casselman et al. (1981) but no significant differences among populations of sympatric lake whitefish were observed for any of the above studies.

If allelic frequency differences among geographically separated samples are assumed to reflect patterns of relative reproductive isolation, then the observed MDH-B1,2 allelic frequency distributions indicated that prior to LWR (1975 samples), at least two different stocks of lake whitefish existed in the area of northern Lake

two stocks were 1) fish using sites in the north basin of Lake Winnipeg for spawning (Grand Rapids and Big Black River), and 2) fish using Little Playgreen Lake and Playgreen Lake for spawning (Warren Landing and Little Playgreen Lake fish). The 1975 Big Black River sample was pooled with the 1975 Grand Rapids sample because there was difficulty in scoring of the gels which led to a conservative estimate for the frequency of the b allele. The grouping of the 1975 Big Black River and Grand Rapids samples is supported by the morphological results which indicated that these two groups have a closer affinity to each other than the rest of the groups. The same geographic separation was observed for the 1989 samples on the basis of MDH-B1,2 allele frequencies except that two additional sites, Two Mile Channel and Gunisao River, were grouped with Big Black River and Grand Rapids.

Since genetic differences indicate distinct stocks whereas similarities do not prove identity and because more genetic differences among the populations may exist than were detected by this study, more than two stocks may be present in the LWR area. Although biochemical results presented here indicate that fish captured in Playgreen Lake proper (at Two Mile Channel) and in the Gunisao River have allele frequencies at the MDH-B1,2 genetic loci similar to Lake Winnipeg stocks, this finding may be the result of coincidence. It seems more likely, based on the geographic distribution of spawning areas, that these samples represent one or two

additional genetic stocks. It seems unlikely that fish spawning in the Gunisao River are part of the same stock as fish spawning on the west or east side of the north basin of Lake Winnipeg.

Genetic relationships among stocks sampled in 1989 as revealed by an UPGMA dendrogram of Nei's (1978) unbiased genetic distances were similar, as expected, to those based on MDH-B1,2 allele frequencies. The Warren Landing - Little Playgreen Lake grouping was separated from the remaining stock at a genetic distance of 0.001 for the 1989 sample (Appendix Ia) and the groupings were separated out at a distance of 0.004 in the 1975 sample (Appendix Ib). The Nei's genetic distances observed in this study were similar to those found between sympatric stocks of lake whitefish by Kristofferson (1978), Casselman et al. (1981) and Ihssen et al. (1981). Kristofferson (1978) found that the average genetic distance between Lake Winnipeg populations was 0.0022. Ihssen et al. (1981) observed genetic distances of 0 to 0.0006 between allopatric lake whitefish populations in Lakes Huron and Ontario and Casselman et al. (1981) observed genetic distances of 0.0002 to 0.0007 among sympatric lake whitefish stocks of Lake Huron.

Mean heterozygosity varied from 0.106 to 0.114 for 1989 samples and from 0.114 to 0.148 for 1975 samples. Therefore, genetic variability did not change substantially between years and the slightly higher heterozygosity observed in the 1975 samples is probably due to the fact that fewer loci

were analyzed for the 1975 samples. These mean heterozygosities are at the high end of the scale for salmonids (0.015 to 0.14) and are higher than observed for populations of Great Lakes lake whitefish (Imhof 1980; Casselman et al. 1981; Ihssen et al. 1981). The reason for this difference is not clear, but may be related to environmental heterogeneity or it might indicate that Lake Winnipeg lake whitefish have a higher degree of fitness than Great Lakes lake whitefish (Allendorf & Utter 1979). The outlet lakes area of Lake Winnipeg and Playgreen Lake may be environmentally more complex than simple large lake systems such as the Great Lakes where most previous studies have been carried out.

Differences in MDH-B1,2 allele frequencies detected among stocks in northern Lake Winnipeg and Playgreen Lake probably reflect some degree of reproductive isolation among stocks and confer biochemical individuality upon the two groupings observed (Kristofferson and Clayton 1990). This isolation could be induced by behavioral differences between populations such as innate homing of lake whitefish to natal spawning grounds. Svärdson (1965) and Lindstrom (1970) have observed that European whitefish populations can be segregated on the basis of behavioral differences related to spawning activities. Behavioral mechanisms such as homing could restrict gene flow between stocks and perpetuate the discrete characteristics of each group. The degree of geographic isolation of stocks by distance also affects reproductive isolation by reducing effective rates of

gene flow resulting from straying between adjacent spawning areas. Richardson et al. (1986) have described three alternative population models which could underlie the formation of genetic stocks of fishes. Data from this study conform to the discrete subpopulation model. In this model, mating occurs at random within each subpopulation, and populations are separated from one another by environmental or behavioral barriers that allow only limited inter-stock migration among stocks. The discrete stock model is supported when intra-stock allele frequencies are homogeneous and in equilibrium, when there are inter-stock differences among stocks in allelic frequencies, and when there are discontinuities in allele frequencies at the same geographical locations for several loci (Richardson et al. 1986). In the case of lake whitefish stocks, probably both distance and homing are acting to cause genetic differences between adjacent stocks.

Similar genetic differences among stocks at the MDH-B1,2 duplicated loci were found to be present in 1975, before LWR, and in 1989, after LWR. The temporal stability of the allele frequencies observed in the polymorphic loci IDHP-4, LDH-4 and G3PDH-1 provided additional evidence of genetic stability before and after LWR. Thus, on the basis of genetic findings, there are no indications of genetic changes in stock structure in the area affected by LWR, therefore the null hypothesis is not rejected. Evidence from the present survey of isozyme loci does not support the supposition that changes in the whitefish fishery, as claimed by commercial fishermen on Playgreen

Lake following LWR construction and operation, are attributable to changes in the genetic stock structure of lake whitefish.

A basic limitation of this study was the relatively small number of sample sites examined before LWR in the north basin of Lake Winnipeg and especially in the area of Playgreen Lake. It should be emphasized, however, that the pre-LWR study was not conducted with the objective of providing a baseline data set against which to measure changes caused by LWR. Chakraborty and Leimar (1987) indicated that large sample sizes (>25 fish) are needed when differences are evaluated between conspecific populations with little electrophoretic divergence and in this study sample sizes consisted of at least 50 fish.

II) MORPHOLOGY

Analysis of morphological differences among spawning aggregations usually allows for the discrimination of more stocks than isozyme genetic techniques (Todd 1981). This was observed in this study as well as studies by Kristofferson (1978), Casselman et al. (1980), Ihssen et al. (1981) and Todd (1981). Kristofferson and Clayton (1990) found that the Grand Rapids, Big Black River, and Little Playgreen spawning stocks were separable on the basis of morphological characteristics. After LWR (1989), morphological characters indicated that three additional spawning stocks, Gunisao River, Warren Landing and Two Mile Channel, were present in the study area.

Discrimination among stocks was greater with morphometric characteristics than with meristic and genetic characters because body shape is heavily influenced by local selective forces and growth rates, whereas genetic and meristic characters are less influenced (Beacham & Withler 1985; MacCrimmon & Claytor 1985; Beacham et al. 1988). Ihssen et al. (1981) observed that morphological variation among stocks of Great Lakes lake whitefish was related to differences in growth rate of the stocks and that stocks with similar growth rates were closely related. Ihssen (1981) observed that dendrograms of meristic counts differed from dendrograms for morphometric measurements. This difference between meristic and morphometric branching patterns was observed in this study. This contrast could be explained by the differential influence of environmental and genetic effects on the meristic counts versus the morphometric characters (Ihssen et al. 1981; Todd 1981).

It is important to fisheries managers to understand the significance and the distribution of these stocks and to know if they are genetically fixed or more labile phenotypic expressions of differing environmental conditions (Casselman et. al. 1981). With respect to differing environmental conditions to which lake whitefish may be exposed, morphometrics are more important in reflecting a change over a longer stage (from egg to alevin to juvenile stage), whereas meristics are only susceptible to changes during the embryonic stage (Tåning 1952). The window for effects is therefore much

shorter for meristics. Meristic traits are fixed after hatching, while morphometric traits are plastic and prone to environmental modification over a longer interval of time.

A relationship between gill raker number and/or length and diet or feeding mode has been observed for lake whitefish (Bodaly 1979; Lindsey 1962; Loch 1974; Svärdson 1952), with pelagic species and small food particle size generally being associated with high gill raker counts or small gill raker spaces (Kliewer 1970). Little Playgreen Lake and Two Mile Channel fish had wide gill raker spacing and fewer upper gill rakers while Big Black River and Grand Rapids fish had tight gill raker spacing and more upper gill rakers.

Little Playgreen Lake and Two Mile Channel fish were distinguished by large body proportions and fewer lateral line scales whereas the Grand Rapids and Big Black River fish had small body proportions and a higher number of lateral line scales. These observations are similar to Kristofferson's (1978) findings for fish sampled in 1975. Differences in foraging patterns, feeding range and/or prey availability at different feeding grounds could affect selection for the characteristics of the gill raker apparatus in the fish and could possibly account for the observed morphological divergence between these populations. However, the extent of environmental modification of these characters among stocks sampled in this study is unknown.

In this study, discriminant function analysis correctly reclassified fish into their original grouping for the morphometric, ratio and combined data sets with great precision. *A posteriori* discriminant function analysis classification for morphometric measurements of Lake Huron lake whitefish correctly assigned 70.8% of the individuals into their original groupings (Casselman et al. 1981). Similar analysis of the meristic data set correctly reclassified only 46 to 67% of the Gunisao River, Warren Landing, Little Playgreen Lake and Big Black River fish into their original groupings and only 4 to 11% of the Grand Rapids and Two Mile Channel fish into their original groupings (Table 9). These low reclassification rates could indicate that the Grand Rapids and Two Mile Channel rearing environments were not distinct compared to the rearing environments present at Warren Landing, Little Playgreen Lake, Big Black River and Gunisao River sites. Two Mile Channel and Grand Rapids spawning sites (Fig.1) are downstream of strong currents that can disperse the whitefish larvae down to the other rearing sites after hatching.

Factors such as temperature, food type, water chemistry and rate of exploitation have been shown to differentially affect growth rate of lake whitefish between different parts of the Lake Winnipeg basin (Kristofferson 1978). The first canonical function which accounted for most of the variability among groups in this study has been supposed to reflect some simple biological variable such as growth rate (Ihssen et al. 1981). In this

study, the Big Black River and Grand Rapids stocks had larger head measurements and smaller body proportions than the other stocks, possibly reflecting growth differences. Casselman et al. (1981) and Ihssen et al. (1981) showed that whitefish stocks farthest removed geographically from the other stocks and with the slowest growth were most different for all traits including morphological and genetic characters. Grand Rapids and Big Black River stocks (1989 and 1975) were the most distant from the other stocks. These two groupings exhibited the slowest growth rates (inferred from large head measurements) and they were isolated spatially from the Little Playgreen Lake, Warren Landing, Gunisao River, and Two Mile Channel groups (Fig.1).

Morphological differences reflect genetic and/or environmental differences, and for the genome sampled by this study we know that the stocks have remained genetically stable. Since only a small portion of the genome was sampled though, the possibility of genetic changes underlying a part of the morphological changes can not be ruled out. Any temporal variation in the morphology of the discerned stocks could indicate natural environmental change and possibly anthropogenically induced environmental perturbations. All populations sampled during both years were found to be distinct phenotypic stocks. The morphological characteristics of groups sampled during 1989 and 1975 (Little Playgreen Lake, Grand Rapids and Big Black River) were significantly different over

time. Whitefish collected in 1975 had more lateral line scales and higher gill raker counts, possibly indicating that an environmental change had occurred between the sampling periods. Whitefish collected in 1975 also had smaller heads and larger body proportions than 1989 fish, indicating that growth rate might have decreased since 1975. The morphological characteristics of the stocks have changed but stock integrity has not changed since construction of LWR. There were distinct inter-stock differences evident during 1975 and 1989. The stock stability of the spawning populations is supported by the results obtained from genetic analysis which indicated that the allele frequencies of stocks were stable over time. The study design utilized the Grand Rapids and Big Black River sites as reference sites that would be relatively unaffected by LWR. The Little Playgreen Lake site was an experimental site because it was: a) downstream of Two Mile Channel and relatively close to the possible environmental perturbations that Two Mile Channel could cause, and b) it was the only site that was sampled in 1975 that was downstream of Two Mile Channel. ANOVA and CDFA results indicate that morphological characters have changed significantly since LWR at all three sites. These findings are contrary to results expected if LWR had altered morphological characteristics. There should have been greater morphological change in the Little Playgreen Lake fish (Lake Winnipeg stocks as controls) since this stock is presumably affected to a greater extent by Two Mile Channel than

the other groups. It is therefore impossible to directly attribute any environmental change to the operation of LWR since similar morphological changes occurred at all three sites in the study area (Appendix III).

Savvaitova et al. (1989) have hypothesized that environmental impacts from hydroelectric developments such as LWR may affect the morphological heterogeneity of fish stocks. Scatter plots of the first and second canonicals indicated that there were no changes in variation between years for Little Playgreen Lake, Big Black River and Grand Rapids. Environmental change did not cause an increase in heterogeneity in this study.

PART II : STABLE ISOTOPE ANALYSIS OF LAKE WHITEFISH

INTRODUCTION

Results from the genetics and morphology sections and from Kristofferson (1978) and Kristofferson & Clayton (1991) indicate that Lake Winnipeg, Playgreen Lake and Little Playgreen Lake are inhabited by distinct stocks of lake whitefish. Some of these lake whitefish stocks exhibit extensive spawning migrations to and from their feeding grounds and spawning shoals whereas other stocks do not migrate far from their feeding grounds in order to spawn (Pollard 1973). Patterns of lake whitefish migration in Lake Winnipeg are not well documented but Ihssen et al. (1981) have observed that lake whitefish in lakes Simcoe and Ontario migrate up to 50 to 80 km from their feeding grounds to return to their natal spawning grounds. The objective of this preliminary study was to determine if the stable isotope ratios of carbon, nitrogen and sulphur could be used to delineate stocks of lake whitefish in the waters of Little Playgreen Lake and Lake Winnipeg. Isotope ratios in animals reflect isotope ratios in the food they ingest (Peterson & Fry 1987). Differing isotope ratios among stocks will be indicative of feeding areas with different isotopic signals and/or different diets which could be interpreted as evidence for distinctiveness of the stocks (Hesslein et al. 1991b). The study design for

the stable isotope analysis component of this study assessed the groups within each sampling period for differentiation among groups.

The elements, carbon (C), nitrogen (N) and sulphur (S) have at least one heavier, but rarer, stable isotope. Isotopic compositions of tissues can be considered indicative of the assimilated diet, reflecting both long-term and short-term diets depending on the metabolic rate of turnover of the tissue measured (Peterson & Fry 1987). Animals are similar in isotopic composition to their diet for S and C isotopes but they tend to be 3 to 5 ‰ (parts per thousand) more enriched in ^{15}N relative to dietary N (Peterson & Fry 1987). Physical and chemical reactions fractionate stable isotopes. Isotope fractionation in most biochemical reactions arises when similar molecules of different mass react at different rates. The alteration of the ratio of heavy to light isotopes or stable isotope fractionation during protein metabolism is the reason why animals are enriched with the heavier ^{15}N isotope. This enrichment in the diet is mainly due to the excretion of ^{14}N in urine. Values of ^{15}N increase by 10 to 15 ‰ in many food webs, usually as a result of 3 to 5 successive trophic transfers, each of which increases the ^{15}N content by 3 to 5 ‰ (Peterson & Fry 1987). The isotopic ratio of an element is indicative of a source or location which a) remains unchanged as the element moves through the food chain thus preserving the record of origin (C, S), or b) changes in a constant manner through each trophic transfer (N). Given certain geographical and geochemical combinations (i.e. distinct

distributions of C, S and N stable isotopes), the C, S and N isotope constitution of lake whitefish can be extremely valuable as a tracer of site integrity, of feeding patterns, in discriminating between sources of food, and in trophic level differentiation in the food web (Hesslein et al. 1991b; Peterson & Fry 1986). We need to show that different areas have different isotope ratios. If this is true, then whitefish samples from different spawning sites should be distinguishable by their C, S and N isotope ratios, if the fish remain together and feed in the same area on the same kind of food. If there are large inter-stock isotopic differences and within-stock variation is small, isotopes could prove to be a powerful tool requiring only a small number of samples to be effective in identifying stock membership of fish caught in commercial fisheries and in defining the feeding ranges of lake whitefish stocks in Lake Winnipeg (Peterson et al. 1985).

PHYSICAL PROPERTIES OF LAKE WINNIPEG

Many properties of lakes are dependent upon and influenced greatly by their terrestrial watersheds. Lake Winnipeg lies on the boundary between the Great Plains and the Canadian Shield (Fig. 8). Rivers to the east of Lake Winnipeg drain the thin soils covering the igneous bedrock of the Precambrian Shield. These watersheds are marked by muskeg swamps and boreal forests. Watersheds to the south and west of Lake Winnipeg are underlain by sedimentary strata and are overlain with glacial sediments

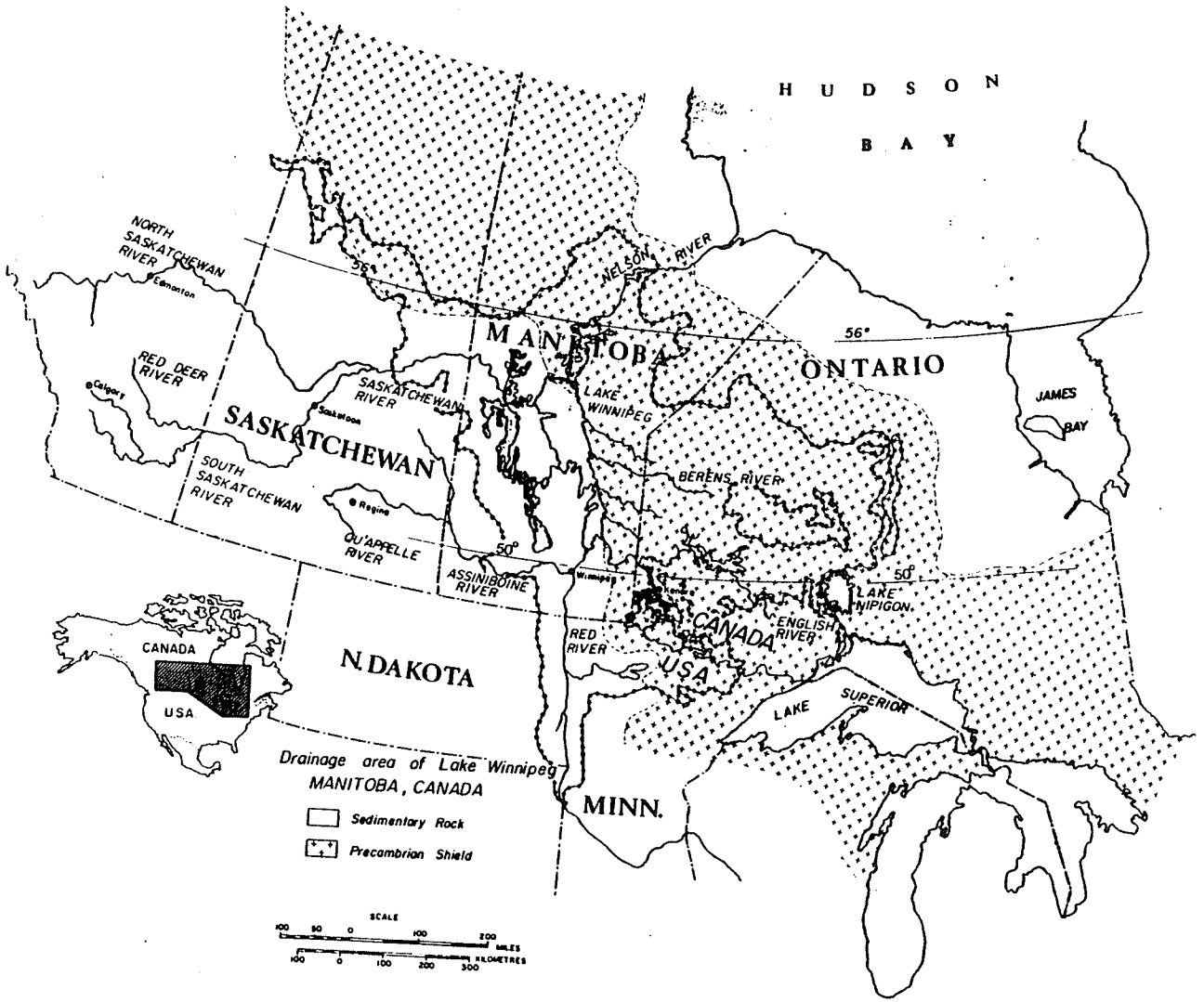

Fig. 8. Map of the major tributaries to Lake Winnipeg and an outline of the Precambrian Shield and sedimentary regions of the Lake Winnipeg watershed (from Brunskill et al. 1980).

and chernozemic soils. Vegetation includes prairie grasses, mixed and coniferous forests (Elson 1967; Davies et al. 1962). The contribution of organic riverine detritus by the tributaries enables Lake Winnipeg to support an abundant and varied aquatic fauna richer than might be expected from levels of primary production in the lake (Brunskill et al. 1979). The water masses from the major watersheds retain substantial source identity and have very different chemical and biological characteristics which result in markedly different limnological characteristics in different areas of Lake Winnipeg (Bajkov 1930; Brunskill et al. 1979; Brunskill & Graham 1979). The relatively short exchange time of 2.9 to 4.3 years (during 1969 to 1974) for Lake Winnipeg (Brunskill et al. 1980) is an important factor in preserving the identity of the water masses. Most of the annual discharge of water and nutrients into Lake Winnipeg comes from the Winnipeg and Red Rivers. The south basin inputs are dominated by the Red and Winnipeg Rivers whereas the north basin inputs are dominated by the Saskatchewan River. Compared to rivers draining the western plains, rivers draining the shield contribute a disproportionate 50% of flow into Lake Winnipeg from only 18% of the drainage area (Brunskill et al. 1980). The greater precipitation, lower evaporation and low water storage capacity found in the shield drainage is converse to the conditions found in prairie watersheds.

The north and south basins of Lake Winnipeg are characteristically different in regards to depth, nutrients, primary productivity and turbidity. Turbulent mixing occurs to all depths and as a result the water masses are well mixed vertically with little or no stratification of temperature, oxygen or of dissolved ions. Surface sediments are frequently resuspended and recirculated as a result of this turbulent mixing (Brunskill et al. 1980). Horizontal gradients of many chemical parameters, nutrients, suspended sediments, algae, zooplankton and benthic biomass tend to occur in the lake. According to Brunskill et al. (1979, 1980), these gradients are caused in part by lake morphometry, major river inflows, wind, geological substrate and technological development in the watersheds. Such differences in source and in basin water could result in different isotope ratios in potential fish food organisms in different parts of the lake.

The flow of total nitrogen from the terrestrial environment to Lake Winnipeg via large rivers such as the Red, Winnipeg and Saskatchewan Rivers is substantial in comparison to the low levels of total nitrogen transported from the smaller prairie and shield rivers (Table 10). High values of total N are present in sediments in the central basin and the western shore of the north basin and at the mouths of the Berens River and the Pigeon River (Brunskill & Graham 1979).

Table 10. Rate of transport of sulphate, carbonate and total nitrogen of major river systems into Lake Winnipeg (mean values for 1969-1974 ($x10^6$ moles yr^{-1}); Brunskill et al. 1980).

		SO_4	HCO_3	total N
South Basin	Red River	8682.5	29842.5	1489.3
	Winnipeg River	2376.3	29577.6	1693.8
North Basin	Dauphin River	4752.0	12176.0	271.2
	Berens & Pigeon Rivers	111.7	1838.0	240.3
	Poplar River	30.8	230.9	65.3
	Saskatchewan River	9792.0	64048.0	1241.0

Sulphate fluxes are greatest from the prairie watersheds and bicarbonate fluxes are greatest from the Saskatchewan River (Table 10). Carbon, nitrogen and sulphur isotope ratios in fresh water organisms vary widely depending on the source of dissolved CO_2 , N and S in the water. Dissolved CO_2 in lake water can originate from weathering of carbonate rock, from mineral springs, from the atmosphere or from metabolism of organic matter (Peterson & Fry 1987). Nitrogen isotope ratios can differ between phytoplankton and terrestrial vegetation and if this occurs the nitrogen isotopes may function as source markers for autochthonous and allochthonous organic matter (Peterson & Fry 1987). Geological weathering of uplifted marine sediments has produced distinct sources with different values for sulphur (Peterson & Fry 1987). It seems likely, based on

limnology and riverine inputs, that water and sediments from different areas of Lake Winnipeg will have different C, N and S ratios .

METHODS

An inter-year comparison of stable isotope ratios in lake whitefish was conducted between the Grand Rapids and the Little Playgreen Lake groups. Big Black River fish from 1975 were not available so Big Black River fish from 1989 were compared temporally with Berens River (1975) fish since Kristofferson (1978; Kristofferson & Clayton 1990) concluded that Big Black River and Berens River fish were morphologically and genetically identical. A sub-sample of three lake whitefish from each of the 1989 Little Playgreen Lake, Big Black River and Grand Rapids collections and four lake whitefish from each of the 1975 Grand Rapids, Little Playgreen Lake, Traverse Bay and Berens River collections were used for isotopic analysis. Eight grams of dorsal muscle from each fish was used to determine C, N and S isotope ratios as described in detail by Hesslein et al. (1989).

In brief, whitefish samples used for sulphur isotope analysis were decomposed to sulphate by nitric acid digestion, nitrate fusion and barium precipitation followed by thermal decomposition to sulphur dioxide. Whitefish samples were dried, decomposed to nitrogen and carbon dioxide by a modified Dumas method and cryogenically separated and trapped, for carbon and nitrogen isotope analysis. All isotopic determinations were

performed on a dual inlet isotope ratio mass spectrometer (VG Micromass 602E) (Hesslein et al. 1991b). Standards used for sulphur originate from the Canyon Diablo meteorite, for carbon from the PeeDee limestone and nitrogen was standardized against atmospheric nitrogen. Over several years of operation, the typical reproducibility of determinations has been found to be at 2 standard deviations; 0.3 ‰ for sulphur, 0.1 ‰ for carbon and 0.4 ‰ for nitrogen (Hesslein et al. 1991b).

The relative abundance of the heavier isotope is expressed in δ notation as parts per mil according to the relationship:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C, ^{15}N or ^{34}S and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$ (Peterson & Fry 1987).

The relationship between the different samples was graphically represented by constructing XY plots using the ^{13}C, ^{15}N or ^{34}S δ values, two at a time, as the X and Y axes. All points for each sample were enclosed by a polygon so that the distribution of each sample could be easily visualized.

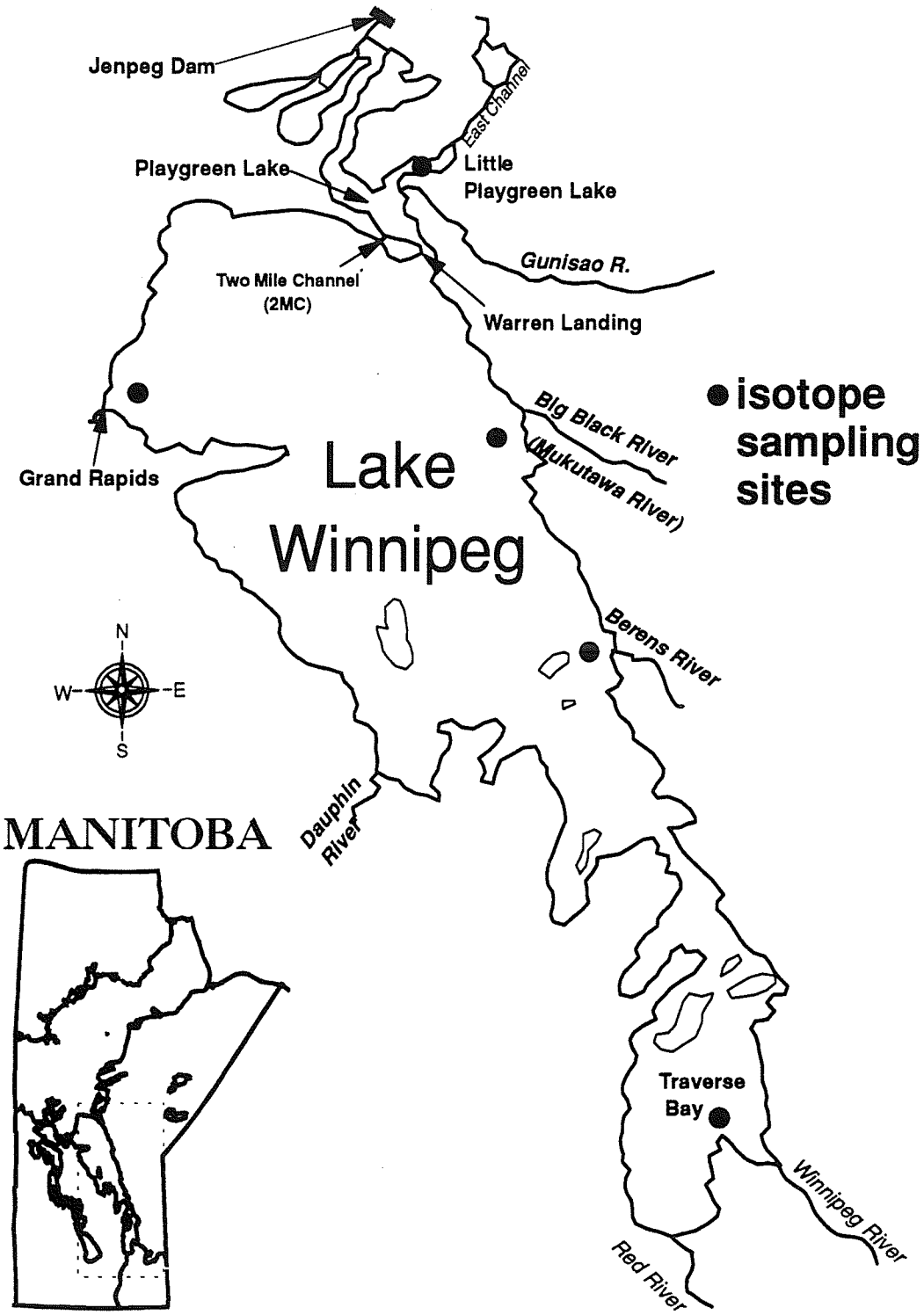

Fig. 9. Location of isotope sampling sites in Lake Winnipeg, Little Playgreen Lake and Playgreen Lake.

RESULTS

Isotopic analysis of muscle tissue samples from individual lake whitefish caught in five different locations during two different sampling periods, showed ranges of -28.5 to -24 ‰ for $\delta^{13}C$, 8.5 to 12 ‰ for $\delta^{15}N$ and -10 to 0 ‰ for $\delta^{34}S$ (Figs. 10-12).

The total range of $\delta^{13}C$ values for lake whitefish collected in 1975 was wider (4 ‰) than in 1989 (2 ‰) (Figs. 9-10). Five of the seven groups showed a relatively narrow range of carbon values while the other two groups (Berens and Grand Rapids (1975)) showed a relatively broad range of C values. All 1975 samples showed overlap with at least one other group, but there appeared to be differences among the groups. Traverse Bay fish were very distinct from the other 1975 fish because they had lower $\delta^{13}C$ values and they displayed a narrow range for $\delta^{13}C$ which was in contrast to the ranges for the other 1975 stocks. All of the 1989 samples exhibited a relatively narrow range of $\delta^{13}C$ values. All 1989 samples showed overlap but Big Black River fish had higher values of $\delta^{13}C$ which differentiated them from the Little Playgreen and Grand Rapids fish which were broadly overlapping (Fig. 10). There seems to have been a temporal change in $\delta^{13}C$ in Grand Rapids fish, while there appeared to be no temporal change in Little Playgreen Lake fish. In general, all 1989 samples are at the high end of the $\delta^{13}C$ scale, whereas all 1975 samples encompass a wide range of $\delta^{13}C$.

Values of $\delta^{34}S$ for fish sampled in 1975 ranged from -10 to -3.5 ‰ and were sufficient to distinguish the Berens River stock, which had the lowest $\delta^{34}S$ values, from the fish taken at Little Playgreen Lake, Traverse Bay and Grand Rapids. There was considerable overlap in these three groups, but most of the Little Playgreen (1975) fish had lower $\delta^{34}S$ values compared to Grand Rapids (1975) and Traverse Bay fish. In contrast, values of $\delta^{34}S$ for fish sampled in 1989 were higher and ranged from only -3 to 0 ‰ with all three stocks clustering together. Samples from 1975 did not overlap with 1989 samples for sulphur isotope ratios (Figs. 10 & 12).

All of the groups except for Traverse Bay showed a relatively narrow range of $\delta^{15}N$. Values of $\delta^{15}N$ for fish sampled in 1975 and 1989 ranged from 0 to 15 ‰ and the 1975 and 1989 samples from the same locations displayed similar values for $\delta^{15}N$. Traverse Bay (3 of the 4 fish) had the highest $\delta^{15}N$ values and were discrete from all the other groups. Both Grand Rapids samples (1989 & 1975) were discernable from all the other north basin (except Traverse Bay which is located in the south basin) samples and had values of $\delta^{15}N$ in the range of 10.5 to 12 ‰ (Figs. 11-12). Berens River, Big Black River and Little Playgreen Lake (1975 & 1989) overlapped considerably and had the lowest $\delta^{15}N$ values of all the groups.

All groups can be separated on the basis of one or more isotopes except perhaps Traverse Bay and Grand Rapids (1975) which overlap somewhat for S, C and N.

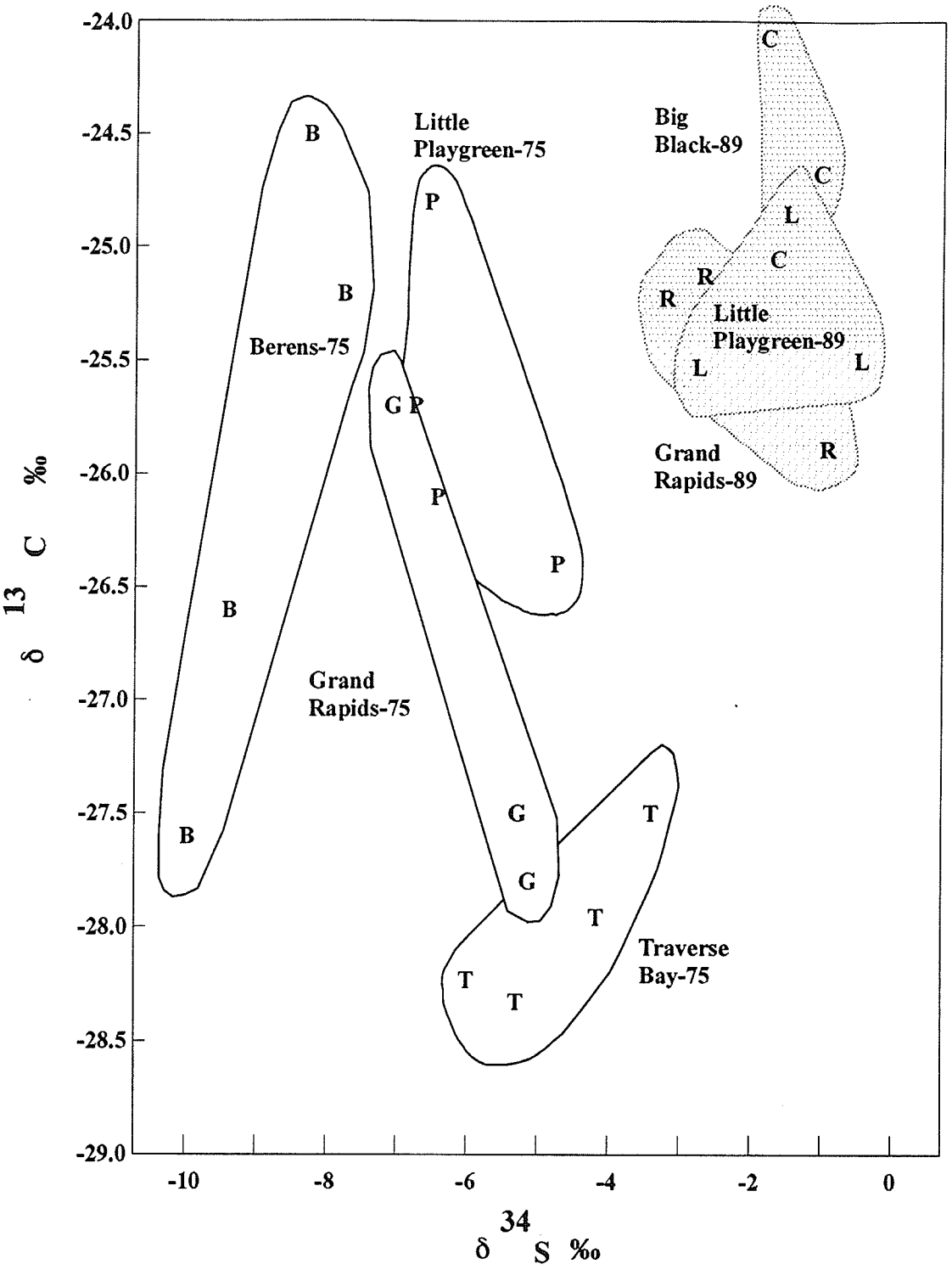

Fig. 10. Concentration of carbon and sulphur isotopes in muscle tissue of lake whitefish caught at various locations in Lake Winnipeg and Little Playgreen Lake (each population is enclosed by an envelope).

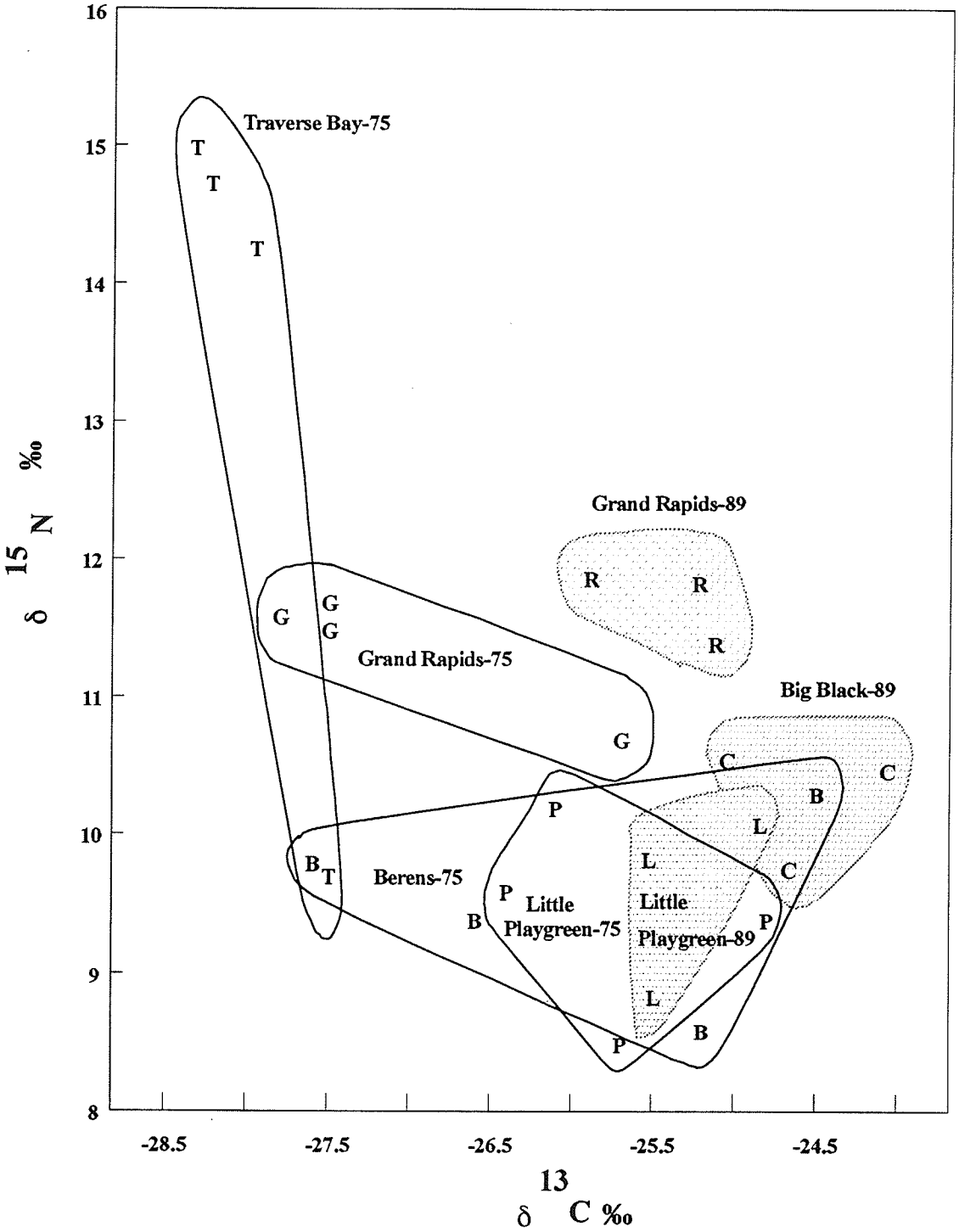

Fig. 11. Concentration of nitrogen and carbon isotopes in muscle tissue of lake whitefish caught in various locations in Lake Winnipeg and Little Playgreen Lake (each population is enclosed by an envelope).

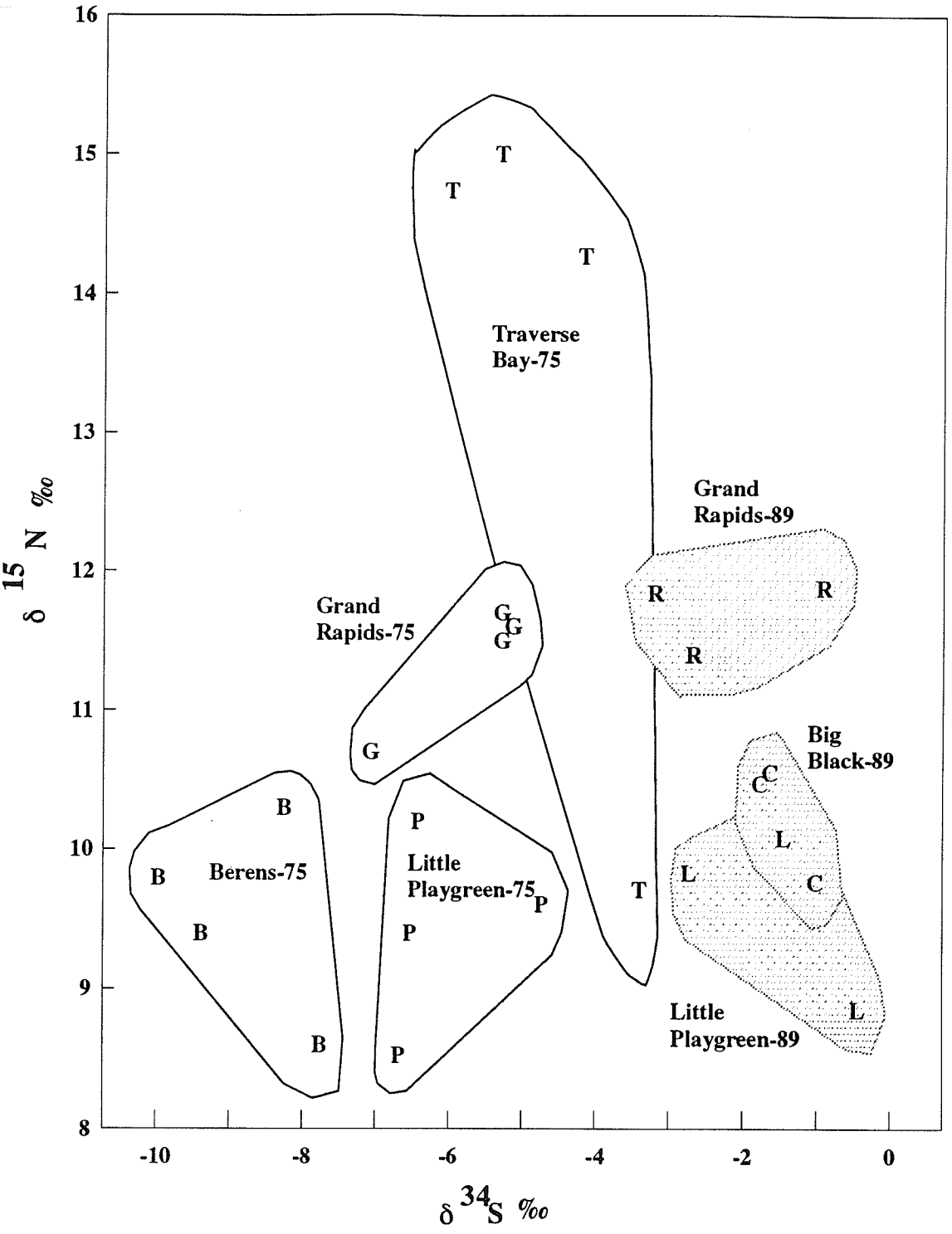

Fig. 12. Concentration of nitrogen and sulphur isotopes in muscle tissue of lake whitefish caught in various locations in Lake Winnipeg and Little Playgreen Lake (each population is enclosed by an envelope).

DISCUSSION

It has been suggested that lake whitefish stocks stay together as a cohesive unit after spawning but it is not known for how long they stay together or if and when they disperse (Pollard 1973). Lake whitefish caught on spawning shoals represent collections of fish which may have been feeding in a variety of habitats, or consuming various kinds and amounts of food items in one habitat, and thus could develop different isotope ratios. Since the isotopic composition of animals is determined by their diet, local isotopic signals could be used as markers to delineate the summer feeding areas of lake whitefish stocks in Lake Winnipeg. Concentrations of SO_4, HCO_3, organic carbon (C_o) and total N differ considerably among the major tributaries of Lake Winnipeg (Table 10). It seems possible that specific isotope ratios may also differ among these major river sources flowing into Lake Winnipeg.

Sulphur is a useful isotope source tracer because amino acids containing sulphur are essential to animals and sulphur does not fractionate in the food chain like nitrogen, thus providing an unaltered signal identical to the food source (Hesslein et al. 1991b). The ^{34}S signal from lake whitefish muscle tissue collected during 1975 and 1989 showed temporal differences, possibly indicating different input of isotopic sulphur into the lake system. Wet conditions present during 1975 and drought conditions experienced during 1985 (Hesslein pers. comm.) might have

affected the input of ^{34}S into Lake Winnipeg. Whitefish samples collected in 1989 could not be differentiated from each other (Figs. 10 & 12). Berens River fish collected in 1975 had the lowest ^{34}S signals and were distinctly different from the other three groups of fish, indicating that they had a different feeding range than the rest of the stocks. Grand Rapids and Little Playgreen Lake fish seem to have the same ^{34}S in 1975 and in 1989, indicating that they possibly feed in the same area. Traverse Bay (1975) fish had the same ^{34}S values as Little Playgreen Lake (1975) and Grand Rapids (1975) but since they are geographically very far apart, the similarity of their ^{34}S signals could be just coincidence. Since the ^{34}S signal seems to change over time, care must be taken if this isotope is to be used to differentiate stocks over time.

Results for ^{13}C indicate that the 1975 Grand Rapids (3 of 4 samples) and Traverse Bays fish can be differentiated from all the other samples (except for one sample from Berens River (1975)). Berens River fish exhibited wide ranging values of ^{13}C that overlapped with ^{13}C values from Grand Rapids and Little Playgreen stocks. An explanation for this phenomenon could be that there were very disparate carbon signal sources in a confined foraging area causing the ^{13}C signals for the Berens stock to be divergent. Differences in feeding habits between individual fish might also explain the differences in ^{13}C within stocks (Hesslein et al. 1991b). The ^{13}C signals for Grand Rapids and Little Playgreen Lake stocks partially

overlapped during both sampling years (Fig. 10). This could suggest that they feed in the same area at some particular time during the summer, but not necessarily at the same time. A narrower range for C isotope ratios found in the 1989 samples, possibly indicates the exact converse - that preferred prey was readily available in one particular area, or that some other constraint was limiting the foraging area of the whitefish during that time period. Values of ^{13}C in 1989 Grand Rapids fish were lower than in 1975 fish and were similar to values of ^{13}C for 1989 Little Playgreen Lake fish, possibly indicating that they shared the same feeding ground.

Three different groupings were evident based on ^{15}N values. Traverse Bay fish (3 out of 4 fish) (1975) had the highest ^{15}N values followed by 1975 and 1975 Grand Rapids fish with the third group comprising all the other fish. These high concentrations of ^{15}N isotopes observed from fish feeding in prairie river plumes (assuming the Traverse Bay fish were influenced to a greater extent by the Red River plume) into Lake Winnipeg, are consistent with results obtained by Hesslein (pers. comm.). These results indicate that the Grand Rapids and Traverse Bay fish may be feeding on a different diet than the rest of the stocks sampled.

It is difficult to demarcate feeding locations without source signals, but the preliminary stable isotope results indicate there are differences in feeding areas. According to these results (Figs. 10-12), adult lake whitefish seem to feed on a range of organisms or in varied locations in Lake

Winnipeg. On examination of C, S and N isotope ratios, all whitefish samples (sites and years) except Traverse Bay and Grand Rapids (1975) and Big Black River (1989) and Little Playgreen Lake (1989) can be distinguished from each other. In brief, this study supplements the argument of stock separation because it suggests that different whitefish stocks feed on different food sources or that they inhabit separate areas of Lake Winnipeg. Nitrogen isotopes indicate trophic status and the location of the isotope source, while carbon and sulphur isotopes only indicate the location of the isotope source. If C and S signals are different, then separate stocks must be feeding in different areas. This is based on the premise that C and S, unlike N, do not fractionate up the food chain so the C and S signal of the whitefish is indicative of the particular area no matter what the fish are eating.

Telemetry and mark-and-recapture techniques should be used to confirm the migratory behaviour of lake whitefish in Lake Winnipeg. There is also the possibility to combine genetic and morphological studies with tagging studies in order to elucidate migration patterns. Genetic data are available but more potential spawning sites need to be sampled to better assess the distribution of genetic stocks. If stable isotope results can be verified with field data, they may provide an alternative or complement to tagging in expensive radio tagging studies. Stable isotope analysis may hold promise as a potential method to delineate stocks from a mixed stock

commercial fishery, but temporal differences warn against assuming temporal stability.

The results obtained in this pilot study are intriguing but there are many more critical questions to be answered before any definite conclusions can be reached. There have not been any studies conducted on the discrimination of fish stocks using stable isotopes other than by Hesslein et al. (1989, 1991a, 1991b). Peterson et al. (1985) analyzed ribbed mussels from a salt marsh and demonstrated that the isotopic composition of these filter feeding mussels was a function of where the organisms grew. Local, non-migrating populations of fish or other sessile organisms such as mussels must be analyzed for C, S and N isotopes so that gradients can be mapped out for Lake Winnipeg. The waters and sediments of Lake Winnipeg need to be investigated to determine the sources of the differing isotopic signals.

GENERAL DISCUSSION

The combination of enzyme genetics, morphological analysis and isotopic analysis provide good discrimination among lake whitefish stocks in Lake Winnipeg, Playgreen Lake and Little Playgreen Lake. Results of this study indicate the presence of at least two genotypic stocks and six phenotypic stocks. Isotopic analysis provided additional evidence that there was discrimination among all stocks sampled. The enzyme genetic differentiation among populations probably reflects migration and genetic drift more than it does local adaptation (Leary & Booke 1990). A significant temporal change in morphological traits in Little Playgreen Lake, Big Black River and Grand Rapids stocks was revealed by morphological analysis. Carbon, sulphur and nitrogen stable isotope ratios of lake whitefish samples were investigated based on the premise that the isotopic composition of the fish is determined by their diet. These isotope ratios also provided good discrimination among spawning aggregations. There was within-year and between-year segregation based on distinct ^{34}S,^{13}C,^{15}N isotope ratios of lake whitefish from Little Playgreen Lake and Grand Rapids, indicating distinct inter-stock feeding areas and environmental change between sampling periods. Morphological and stable isotope analyses were independent techniques that implied an environmental change between sampling periods

(1989 & 1975) that caused a possible feeding area shift and a body shape transformation in lake whitefish stocks.

This multiple approach study confirms the presence of multiple stocks of lake whitefish but fails to reject the null hypothesis that LWR has not altered the stock structure of lake whitefish in the north basin of Lake Winnipeg, Playgreen Lake and Little Playgreen Lake. Effects on the distinctness and genetic composition of lake whitefish stocks from the construction and operation of Two Mile Channel by Manitoba Hydro were not detected by this study. Environmental change was inferred but the possible effects of environmental change attributable to LWR could not be partitioned from the effects of total environmental change. This does not rule out the possibility that human alterations to the lake affected the distinctness of lake whitefish stocks but it is difficult to delineate the effect on stock structure, especially in the presence of naturally fluctuating environmental conditions, fishing pressure, sparse physical data, short monitoring periods and a limited number of samples and sampling sites. O'Connor (1982) examined commercial catch statistics for lake whitefish in Playgreen Lake between 1975 and 1981 to investigate whether changes in population characteristics had occurred as a result of LWR. He concluded, in similar fashion to this study, that there was a decline in mean weight and growth rate of Playgreen Lake whitefish but these changes could not be attributed to LWR and were probably due to fishing gear selectivity. Stock

characteristics such as growth and abundance were not examined in this study, but stock integrity was examined and it did not change as a result of LWR.

Stocks are the natural and logical unit of management for fisheries and each stock should, in theory, be harvested as a distinct unit because each stock can have a distinct rate of growth, reproduction and mortality. Before effective stock management can be implemented, more information is needed on the stock discreteness and stock structure of lake whitefish during the summer feeding period and on the productivity of the lake whitefish stocks. The use of telemetry studies, mark-and-recapture techniques and additional stable isotope, genetic and morphological information would greatly clarify this problem of uncharted migration routes between feeding and spawning sites and stock structure during the summer. Todd (1990) suggests that stocking fish which have unique genetic marks (Billington & Hebert 1988) could not only provide an excellent way of tracing the movements of these tagged fish, but could also provide a measure of the contribution of their gametes to various spawning populations. Differential vulnerability and recruitment to the whitefish fishery should also be resolved while the fishery itself should be regulated so that the harvesting of each stock can be individually controlled. Fishing efforts should be distributed in accordance to the productivity of each stock since haphazard mixed harvesting of stocks can lead to inappropriate

harvest rates of certain local stocks and the possible extirpation of some populations.

If the size of the local spawning sub-populations (stocks) of lake whitefish was to decrease, then there would be risk that genetic variation will be lost due to genetic drift. It might be disastrous to lose the genetic variability present in the northern Lake Winnipeg and Little Playgreen Lake area since this area seems rich in spawning grounds. According to local fishermen, one of the whitefish stocks in Playgreen Lake is known to grow to a large size and has been the mainstay of the smoker whitefish fishery (Flett pers. comm.). As of the 1992 season, commercial catches of this large smoker whitefish have been very rare and the Lake Winnipeg smoker trade has plummeted.

The combination of genetics, morphology and stable isotopes techniques proved to be a good approach in delineating stocks of lake whitefish. Genetic traits are more stable over time, morphological characters show better resolution and stable isotope analysis shows promise as a technique for mapping distribution patterns of lake whitefish, but these techniques are not as powerful if used in unison. This study provides results that indicate that lake whitefish populations from the six different spawning assemblages be considered and managed as distinct stocks in order to conserve the populations of lake whitefish for the continued existence and productivity of the fishery in northern Lake Winnipeg and

Playgreen Lake. In order for this recommendation to be implemented, we need to have more information on the size of the stocks and the movement patterns of the stocks.

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Appendix Ia. Matrix of Nei's unbiased genetic distances, cluster analysis and goodness of fit statistics for 1989 lake whitefish samples.

Below diagonal: Nei (1978) unbiased genetic distance

Population	1	2	3	4	5	6
1 WARREN LDN.	*****					
2 GUNISAO R.	.001	*****				
3 L.PLAYGREEN L.	.001	.000	*****			
4 2MC	.002	.000	.000	*****		
5 B.BLACK R.	.002	.000	.001	.000	*****	
6 G.RAPIDS	.002	.000	.002	.000	.000	*****

Cluster analysis using unweighted pair group method
Coefficient used: Nei (1978) unbiased genetic distance

Population or cluster numbers joined		Clustering level	Cycle
2	4	.00000	1
2	5	.00000	2
2	6	.00007	3
1	3	.00063	4
1	2	.00137	5

Goodness of fit statistics
Farris (1972) "f" = .006
Prager and Wilson (1976) "F" = 53.999
Percent standard deviation (Fitch and Margoliash,1967) =137.063
Cophenetic correlation = .737

Appendix Ib. Matrix of Nei's unbiased genetic distances, cluster analysis and goodness of fit statistics for 1975 lake whitefish samples.

Below diagonal: Nei (1978) unbiased genetic distance

Population	1	2	3	4
1 L. PLAYGREEN L.	*****			
2 WARREN LDN.	.001	*****		
3 GRAND RAPIDS	.005	.006	*****	
4 BIG BLACK R.	.005	.007	.007	*****

Cluster analysis using unweighted pair group method
Coefficient used: Nei (1978) unbiased genetic distance

Population or cluster numbers joined		Clustering level	Cycle
1	2	.00004	1
1	4	.00443	2
1	3	.00484	3

Goodness of fit statistics
Farris (1972) "f" = .003
Prager and Wilson (1976) "F" = 14.116
Percent standard deviation (Fitch and Margoliash,1967) =16.357
Cophenetic correlation = .938

Appendix IIa. Total sample standardized canonical coefficients for residual morphometric values for 1989 lake whitefish samples.

	DISCRIMINANT AXIS				
VARIABLE	***CAN 1***	***CAN 2***	***CAN 3***	***CAN 4***	***CAN 5***
RESIDUALS					
POL	0.550	--0.004	0.095	--0.329	--0.368
OOL	0.019	--0.492	0.221	0.397	0.575
PSL	--0.487	0.280	--0.224	0.385	--0.094
TTL	--0.439	--0.172	--0.349	0.358	0.004
DOL	--0.245	--0.192	--0.066	0.200	--0.057
LUL	--0.198	--0.286	0.154	0.128	--0.375
ANL	0.172	0.175	--0.340	0.039	--0.074
CPL	--0.221	0.312	--0.124	0.114	0.287
HDD	--0.565	0.477	--0.259	--0.204	--0.129
BDD	0.587	0.008	--0.278	0.527	--0.307
CPD	0.001	--0.319	--0.486	--0.365	0.228
IOW	0.214	--0.346	--0.104	--0.246	--0.023
MXL	0.349	0.152	0.168	0.306	0.478
MXW	0.067	--0.283	--0.013	0.319	--0.246
PCL	0.043	--0.034	--0.134	0.307	0.142
PVL	0.114	0.122	0.210	--0.525	0.273
ADL	0.096	0.354	0.235	0.097	--0.522
GRL	0.234	0.356	0.095	--0.069	--0.095
LAL	--0.024	0.043	0.047	--0.015	--0.255
GRS	--0.040	0.278	0.381	0.016	0.172
FRS	0.111	0.112	0.002	--0.139	0.307
λ	1.565	0.316	0.278	0.175	0.042
% of total	65.85	13.31	11.70	7.37	1.77
Significance[a]	***	***	***	**	N.S.

[a]NS, nonsignificant at P>0.05; ***=P<0.001; **=P<0.01; *=P<0.05.

Appendix IIb. Total sample standardized canonical coefficients for ratio morphometric and meristic variables for 1989 lake whitefish samples.

RATIOS	CAN 1	CAN 2	CAN 3	CAN 4	CAN 5
POL	0.709	-0.428	0.101	0.097	-0.280
OOL	0.124	1.028	0.014	-0.056	0.250
PSL	-0.228	-0.106	0.174	-0.523	-0.116
TTL	-0.032	0.144	-0.175	-0.076	0.157
DOL	-0.201	0.173	-0.106	-0.108	0.364
LUL	-0.169	0.216	-0.165	0.195	-0.296
ANL	0.231	-0.055	0.183	-0.311	0.086
CPL	-0.179	-0.124	0.135	-0.307	-0.041
HDD	-0.568	-0.461	0.072	0.056	-0.168
BDD	0.568	0.213	0.189	-0.232	-0.648
CPD	0.134	0.074	-0.634	-0.062	0.363
IOW	0.433	-0.149	-0.072	0.006	0.199
MXL	0.255	-0.118	0.386	-0.023	0.625
MXW	0.034	0.378	-0.521	0.002	-0.615
PCL	0.128	-0.162	-0.026	0.138	-0.150
PVL	0.004	0.179	0.094	-0.170	0.419
ADL	0.135	0.100	0.360	-0.128	-0.060
LAL	-0.108	0.051	0.142	0.016	-0.077
GRL	0.143	-0.344	0.328	0.049	-0.067
FRS	-0.001	0.008	0.015	0.348	0.343
GRS	-0.029	-0.071	0.422	0.520	-0.123
λ	1.126	0.351	0.242	0.100	0.026
% of total	60.96	19.03	13.12	5.45	1.44
Significance[a]	***	***	**	N.S.	N.S.

[a]NS, nonsignificant at P>0.05; ***=P<0.001; **=P<0.01; *=P<0.05.

MERISTICS	CAN 1	CAN 2	CAN 3	CAN 4	CAN 5
LLS	0.506	0.690	0.079	0.061	-0.560
SPS	0.224	0.019	0.053	-0.331	-0.094
ULS	0.177	0.385	0.435	-0.589	0.394
DRC	-0.027	0.512	-0.186	0.357	0.428
ARC	-0.396	0.401	-0.057	0.077	0.061
PRC	0.241	-0.408	0.518	0.522	0.269
VRC	0.140	-0.113	0.304	0.168	-0.018
UGR	0.599	-0.096	-0.509	-0.105	0.590
LGR	0.207	-0.295	-0.096	0.043	-0.315
λ	0.201	0.131	0.028	0.026	0.010
% of total	51.35	33.48	7.33	6.80	1.04
Significance [a]	***	*	N.S.	N.S.	N.S.

[a]NS, nonsignificant at P>0.05; ***=P<0.001; **=P<0.01; *=P<0.05.

Appendix IIc. Total sample standardized canonical coefficients for combined ratio morphometric and meristic variables for 1989 lake whitefish samples.

COMBINATION	*CAN 1*	*CAN 2*	*CAN 3*	*CAN 4*	*CAN 5*
POL	0.486	0.110	0.110	-0.379	-0.179
OOL	0.060	0.082	-0.514	0.273	0.357
PSL	-0.443	-0.078	0.225	0.478	-0.112
TTL	-0.321	-0.281	-0.226	0.400	-0.135
DOL	-0.127	-0.162	-0.260	0.283	-0.001
LUL	-0.238	0.188	-0.324	0.048	-0.305
ANL	0.133	-0.305	0.232	0.343	-0.039
CPL	-0.259	-0.092	0.269	0.051	0.085
HDD	-0.588	-0.180	0.433	-0.044	-0.131
BDD	0.600	-0.200	0.128	0.541	-0.214
CPD	-0.006	-0.458	-0.215	-0.499	-0.181
IOW	0.220	-0.027	-0.253	-0.306	0.007
MXL	0.296	0.196	0.165	0.299	0.263
MXW	0.100	-0.098	-0.299	0.192	-0.100
PCL	0.108	-0.234	-0.072	0.269	0.008
PVL	0.021	0.264	0.164	-0.481	0.170
ADL	0.169	0.202	0.209	0.148	-0.373
GRL	0.198	0.118	0.362	-0.044	-0.153
LAL	1.360	0.806	-2.254	1.088	-3.109
GRS	-1.813	-0.499	3.047	-1.278	3.855
FRS	0.065	-0.021	0.111	-0.127	0.200
LLS	-0.202	0.222	0.166	0.046	-0.430
SPS	-0.053	-0.024	-0.057	-0.145	-0.097
ULS	-0.182	0.114	-0.091	-0.066	0.109
DRC	-0.124	0.291	0.140	-0.005	-0.230
ARC	-0.027	0.402	-0.161	-0.143	-0.151
PRC	0.034	-0.211	0.004	0.160	0.564
VRC	-0.101	-0.108	0.228	-0.209	0.160
UGR	-0.067	-0.297	0.060	-0.335	-0.405
LGR	-1.308	-0.628	2.114	-0.946	2.964
λ	1.830	0.432	0.391	0.221	0.124
% of total	61.02	14.40	13.05	7.38	4.14
Significance[a]	***	***	***	*	N.S.

[a]NS, nonsignificant at P>0.05; ***=P<0.001; **=P<0.01; *=P<0.05.

Appendix IIIa Temporal ANOVA of 8 meristic counts and 18 morphometric measurements for Little Playgreen Lake.

UNIVARIATE F TESTS

VARIABLE	SS	DF	MS	F	P
AND	0.008	1	0.008	17.5	0.000
ERROR	0.045	99	0.000		
ARC	0.793	1	0.793	1.6	0.204
ERROR	47.959	99	0.484		
BDWD	0.001	1	0.001	3.3	0.072
ERROR	0.023	99	0.000		
CPD	0.001	1	0.001	53.4	0.000
ERROR	0.002	99	0.000		
DLAD	0.003	1	0.003	2.1	0.143
ERROR	0.139	99	0.001		
DRC	0.002	1	0.002	0.0	0.938
ERROR	33.047	99	0.334		
FRS	0.001	1	0.001	3.5	0.061
ERROR	0.017	99	0.000		
GRL	0.000	1	0.000	55.0	0.000
ERROR	0.000	99	0.000		
GRN	8.539	1	8.539	6.3	0.013
ERROR	132.906	99	1.342		
HDD	0.079	1	0.079	1436.3	0.000
ERROR	0.005	99	0.000		
IOW	0.000	1	0.000	0.1	0.676
ERROR	0.002	99	0.000		
LLS	25.625	1	25.625	2.6	0.109
ERROR	967.702	99	9.775		
VLO	0.000	1	0.000	0.1	0.659
ERROR	0.112	99	0.001		
MXL	0.001	1	0.001	44.9	0.000
ERROR	0.001	99	0.000		
MXW	0.000	1	0.000	2.5	0.113
ERROR	0.000	99	0.000		
PCL	0.000	1	0.000	0.6	0.414
ERROR	0.014	99	0.000		
PCO	0.000	1	0.000	1.1	0.279
ERROR	0.039	99	0.000		
PNCS	7.907	1	7.907	10.6	0.002
ERROR	73.400	99	0.741		
PPO	0.000	1	0.000	5.8	0.017
ERROR	0.005	99	0.000		
PRC	0.016	1	0.016	0.0	0.842
ERROR	40.657	99	0.411		
PSL	0.002	1	0.002	57.3	0.000
ERROR	0.004	99	0.000		
PVL	0.000	1	0.000	0.1	0.750
ERROR	0.045	99	0.000		
SPS	3.173	1	3.173	19.8	0.000
ERROR	15.837	99	0.160		
TTL	0.006	1	0.006	20.6	0.000
ERROR	0.030	99	0.000		
ULS	27.370	1	27.370	127.0	0.000
ERROR	21.324	99	0.215		
VLAL	0.002	1	0.002	2.3	0.132
ERROR	0.089	99	0.001		

MULTIVARIATE TEST STATISTICS

WILKS' LAMBDA =	0.030					
F-STATISTIC =	88.7	DF =	26,	73	PROB =	0.000
PILLAI TRACE =	0.970					
F-STATISTIC =	88.7	DF =	26,	73	PROB =	0.000
HOTELLING-LAWLEY TRACE =	32.832					
F-STATISTIC =	88.7	DF =	26,	73	PROB =	0.000

Appendix IIIb Temporal ANOVA of 8 meristic counts and 18 morphometric measurements for Grand Rapids.

UNIVARIATE F TESTS

VARIABLE	SS	DF	MS	F	P
AND	0.018	1	0.018	118.5	0.000
ERROR	0.015	102	0.000		
ARC	3.228	1	3.228	6.5	0.012
ERROR	50.157	102	0.492		
BDWD	0.001	1	0.001	4.7	0.032
ERROR	0.024	102	0.000		
CPD	0.006	1	0.006	432.6	0.000
ERROR	0.001	102	0.000		
DLAD	0.018	1	0.018	31.4	0.000
ERROR	0.060	102	0.001		
DRC	1.816	1	1.816	5.8	0.017
ERROR	31.713	102	0.311		
FRS	0.024	1	0.024	2.1	0.147
ERROR	1.169	102	0.011		
GRL	0.000	1	0.000	7.3	0.008
ERROR	0.000	102	0.000		
GRN	0.013	1	0.013	0.0	0.927
ERROR	153.333	102	1.503		
HDD	0.116	1	0.116	1654.7	0.000
ERROR	0.007	102	0.000		
IOW	0.001	1	0.001	92.8	0.000
ERROR	0.001	102	0.000		
LLS	29976.241	1	29976.241	1.8	0.173
ERROR	1625644.980	102	15937.696		
VLO	0.017	1	0.017	29.8	0.000
ERROR	0.060	102	0.001		
MXL	0.000	1	0.000	9.6	0.002
ERROR	0.001	102	0.000		
MXW	0.000	1	0.000	8.6	0.004
ERROR	0.000	102	0.000		
PCL	0.000	1	0.000	2.4	0.120
ERROR	0.012	102	0.000		
PCO	0.008	1	0.008	44.2	0.000
ERROR	0.019	102	0.000		
PNCS	1.023	1	1.023	1.4	0.233
ERROR	72.361	102	0.709		
PPO	0.002	1	0.002	25.7	0.000
ERROR	0.006	102	0.000		
PRC	2.910	1	2.910	6.9	0.010
ERROR	42.628	102	0.418		
PSL	0.003	1	0.003	15.0	0.000
ERROR	0.018	102	0.000		
PVL	0.000	1	0.000	5.1	0.026
ERROR	0.008	102	0.000		
SPS	6.626	1	6.626	29.3	0.000
ERROR	23.028	102	0.226		
TTL	0.004	1	0.004	3.6	0.058
ERROR	0.099	102	0.001		
ULS	483.010	1	483.010	2.2	0.140
ERROR	22291.980	102	218.549		
VLAL	0.001	1	0.001	2.7	0.100
ERROR	0.042	102	0.000		

MULTIVARIATE TEST STATISTICS

WILKS' LAMBDA =	0.025						
F-STATISTIC =	111.7	DF =	26,	76	PROB =	0.000
PILLAI TRACE =	0.975						
F-STATISTIC =	111.7	DF =	26,	76	PROB =	0.000
HOTELLING-LAWLEY TRACE =	39.698						
F-STATISTIC =	111.7	DF =	26,	76	PROB =	0.000

Appendix IIIc Temporal ANOVA of 8 meristic counts and 18 morphometric measurements for Big Black River.

UNIVARIATE F TESTS

VARIABLE	SS	DF	MS	F	P
AND	0.054	1	0.054	371.5	0.000
ERROR	0.015	103	0.000		
ARC	0.019	1	0.019	0.0	0.837
ERROR	47.409	103	0.460		
BDWD	0.028	1	0.028	345.2	0.000
ERROR	0.008	103	0.000		
CPD	0.002	1	0.002	124.6	0.000
ERROR	0.002	103	0.000		
DLAD	0.025	1	0.025	52.0	0.000
ERROR	0.049	103	0.000		
DRC	0.000	1	0.000	0.0	1.000
ERROR	33.200	103	0.322		
FRS	0.001	1	0.001	13.1	0.000
ERROR	0.009	103	0.000		
GRL	0.000	1	0.000	12.9	0.000
ERROR	0.000	103	0.000		
GRN	1.145	1	1.145	0.7	0.389
ERROR	157.845	103	1.532		
HDD	0.066	1	0.066	1180.2	0.000
ERROR	0.006	103	0.000		
IOW	0.000	1	0.000	36.2	0.000
ERROR	0.001	103	0.000		
LLS	23.501	1	23.501	2.1	0.145
ERROR	1121.489	103	10.888		
VLO	0.003	1	0.003	1.9	0.161
ERROR	0.141	103	0.001		
MXL	0.000	1	0.000	0.3	0.579
ERROR	0.004	103	0.000		
MXW	0.000	1	0.000	9.9	0.002
ERROR	0.001	103	0.000		
PCL	0.000	1	0.000	1.4	0.231
ERROR	0.013	103	0.000		
PCO	0.001	1	0.001	7.8	0.006
ERROR	0.017	103	0.000		
PNCS	0.500	1	0.500	0.6	0.431
ERROR	82.262	103	0.799		
PPO	0.002	1	0.002	24.0	0.000
ERROR	0.009	103	0.000		
PRC	5.586	1	5.586	4.3	0.039
ERROR	132.262	103	1.284		
PSL	0.006	1	0.006	132.5	0.000
ERROR	0.004	103	0.000		
PVL	0.001	1	0.001	6.9	0.010
ERROR	0.008	103	0.000		
SPS	68.119	1	68.119	0.8	0.355
ERROR	8114.929	103	78.786		
TTL	0.002	1	0.002	6.5	0.012
ERROR	0.028	103	0.000		
ULS	19.617	1	19.617	76.2	0.000
ERROR	26.516	103	0.257		
VLAL	0.040	1	0.040	32.3	0.000
ERROR	0.126	103	0.001		

MULTIVARIATE TEST STATISTICS

WILKS' LAMBDA =	0.032					
F-STATISTIC =	87.2	DF =	26,	77	PROB =	0.000
PILLAI TRACE =	0.968					
F-STATISTIC =	87.2	DF =	26,	77	PROB =	0.000
HOTELLING-LAWLEY TRACE =	30.611					
F-STATISTIC =	87.2	DF =	26,	77	PROB =	0.000

Appendix IV MDH-B1,2 phenotypic counts for 1989 and 1975 lake whitefish samples.

Year & Location	MDH-B1,2 Phenotypes				
	BBBB	BBBA	BBAA	BAAA	AAAA
1989					
Warren Landing	6	16	18	7	1
Gunisao River	7	22	14	6	1
Little Playgreen L.	5	12	20	12	1
Two Mile Channel	9	17	17	7	0
Big Black River	11	13	16	9	1
Grand Rapids	5	19	21	5	0
1975					
Warren Landing	5	15	21	10	1
Little Playgreen L.	6	12	20	10	1
Big Black River	9	13	20	11	2
Grand Rapids	8	20	21	6	0