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

BY

WILLIAM V. MAVROS

A Thesis

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in Partial Fulfilment of the Requirements
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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.

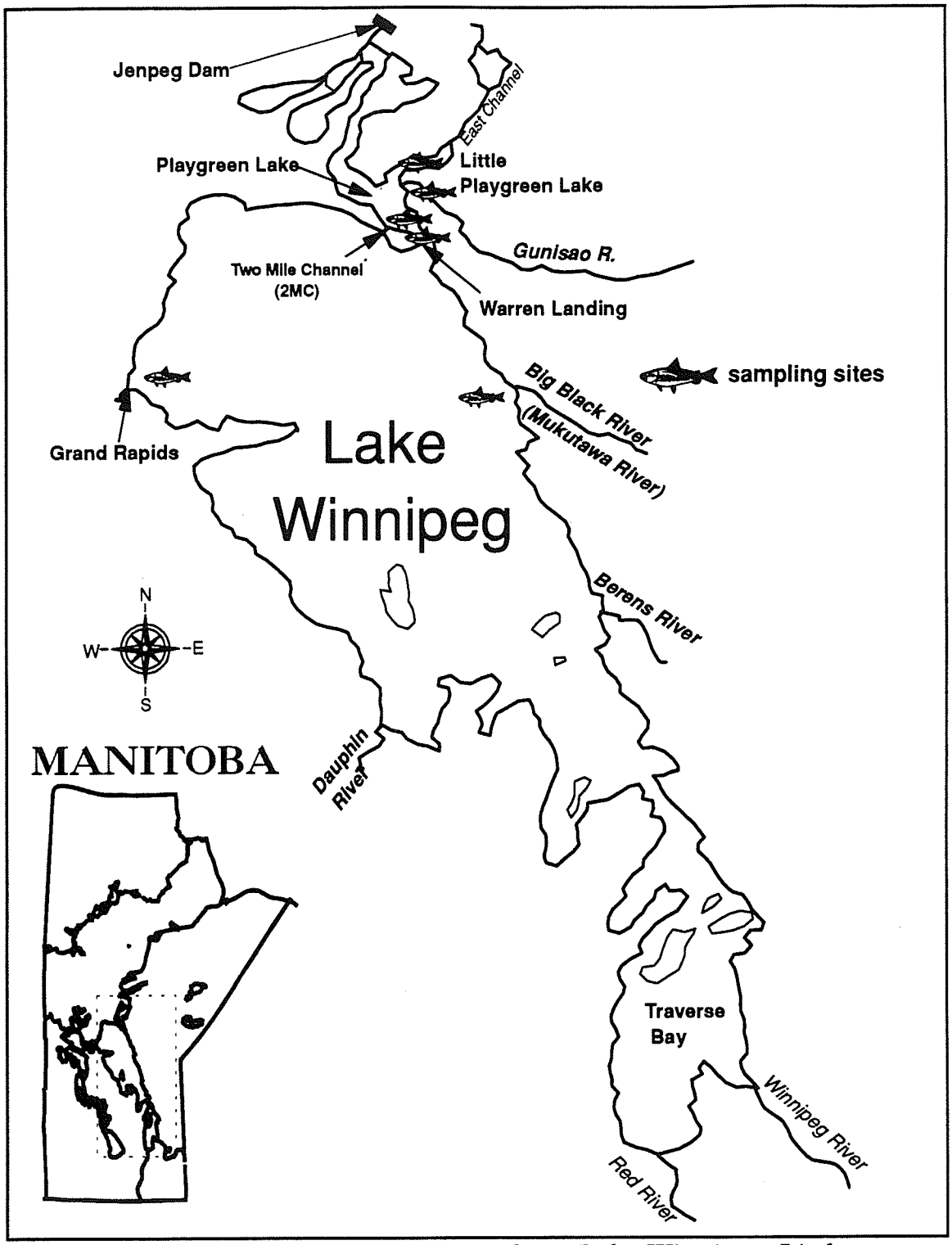

Fig. 1. Location of sampling sites in northern Lake Winnipeg, Little Playgreen Lake and Playgreen Lake.

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