Ecology and evolution of the deepwater sculpin *(Myoxocephalus thompsonii)*: conservation of a glacial relict

Tom A. Sheldon

Submitted for the Master of Science Degree
Department of Zoology
University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

Ecology and evolution of the deepwater sculpin *(Myoxocephalus thompsonii)*: conservation of a glacial relict

BY

Tom A. Sheldon

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

OF

MASTER OF SCIENCE

Abstract

The deepwater sculpin, *Myoxocephalus thompsonii*, is a North American lake-dwelling fish species whose distribution, biology, habitat and taxonomic structure are poorly understood. A survey of deepwater sculpin across its range resulted in four new distribution records. In some lakes where deepwater sculpin had been previously reported, none were collected despite intensive effort. Diet analyses revealed the importance of *Diporeia* spp., *Mysis* and chironomid larvae to deepwater sculpin, while age analyses suggest that deepwater sculpin are much longer-lived than previously thought. Habitat analyses suggest that the presence of deepwater sculpin is intimately linked to highly oligotrophic lakes and, more specifically, extremely low (<7 °C) benthic water temperatures. Phylogenetic analyses of the mitochondrial DNA control region and ATPase6,8 sequences of *Myoxocephalus thompsonii* from 23 inland lakes across Canada, as well as marine and freshwater fourhorn sculpin (*M. quadricornis*) from 11 locations across the arctic support the distinction of deepwater sculpin and fourhorn sculpin (including freshwater fourhorn sculpin) as two distinct species. Additionally, three well-defined mitochondrial haplotype lineages of deepwater sculpin within Canada support the historical isolation of deepwater sculpin in Mississippian, Southwestern, and Atlantic refugia.

Acknowledgements

First and foremost, Dr. Nathan Lovejoy was a great advisor. He was always relaxed, helpful and, most importantly, insightful. I could not have asked for a better person to work with throughout the duration of my graduate studies. I also thank Dave Boguski, who spent an entire summer assisting with the collection of deepwater sculpin across Canada. Sometimes a thankless job with countless hours of field work and travel, he was able to put up with me for over four months. I hope that the sights of Canada, its incredible lakes, and unique regions made the summer worthwhile. Dr. Nick Mandrak was also always willing to take time out of a busy schedule to help in any way possible whenever asked. He was instrumental in the completion of my degree. Dr. Chris Wilson was also kind enough to provide advice and help. I am also thankful to Doug Watkinson and Dr. William Franzin, who got me started with fisheries work during my undergraduate years. They continued to provide both support and advice throughout the course of my graduate research. I especially thank them for lending a boat for an entire summer. Dr. Ken Mills also generously provided his time and energy throughout my graduate work. Dr. Ken Stewart was a fountain of wisdom and knowledge. DFO, the Great Lakes Fishery Commission, NSERC, and the Northern Scientific Training Program provided financial support. Most importantly, however, my family has provided continual support for 24 years. Thank you Mandy, for being such an important part of my life. I look forward to the rest of our lives sharing great experiences. Mom, Dad, Don, Jacq and Pat: thank you. Everything good that I may be lucky enough to achieve in life is a reflection of our family and the roots from which I've grown. I hope that I can make a difference, as you have in my life.

Preface

Deepwater sculpin are the unifying element of the thesis. However, the manuscript format of the thesis results in three central chapters, each intended to be an independent unit that has been, or will be, submitted to a peer-reviewed journal for publication. As such, each central chapter has a separate abstract, introduction, results and discussion. Because of the manuscript style of the thesis, some content may be reviewed in more than one chapter, where appropriate.

The introduction to the thesis reviews the limited available information on deepwater sculpin, identifies current gaps in knowledge, and introduces the topics and issues addressed by my research. The three central chapters address the (1) distribution, (2) biology and habitat and (3) evolution of deepwater sculpin. The thesis discussion synthesizes the three central chapters and addresses conservation implications in light of the newly available data.

Table of Contents

Thesis Introduction

Freshwater ecosystems within Canada are an essential resource. Further, Canada is often viewed as being synonymous with vast amounts of wilderness, particularly our freshwater ecosystems and the diversity of species within them. Many Canadian lakes and their resources have been studied extensively. However, the top predators within aquatic systems garner the most attention. The mid-level consumers that are important for overall lake health, food web dynamics, and energy transfer to higher trophic levels are sometimes ignored or overlooked. A good understanding of these relatively unknown resources is important for ensuring the long-term sustainability of freshwater resources and the preservation of biodiversity.

The deepwater sculpin, *Myoxocephalus thompsonii* (Girard 1852) is generally viewed as an under-studied, mid-trophic level, freshwater fish species within Canada (Scott and Crossman 1973). The range of deepwater sculpin in Canada stretches from the Gatineau region of Quebec through the Laurentian Great Lakes, continuing through Manitoba and Saskatchewan, and northwest to Great Slave and Great Bear Lakes (Parker 1988; Scott and Crossman 1973). An additional population occurs in the Waterton Lakes of southwestern Alberta (Parker 1988; Scott and Crossman 1973; McAllister and Ward 1972). Within its range, the deepwater sculpin is restricted to the bottoms of the deepest lakes (Stewart and Watkinson 2004).

The deepwater sculpin has been studied in the Laurentian Great Lakes; including habitat (Selgeby 1988; Brandt 1986), diet (Selgeby 1988; Brandt 1986; Kraft and Kitchell

1986) and age analyses (Selgeby 1988). However, owing to logistical constraints, few studies have investigated the biology of the species outside the Laurentian Great Lakes. An exception is a single study on the parasites of deepwater sculpin from Burchell Lake, ON (Black and Lankester 1981). The limited investigations of deepwater sculpin are largely due to the enormous logistics and effort required to catch small benthic fish that reside at great depths within lakes. These significant logistical constraints are further compounded by the remoteness of many lakes where deepwater sculpin occur. Part of the thesis work described here was designed to fill in the knowledge gaps about deepwater sculpin biology and habitat.

Even with the lack of knowledge of the biology and habitat of deepwater sculpin, speculation on both the origins and taxonomy of deepwater sculpin has been on-going for fifty years (Scott and Crossman 1973; McAllister 1961). The information published on this topic, however, is still limited. Deepwater sculpin have attracted the attention of zoogeographers because they are considered to be a "glacial relict" that originated from arctic marine waters. Morphometrics, combined with incomplete knowledge of distribution patterns has accounted for the vast majority of the zoogeographic studies on deepwater sculpin (Scott and Crossman 1973). These studies have resulted in speculations regarding deepwater sculpin origins and debates regarding the proper taxonomy and nomenclature of the species (McAllister 1961). The few studies published have focused on the relationship between deepwater sculpin and its closest relative, the marine fourhorn sculpin, *M. quadricornis* (Linnaeus 1758). Despite the potential usefulness of genetic data for resolving these issues and for understanding glacial zoogeography, there has been only a single study that has investigated the phylogeography of deepwater sculpin. This thesis considerably expands upon the limited available information to significantly improve our understanding of deepwater sculpin origins, phylogeography and post-glacial dispersal.

Despite the lack of overall knowledge of deepwater sculpin, the species is thought to be an important part of the benthic community and deepwater lacustrine food chains (Scott and Crossman 1973). It has been shown that deepwater sculpin are an integral food item in the diet of deepwater piscivores, such as lake trout (*Salvelinus namaycush*), burbot (*Lota lota*) and alewife (*Alosa pseudoharengus*) (Stewart and Watkinson 2004; Murray et al. 2003; Madenjian et al. 2002; Day 1983). Most importantly, however, the deepwater sculpin is thought to be an excellent indicator of the health or well-being of deepwater fish communities and habitat (Casselman and Scott 2003; Mills et al. 2003). Thus, improved understanding of the species will be of significant interest for biologists concerned with the health and conservation of Canadian biodiversity.

To fill gaps in our limited knowledge of deepwater sculpin, an extensive survey of the species was completed across Canada in 2004. Following the survey, studies were completed to enhance our understanding of the biology, habitat, and evolution of the species. These are described in the following three chapters.

The first chapter of this thesis reviews the 2004 survey of deepwater sculpin across Canada. A trap specifically designed to capture deepwater sculpin is described.

The chapter reviews locations where deepwater sculpin were collected for the first time as well as locations where deepwater sculpin were not collected despite previous reports of their presence. Possible explanations for their apparent absence from lakes where they were previously reported are briefly explored. The updated distribution of deepwater sculpin is then used to explore the origins and current conservation of the species.

The second chapter of this thesis describes the biology, habitat and conservation of deepwater sculpin throughout its range. The habitat of deepwater sculpin is quantified using multivariate statistical techniques that compare the benthic physicochemical parameters of lakes where deepwater sculpin were captured during the 2004 survey and lakes where sampling failed to detect the species presence. Results suggest that slight eutrophication of lakes could result in the extirpation of deepwater sculpin populations.

The third chapter of this thesis describes the evolution and phylogeographic origins of deepwater sculpin. Mitochondrial DNA is used to determine the relationship of deepwater sculpin and its close relative, the marine fourhorn sculpin. Deepwater sculpin origin(s) and dispersal mechanisms are reviewed in light of the genetic data. Finally, phylogeographic analyses of deepwater sculpin are used to investigate the presence of haplotype lineages throughout the species range. Hypotheses of the mechanisms of diversification and subsequent dispersal are then discussed.

A final discussion summarizes the findings of the thesis and provides recommendations for the conservation and monitoring of deepwater sculpin throughout its range. Suggestions for future research directions are proposed.

References

Black, G.A., and M.W. Lankester. 1981. The biology and parasites of deepwater sculpin, *Myoxocephalus quadricornis thompsonii* (Girard), in Burchell Lake, Ontario. Can. J. Zool. 59:1454-1457.

Brandt, S.B. 1986. Disappearance of the deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Ontario: the keystone predator hypothesis. J. Great Lakes Res. 12(1):18-24.

Casselman, J.M., and K.A. Scott. 2003. Fish-community dynamics of Lake Ontario - Long-term trends in the fish populations of eastern Lake Ontario and the Bay of Quinte. *In* State of Lake Ontario: Past, present and future. *Edited by* M. Munawar. Ecovision World Monograph Series, Aquatic Ecosystem Health & Management Society. pp. 349-383.

Day, S.C. 1983. Biological population characteristics, and interactions between an unexploited Burbot (*Lota lota*) population and an exploited lake trout (*Salvelinus namaycush*) population from Lake Athapapuskow, Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Girard, G. 1852. Contributions to the natural history of the freshwater fishes of North America. I. A monograph of the cottids. Smiths. Contrib. Knowl. 3 (article 3). 80 pp.

Kraft, C.E., and J.F. Kitchell. 1986. Partitioning of food resources by sculpins in Lake Michigan. Environ. Biol. Fishes 16(4):309-316.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis, Laurentii Salvii, Holmiae, 12th Edition, Volume 1.

Madenjian, C.R., G.L. Fahnenstiel, T.H. Johengen, T.F. Nalepa, H.A. Vanderploeg, G.W. Fleischer, P.J. Schneeberger, D.M. Benjamin, E.B. Smith, J.R. Bence, E.S. Rutherford, D.S. Lavis, D.M. Robertson, D.J. Jude, and M.P. Ebener. 2002. Dynamics of the Lake Michigan food web, 1970-2000. Can. J. Fish. Aquat. Sci. 59:736-753.

McAllister, D.E. 1961. The origin and status of the deepwater sculpin, *Myoxocephalus thompsonii*, a Nearctic glacial relict. Nat. Mus. Can. Bull. 172:44-65.

McAllister, D.E., and J.C. Ward. 1972. The deepwater sculpin, *Myoxocephalus quadricornis thompsoni*, new to Alberta, Canada. J. Fish. Res. Bd. Can. 29:344-345.

Mills, E.L., J.M. Casselman, R. Dermott, J.D. Fitzsimons, G. Gal, K.T. Holeck, J.A. Hoyle, O.E. Johannsson, B.F. Lantry, J.C. Makarewicz, E.S. Millard, I.F. Munawar, M. Munawar, R. O'Gorman, R.W. Owens, L.G. Rudstam, T. Schaner, and T.J. Stewart. 2003. Lake Ontario: food web dynamics in a changing ecosystem (1970-2000). Can. J. Fish. Aquat. Sci. 60:471-490.

Murray, L., M.H. Papst, and J.D. Reist. 2003. First record of the Deepwater Sculpin, *Myoxocephalus thompsonii*, from George Lake in Whiteshell Provincial Park, Manitoba. Can. Field-Nat. 117(4):642-644.

Parker, B.J. 1988. Status of the deepwater sculpin, *Myoxocephalus thompsoni*, in Canada. Can. Field-Nat. 102(1):126-131.

Scott, W.B., and E.J. Crossman. 1973. The freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184. 966 pp.

Selgeby, J.H. 1988. Comparative biology of the sculpins of Lake Superior. J. Great Lakes Res. 14:44-51.

Stewart, K.W., and D.A. Watkinson. 2004. The freshwater fishes of Manitoba. The University of Manitoba Press, Winnipeg, Manitoba. 276 pp.

Chapter 1: Current distribution of the deepwater sculpin (*Myoxocephalus thompsonii*) including four new locality records.

Abstract

Although the freshwater icthyofauna of Canada is well-studied, the distribution and biology of the deepwater sculpin (*Myoxocephalus thompsonii*) remains poorly understood. Information gaps for the species are due, in part, to the remote locations and associated logistic challenges of sampling ecologically suitable lakes. This is compounded by the isolation of the species at great depths within these lakes. To investigate the distribution of the deepwater sculpin, an intensive field sampling program was carried out between May and October 2004. Fishes were collected using a variety of sampling gear, including a trap that was specifically designed to capture deepwater sculpin. The survey included 35 lakes across Canada, including both localities where deepwater sculpin had previously been captured, and localities where deepwater sculpin had not yet been reported but were expected to be present based on lake bathymetry and postglacial history. A total of 155 specimens were collected from 20 lakes. In some lakes where deepwater sculpin had previously been reported, none were collected despite intensive effort. However, deepwater sculpin were collected for the first time in four lakes in northwestern Ontario (Teggau and Eagle lakes) and Manitoba (Clearwater and Second Cranberry lakes). The collection of deepwater sculpin from Second Cranberry Lake (MB) represents the first record of deepwater sculpin from the Nelson River drainage.

Keywords

Deepwater sculpin, *Myoxocephalus thompsonii*, distribution, Canada, zoogeography, Pleistocene, glaciation, conservation, minnow trap

Introduction

The deepwater sculpin (*Myoxocephalus thompsonii*) is one of the most poorly understood freshwater fishes in Canada. It lives at the bottom of very deep, cold lakes. The deepest recorded specimens have been captured at 366 m from Great Bear Lake and Lake Superior (Scott and Crossman 1973). The deepwater sculpin is both long-lived and slow growing, and is only known to occur in unproductive oligotrophic lakes (see Chapter 2). The species can be an important part of the benthic community and deepwater lacustrine food chains (Scott and Crossman 1973). It is a primary consumer of *Diporeia* spp., *Mysis* spp., and chironomid larvae (see Chapter two; Geffen and Nash 1992; Selgeby 1988; Kraft and Kitchell 1986; Brandt 1986; Black and Lankester 1981). It is also an integral food item in the diet of deepwater piscivores, such as lake trout (*Salvelinus namaycush*), burbot (*Lota lota*) and alewife (*Alosa pseudoharengus*) (Stewart and Watkinson 2004; Murray et al. 2003; Madenjian et al. 2002; Day 1983). One of the primary determinants of deepwater sculpin abundance in Lake Michigan is predation levels by burbot and alewife (Madenjian et al. 2005; Madenjian et al. 2002). Perhaps most importantly, the deepwater sculpin is thought to be an excellent indicator of the health or well-being of deepwater fish community and habitat (Casselman and Scott

2003; Mills et al. 2003). The deepwater sculpin is also thought to be negatively affected by contaminants and eutrophication within lakes (see chapter 2; Parker 1988).

The range of deepwater sculpin in Canada stretches from the Gatineau region of Quebec through the Laurentian Great Lakes, continuing through Manitoba and Saskatchewan, and northwest to Great Slave and Great Bear Lakes (Parker 1988; Scott and Crossman 1973). An additional population occurs in the Waterton Lakes of southwestern Alberta (Parker 1988; Scott and Crossman 1973). Distribution records indicate that deepwater sculpin are almost entirely endemic to Canada. Prior to this study, deepwater sculpin had been reported from 57 lakes in Canada (Appendix) and only four from the United States. Deepwater sculpin have been recorded from all of the Laurentian Great Lakes; however, the presence of a reproducing population within Lake Erie has never been reported. Juveniles recorded within Lake Erie are most likely due to larval drift from Lake Huron as Lake Erie is probably too shallow and eutrophic to sustain reproducing populations of deepwater sculpin (Roseman et al. 1998). Deepwater sculpin were also thought to be extirpated from Lake Ontario, but have since been reported in extremely low numbers (Parker 1998; Casselman and Scott 1993; Brandt 1986).

The deepwater sculpin has been called a "glacial relict" because of its origins and close relationship to the marine fourhorn sculpin, *Myoxocephalus quadricornis* (Linnaeus 1758). It is thought to have been derived from the fourhorn sculpin during the Pleistocene glaciations (Kontula and Vainola 2003; Dadswell 1972; McAllister 1961).

The proglacial lakes, which formed at the foot of the large receding glaciers during the Pleistocene, facilitated the dispersal of deepwater sculpin throughout their range. Therefore, the current distribution of the species is a product of historical connections among proglacial lakes and the present environmental conditions of the lakes themselves (Dadswell 1974). The highly disjunct nature of deepwater sculpin distribution is most likely a result of the patchy occurrence of lakes with suitable environmental conditions (very deep and cold) that were also accessable via proglacial routes of dispersal (Parker 1988).

Despite the interesting zoogeography of the deepwater sculpin, the distribution of the species across its range remains poorly understood, and the known localities of deepwater sculpin outside the Laurentian Great Lakes may not adequately reflect their full distribution. Information gaps are due, in part, to the remote locations and associated logistic challenges of sampling ecologically suitable lakes, as well as the isolation of the species at great depths within lakes (Murray et al. 2003). Because of this, many records of the species are incidental catch reports. Furthermore, many of these reports are from the 1960s and 1970s with little to no re-sampling of lakes specifically for deepwater sculpin to verify the continued presence of the species (Dadswell 1974; Dadswell 1972; McAllister and Ward 1972; Delisle and Van Vliet 1968). Consequently, distributional information for deepwater sculpin outside the Laurentian Great Lakes is limited, in many cases, to data that are 30 to 40 years old.

In light of the importance of deepwater sculpin as an indicator of the health of deepwater fish communities and habitat, as well as the limited current distributional knowledge of the species throughout its entire range, we conducted a survey for deepwater sculpin across Canada in 2004. The goal of the survey was to confirm historical records and discover new localities for deepwater sculpin outside the Laurentian Great Lakes. At the same time, we tested the capture efficiency of a newly designed, collapsible, square minnow trap.

Methods

We conducted an intensive field sampling program targeting deepwater sculpin between May and October, 2004. Our sampling effort spanned most of the known distribution of deepwater sculpin, ranging from Alexie Lake in the Northwest Territories in the northwestern portion of its range, to Thirty-One Mile Lake in Quebec in the extreme east, and Upper Waterton Lake in Alberta in the extreme southwest (Figure 1.1). The survey included two categories of lakes: (a) those where deepwater sculpin had previously been reported; and, (b) those without historical records of deepwater sculpin, but with bathymetry and postglacial history that suggested the possible presence of the species.

Equipment and procedures were designed to specifically target deepwater sculpin. Previous sampling efforts relied largely on trawling (Dadswell 1974; Dadswell 1972), with varying degrees of success. We deployed several complementary sampling methods, with greatly increased capture efficiency.

To specifically target benthic fish species such as the deepwater sculpin, we designed collapsible minnow traps with quarter inch galvanized steel mesh. These benthic minnow traps were designed to lie flat on the bottom and offer the maximum possible catchment area at 0-15 cm above the lake bed. It was important that the benthic minnow traps be collapsible in order to reduce volume for both charter and commercial flights. Their dimensions were 90 cm length x 45 cm width x 15 cm height (Figure 1.2). The mouth of the catchment area was 45 cm wide by 15 cm high, and this funneled into the trap with a final entrance size of 6.25 x 5 cm. The funnel length was 25 cm. The reduced height of the trap, combined with the length of the funnel, caused the slope angle of the funnels to be 11.5°. This is extremely low and minimized the distance fishes needed to travel vertically. We predicted that this, along with the large trap catchment would significantly increase catch per unit effort of deepwater sculpin relative to traditional basket minnow traps. During a trial period, equal numbers of these benthic minnow traps and traditional basket minnow traps were deployed randomly throughout the sampling area in the first 11 lakes. Of 46 deepwater sculpin captured in the traps, 40 were captured in the benthic minnow traps. Subsequent to this trial period, only benthic minnow traps were used.

In each lake, fifteen to thirty traps were baited with dog biscuits and cyalume (glow) sticks, and set for at least 12 hours. These traps were re-set up to five times, with each set lasting approximately 12 hours, until a minimum of five deepwater sculpin were captured. A 1.0 cm stretched mesh gillnet (1 m high x 15 m long panel) was also set for

12 hours and a minimum of two bottom trawls of 10 minutes in duration were also conducted in each lake, weather permitting. Trawling methods were modeled after Dadswell (1974), using an exact replicate of a small otter trawl that was successfully employed during the course of his work on benthic crustaceans and fishes in eastern North America. In our study, a 4.9 m (16 foot) boat equipped with a 25 horsepower engine was used to tow the trawl across the lake bottom at approximately 3-5 km/h. A ratio of approximately 3:1 was used to gauge tow rope length to trawling depth (Dadswell 1974). All sampling was conducted in the deeper regions of each lake as indicated by bathymetric maps and local knowledge. Depths were confirmed using a Garmin sonar depth finder. Trawls were conducted in two transects across the deepest part of each lake, whenever possible.

Upon capture, deepwater sculpin were removed from fishing gear and anaesthetized in 0.5-0.6 mL/L of 2-Phenoxyethanol. Gill and fin clips were then taken from all deepwater sculpin for genetic analyses. Deepwater sculpin were subsequently preserved in a 10% buffered formalin solution and then transferred to 70% ethanol for long-term storage. This allowed immediate preservation of gut contents, while ensuring the otoliths remained undamaged. Complete necropsies were done for other studies on all individuals. Where possible, one female and one male from each locality were deposited in the Royal Ontario Museum.

Results

We collected a total of 155 deepwater sculpin specimens. The species was recorded from 20 out of the 35 lakes sampled. Table 1.1 summarizes the results of the 2004 survey. Deepwater sculpin were collected in lakes throughout their range within Canada, outside of the Laurentian Great Lakes. Lakes where deepwater sculpin were captured near the boundary of the species range included Alexie Lake in the Northwest Territories in the northwestern portion of its range, Thirty-One Mile Lake in Quebec in the extreme east, and Upper Waterton Lake in Alberta in the extreme southwest (Figure 1.1). In addition, deepwater sculpin were discovered in four lakes where they have not previously been recorded: Eagle and Teggau lakes in northwestern Ontario, and Clearwater and Second Cranberry lakes in northwestern Manitoba (Figure 1.1). The occurrence of deepwater sculpin in Second Cranberry Lake is the first record of deepwater sculpin from the Nelson River watershed of Manitoba. Results of the 2004 survey, combined with historical distribution records, bring the total number of Canadian lakes where deepwater sculpin have been found to 61.

Our sampling failed to confirm the presence of deepwater sculpin in some lakes where it had previously been documented. These included Lac des Iles and Heney Lake in the Gatineau region of Quebec, Cedar Lake in Algonquin Provincial Park in Ontario, Lake 310 and Lake of the Woods in northwestern Ontario, and Mirond Lake and Lac La Ronge in northeastern Saskatchewan.

Discussion

Our survey confirms that deepwater sculpin are restricted to deep, oligotrophic lakes in areas that were formerly covered by ice sheets as well as proglacial lakes or their outlets. Confirmation of historical distribution records and identification of new distribution records during the 2004 survey re-affirmed this pattern. Each of the four lakes where deepwater sculpin were newly discovered in the 2004 survey were in areas formerly occupied by both the Wisconsinan ice sheet and glacial Lake Agassiz (Dyke and Prest 1986). These four lakes were oligotrophic and ranged from a maximum depth of 33 m (Eagle Lake) to 165 m (Teggau Lake). All lakes where historical records of deepwater sculpin were confirmed were also oligotrophic.

The new records of deepwater sculpin from Eagle Lake in northwestern Ontario, as well as Clearwater and Second Cranberry lakes in northwestern Manitoba, suggest that deepwater sculpin may be present in fairly accessible and popular fishing lakes. Each of these lakes has a substantial number of fishing lodges, some of which are active for over five months of the year. However, deepwater sculpin had remained undetected in all three lakes. This was most likely due to emphasis on sport-fishing species, such as lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) and the difficulties associated with the identification of sculpin species from the gut contents of these deepwater piscivores, if gut contents were examined at all. The presence of deepwater sculpin in both Teggau and Eagle lakes within the Experimental Lakes Area of northwestern Ontario suggests that deepwater sculpin may remain undetected in lakes where targeted sampling has not occurred. This is most likely due to the difficulty inherent in catching smaller fish at the bottom of these very deep lakes. Scientific fish surveys have been carried out in both of these lakes, yet deepwater sculpin have not been recorded in either (K. Mills pers. comm.). This indicates that sampling to detect the presence of deepwater sculpin must be targeted, as general fish surveys without the appropriate sampling equipment may not yield true indications of presence or absence. These four new distribution records also suggest that the presence of deepwater sculpin in other deep, remote lakes throughout their range is a strong possibility.

During the 2004 survey, we failed to capture deepwater sculpin in seven lakes from where they were previously reported. There are a number of possible explanations for this failure to detect presence. First, deepwater sculpin may persist in these lakes, but sampling during the 2004 survey may have been inadequate to re-confirm their presence (false negatives). This may be the case for lakes such as Lac La Ronge and Mirond Lake, which still seem suitable for deepwater sculpin. Sampling of these lakes occurred in a restricted area of less than 2 km^2 due to extremely high winds. Because of this, sampling may not have been in an area with adequate numbers of deepwater sculpin to achieve detection with the search effort invested. Both lakes, especially Lac La Ronge, are very large and sampling a small proportion of lake area may simply indicate the absence of deepwater sculpin from that specific area rather than the lake as a whole.

A more concerning explanation for the absence of deepwater sculpin from some lakes in which they were previously documented may be that changing lake conditions have resulted in their extirpation. Generally, deepwater sculpin are restricted to deep, cold, oligotrophic lakes (see Chapter 2; Stewart and Watkinson 2004; Scott and Crossman 1972). In fact, all lakes where deepwater sculpin occur have relatively low nutrient concentrations and low biological production rates. This is reflected in increased dissolved oxygen levels and secchi disc visibilities as well as decreased temperature and total dissolved solids in the benthic environment (described in more detail in Chapter 2). However, two lakes sampled in the Gatineau region of Quebec were relatively eutrophic or mesotrophic. Lac des Iles and Heney Lake were sampled extensively for deepwater sculpin, without success. Both of these lakes had benthic temperatures (8.3 °C and 7.2 °C respectively) and dissolved oxygen levels (6.07 and 3.18 mg/L respectively) that were outside the ranges recorded from the 20 lakes where deepwater sculpin were found (3.15 - 6.93 °C and 6.74 - 14.44 mg/L) during the survey (see Chapter 2). These data suggest that the conditions within Lac des Iles and Heney Lake have gravitated towards eutrophication and, as a result, are less suitable for deepwater sculpin. Although it is possible that deepwater sculpin are still present within these lakes and sampling during the 2004 survey was a false negative, the habitat conditions are alarming. These results indicate that deepwater sculpin may have been extirpated from these lakes, or possibly that deepwater sculpin are stressed within these lakes and population levels may be declining, eventually to the point of extirpation, if action is not taken to reverse the effects of eutrophication.

Finally, the failure to collect deepwater sculpin from some lakes where they were previously recorded may be due to previously inaccurate species identifications, with other species of sculpin mistakenly identified as deepwater sculpins. This possibility seems likely in Cedar Lake, ON, where a single juvenile deepwater sculpin, 10 mm in length, was captured in a bottom trawl over 40 years ago and none have been captured since (Martin and Chapman 1965). Intense sampling of the lake over a three-day period in August 2004 yielded 113 sculpins, all of which were spoonhead sculpins (*Cottus ricei*).

The 2004 survey strengthens the previously held theory that the origin and distribution of the deepwater sculpin is the result of Pleistocene glaciations and dispersal via large proglacial lakes (Kontula and Vainola 2003; McAllister and Ward 1972; McPhail and Lindsey 1970). Aside from the isolated population in Upper Waterton Lake, AB, the remaining populations of deepwater sculpin show a relatively continuous distribution in ecologically suitable lakes across Canada in an area once covered by the Wisconsinan ice sheet (Mandrak and Crossman 1992). Furthermore, the 2004 survey confirms all lakes with deepwater sculpin (except Upper Waterton Lake), including the new distribution records, were also in areas that were occupied by proglacial lakes McConnell, Agassiz, Algonquin, Ojibway, or were in close proximity to them and directly connected to their former outlets (Barnett and Bajc 2002; Dyke and Prest 1986; Eschman and Karrow 1985; Karrow and Calkin 1985). This confirms that the current distribution of deepwater sculpin is intimately tied to the Pleistocene glaciations and proglacial lake formations.

The renewed account of deepwater sculpin distribution also suggests the complete absence of dispersal beyond glacial lake boundaries throughout Canada, subsequent to the Wisconsinan proglacial lake phase. Dispersal of deepwater sculpin between lakes has most likely not occurred since the late stages of the proglacial lake phase of the Wisconsinan glaciation. Rare drift of pelagic larvae has been shown to occur downstream of Lake Huron through the St. Clair River to Lake Erie (Roseman et al. 1998). However, reproducing populations of deepwater sculpin have not been established in Lake Erie (Roseman et al. 1998). This further emphasizes that the distribution of deepwater sculpin throughout their range is entirely due to dispersal through proglacial lakes and their outlets, and the current distribution of deepwater sculpin in Canada is static, rather than dynamic. Dispersal from lake to lake resulting in newly founded reproducing populations is highly unlikely. This suggests that deepwater sculpin are unable to expand their range or exploit newly suitable habitat, should it become available. This makes the species extremely vulnerable to local extirpation should their current habitats become eutrophied or otherwise disturbed.

The 2004 survey has enhanced understanding of the present distribution of deepwater sculpin. The discovery of four new locality records is important, as current information about the species outside the Laurentian Great Lakes basin is limited. The survey also revealed the possible disappearance, or stress, of deepwater sculpin from certain lakes within their range due to eutrophication. Finally, the survey confirms that dispersal of deepwater sculpin has likely not occurred since the late stages of the proglacial lake phase, nor is it expected to occur in the future. Thus, deepwater sculpin offer a tremendous opportunity to study the impacts of glaciation on a widespread fish species within Canada. However, without the proper conservation measures, this opportunity may be lost. It is hoped that this study will provide a baseline for more effective research on the history, management, and conservation of the deepwater sculpin.

References

Barnett, P.J., and A.F. Bajc. 2002. Quaternary geology. *In* The Physical Environment of the City of Greater Sudbury. *Edited by* D.H. Roussel and K.J. Jansons. Ont. Geol. Surv. Spec. Vol. 6. pp. 56-85.

Black, G.A., and M.W. Lankester. 1981. The biology and parasites of deepwater sculpin, *Myoxocephalus quadricornis thompsonii* (Girard), in Burchell Lake, Ontario. Can. J. Zool. 59:1454-1457.

Brandt, S.B. 1986. Disappearance of the deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Ontario: the keystone predator hypothesis. J. Great Lakes Res. 12(1):18-24.

Casselman, J.M., and K.A. Scott. 2003. Fish-community dynamics of Lake Ontario - Long-term trends in the fish populations of eastern Lake Ontario and the Bay of Quinte. *In* State of Lake Ontario: Past, present and future. *Edited by* M. Munawar. Ecovision World Monograph Series, Aquatic Ecosystem Health & Management Society. pp. 349-383.

Dadswell, M.J. 1972. Post-glacial dispersal of four freshwater fishes on the basis of new distribution records from eastern Ontario and western Quebec. J. Fish. Res. Bd. Can. 29:545-553.

Dadswell, M.J. 1974. Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. Nat. Mus. Nat. Sci. Pub. Zool. 11:1-110.

Day, S.C. 1983. Biological population characteristics, and interactions between an unexploited Burbot (*Lota lota*) population and an exploited lake trout (*Salvelinus* *namaycush*) population from Lake Athapapuskow, Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Delisle, C. and W. Van Vliet. 1968. First records of the sculpins *Myoxocephalus thompsonii* and *Cottus ricei* from the Ottawa Valley, southwestern Quebec. J. Fish. Res. Bd. Can. 25:2733-2737.

Dyke, A.S., and V.K. Prest. 1987. Late Wisconsinan and Holocene history of the Laurentide Ice Sheet. Geogr. Phys. Quat. 41:237-263.

Eschman, D.F. and P.F. Karrow. 1985. Huron basin glacial lakes: a review. *In* Quaternary Evolution of the Great Lakes. *Edited by* P.F. Karrow and P.E. Calkin. Geol. Assoc. Can. Spec. Pap. No. 30. pp. 79-93.

Geffen, A.J., and R.D.M. Nash. 1992. The life-history strategy of deepwater sculpin, *Myoxocephalus thompsoni* (Girard), in Lake Michigan: dispersal and settlement patterns during the first year of life. J. Fish Biol. 41 (supplement B): 101-110.

Girard, G. 1852. Contributions to the natural history of the freshwater fishes of North America. I. A monograph of the cottids. Smiths. Contrib. Knowl. 3 (article 3). 80 pp.

Karrow, P.F., and P.E. Calkin. 1985. Quaternary evolution of the Great Lakes. Geol. Assoc. Can. Spec. Pap. 30. 258 pp.

Kontula, T., and R. Vainola. 2003. Relationships of Palearctic and Nearctic 'glacial relict' *Myoxocephalus* sculpins from mitochondrial DNA data. Mol. Ecol. 12:3179-3184.

Kraft, C.E., and J.F. Kitchell. 1986. Partitioning of food resources by sculpins in Lake Michigan. Environ. Biol. Fishes 16(4):309-316.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis, Laurentii Salvii, Holmiae, 12th Edition, Volume 1.

Madenjian, C.R., G.L. Fahnenstiel, T.H. Johengen, T.F. Nalepa, H.A. Vanderploeg, G.W. Fleischer, P.J. Schneeberger, D.M. Benjamin, E.B. Smith, J.R. Bence, E.S. Rutherford, D.S. Lavis, D.M. Robertson, D.J. Jude, and M.P. Ebener. 2002. Dynamics of the Lake Michigan food web, 1970-2000. Can. J. Fish. Aquat. Sci. 59:736-753.

Madenjian, C.R., D.W. Hondorp, T.J. Desorcie, and J.D. Holuszko. 2005. Sculpin community dynamics in Lake Michigan. J. Great Lakes Res. 31:267-276.

Mandrak, N.E., and E.J. Crossman. 1992. Postglacial dispersal of freshwater fishes into Ontario. Can. J. Zool. 70:2247-2259.

Martin, N.V., and L.J. Chapman. 1965. Distribution of certain crustaceans and fishes in the region of Algonquin Park, Ontario. J. Fish. Res. Bd. Can. 22(4):969-976.

McAllister, D.E. 1961. The origin and status of the deepwater sculpin, *Myoxocephalus thompsonii*, a Nearctic glacial relict. Nat. Mus. Can. Bull. 172:44-65.

McAllister, D.E., and J.C. Ward. 1972. The deepwater sculpin, *Myoxocephalus quadricornis thompsoni*, new to Alberta, Canada. J. Fish. Res. Bd. Can. 29:344-345.

McPhail, J.D., and C.C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Bd. Can. Bull. 173. pp. 319-323.

Mills, E.L., J.M. Casselman, R. Dermott, J.D. Fitzsimons, G. Gal, K.T. Holeck, J.A. Hoyle, O.E. Johannsson, B.F. Lantry, J.C. Makarewicz, E.S. Millard, I.F.

Munawar, M. Munawar, R. O'Gorman, R.W. Owens, L.G. Rudstam, T. Schaner, and T.J. Stewart. 2003. Lake Ontario: food web dynamics in a changing ecosystem (1970-2000). Can. J. Fish. Aquat. Sci. 60:471-490.

Murray, L., M.H. Papst, and J.D. Reist. 2003. First record of the Deepwater Sculpin, *Myoxocephalus thompsonii*, from George Lake in Whiteshell Provincial Park, Manitoba. Can. Field-Nat. 117(4):642-644.

Parker, B.J. 1988. Status of the deepwater sculpin, *Myoxocephalus thompsoni*, in Canada. Can. Field-Nat. 102(1):126-131.

Roseman, E.F., D.J. Jude, M.K. Raths, T.G. Coon, and W.W. Taylor. 1998. Occurrence of the deepwater sculpin (*Myoxocephalus thompsoni*) in western Lake Erie. J. Great Lakes Res. 24(2):479-483.

Scott, W.B., and E.J. Crossman. 1973. The freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184. 966 pp.

Selgeby, J.H. 1988. Comparative biology of the sculpins of Lake Superior. J. Great Lakes Res. 14:44-51.

Stewart, K.W., and D.A. Watkinson. 2004. The freshwater fishes of Manitoba. The University of Manitoba Press, Winnipeg, Manitoba. 276 pp.

Tables and Table Captions

Table 1.1. Results of 2004 survey for deepwater sculpin from inland lakes across their range. Historical presence constitutes a minimum of one record for deepwater sculpin prior to 2004 survey. "No." corresponds to numbers on Figure 1.1. "N" indicates number of deepwater sculpin captured in 2004. *Indicates new distribution record of deepwater sculpin.

No.	Lake	Region	Latitude (N)	Longitude (W)	Historical presence	2004 survey presence	N
1	Roddick Lake	Quebec	46 14' 54.4"	75 53' 30.9"	Yes	Yes	8
2	Lac des Iles		46 27' 36.0"	75 31' 59.2"	Yes	No	0
3	Thirty-One mile		46 12' 43.1"	75 48' 46.4"	Yes	Yes	6
4	Heney Lake		46 01' 16.4"	75 55' 29.2"	Yes	No	0
5	Lake 259 (ELA)	Ontario	49 41' 19.9"	93 47' 08.2"	Yes	Yes	6
6	Teggau (ELA)*		49 42' 07.7"	93 38' 53.1"	No	Yes	2
7	Lake 310 (ELA)		49 39' 42.3"	93 38' 13.6"	Yes	No	0
8	Lake 258 (ELA)		49 41' 41.6"	93 48' 02.9"	No	No	0
9	Eagle Lake*		49 46' 15.5"	93 36' 44.0"	No	Yes	11
10	Burchell Lake		48 35' 07.6"	90 37' 37.6"	Yes	Yes	17
11	Fairbank Lake		46 27' 35.0"	81 25' 37.0"	Yes	Yes	6
12	Cedar Lake		46 02' 46.7"	78 33' 11.9"	Yes	No	0
13	Saganaga Lake		48 14' 32.7"	90 56' 02.7"	Yes	Yes	10
14	Lake Nipigon		49 27' 37.0"	88 09' 57.6"	Yes	Yes	2
15	High Lake	Manitoba/Ontario	49 42' 05.2"	95 08' 01.2"	No	No	0
16	Westhawk Lake	Manitoba	49 45' 32.0"	95 11' 28.0"	Yes	Yes	6
17	George Lake		50 15' 49.6"	95 28' 16.2"	Yes	Yes	1
18	Lake of the Woods		49 41' 28.7"	94 48' 53.3"	Yes	No	0
19	Clearwater Lake*		54 04' 05.5"	101 05' 33.7"	No	Yes	5
20	Second Cranberry Lake*		54 39' 08.5"	101 09' 58.2"	No	Yes	18
21	Lake Athapapuskow		54 33' 01.2"	101 39' 05.4"	Yes	Yes	9
22	Mirond Lake	Saskatchewan	55 07' 20.3"	102 48' 07.6"	Yes	No	0
23	Lac La Ronge		55 12' 06.9"	105 03' 59.2"	Yes	No	0
24	Reindeer Lake		56 23' 34.7"	102 58' 22.2"	Yes	Yes	4
25	Wollaston Lake		58 14' 59.3"	103 29' 44.4"	Yes	Yes	4

26	Lac La Plonge		55 08' 16.8"	107 15' 43.2"	Yes	Yes	2
27	Chitty Lake	Northwest Territories	62 43' 42.0"	114 07 57.2"	No	No	0
28	Alexie Lake		62 40' 36.0"	114 06' 08.0"	Yes	Yes	1
29	Great Slave Lake		62 29' 15.0"	110 52' 44.0"	Yes	Yes	9
30	Cold Lake	Alberta	54 31' 23.0"	110 06' 30.8"	No	No	0
31	Peerless Lake		56 40' 23.0"	114 41' 04.0"	No	No	0
32	Upper Waterton Lake		49 00' 17.9"	113 54' 16.8"	Yes	Yes	28
33	Upper Kananaskis		50 36' 41.4"	115 09' 55.9"	No	No	0
34	Lake Minnewanka		51 16' 02.2"	115 25' 57.4"	No	No	0
35	Emerald Lake	British Columbia	51 26' 25.1"	116 31' 39.8"	No	No	0

Figure Captions

Figure 1.1. Results of 2004 survey for deepwater sculpin compared to historical records.

Figure 1.2. Design of the collapsible square minnow trap used to catch deepwater sculpin during the 2004 survey.

Figure 1.1.

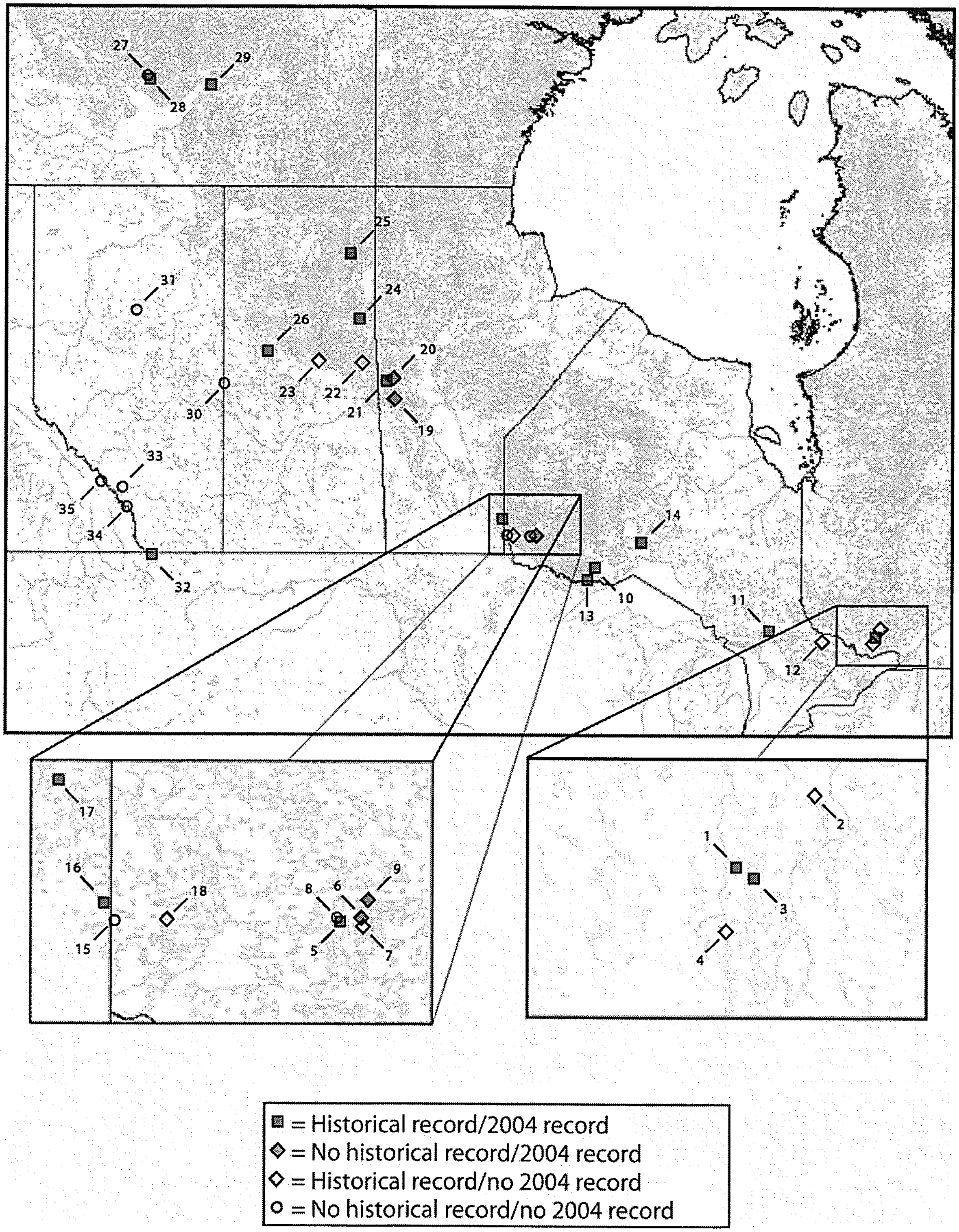
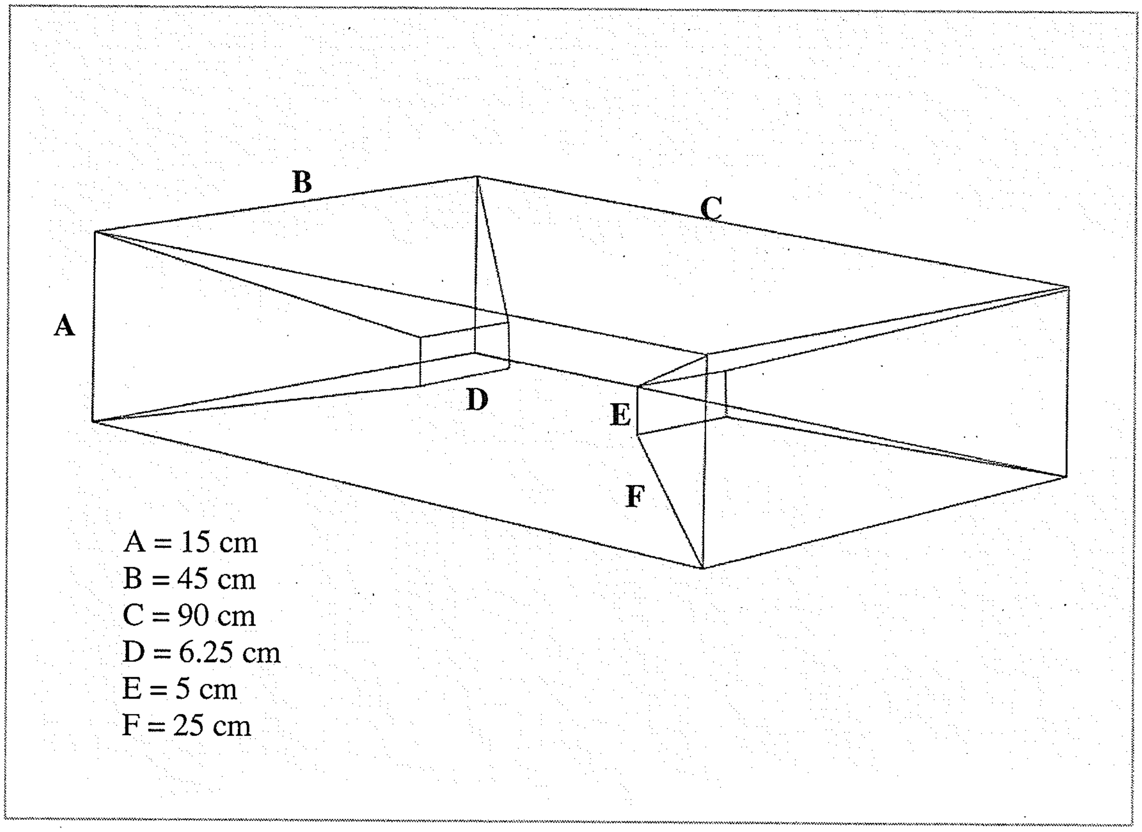

27
29
28
25
31
24
26
20
23
22
21
30
19
33
35
34
14
32
10
13
11
12
17
16
18
9
8
6
15
5
7
2
1
3
4
■ = Historical record/2004 record
◆ = No historical record/2004 record
◇ = Historical record/no 2004 record
○ = No historical record/no 2004 record

Figure 1.2.



Appendix

All locations where deepwater sculpin have been recorded in Canada.

Region	Lake	Latitude (N)	Longitude (W)
NT	Great Slave Lake	62 29 15.0	110 52 44.0
	Lac La Martre	63 21' 48"	117 57' 47.2"
	Keller Lake	63 57' 00"	121 35' 00"
	Great Bear Lake	65 50' 00"	120 45' 00"
	Alexie Lake	62 40 36.0	114 06 08.0
Alberta	Upper Waterton Lake	49 00' 17.9"	113 54' 16.8"
Saskatchewan	Reindeer Lake	56 23' 34.7"	102 58' 22.2"
	Wollaston Lake	58 14' 59.3"	103 29' 44.4"
	Lac La Ronge	55 12' 06.9"	105 03' 59.2"
	Lac La Plonge	55 08' 16.8"	107 15' 43.2"
	Mirond Lake	55 07' 20.3"	102 48' 07.6"
	Lake Athabasca	59 00' 00"	110 30' 00"
	Black Lake	59 10' 00"	105 20' 00"
	Riou Lake	59 07' 00"	106 25' 00"
	Beaverlodge Lake	59 31' 00"	108 35' 00"
	Canoe Lake	55 10' 00"	108 15' 00"
	East Lake	58 17' 00"	103 38' 00"
	Hatchet Lake	58 38' 00"	103 34' 00"
	Laonil Lake	55 41' 00"	103 38' 00"
	Mackay Lake	55 27' 00"	104 56' 00"
	McLennan Lake	55 53' 00"	104 22' 00"
	Milliken Lake	59 27' 00"	108 45' 00"
	Waterbury Lake	58 10' 00"	104 22' 00"
	Yalowega Lake	57 48' 00"	104 53' 00"
	Lake C1	58 19' 00"	104 02' 00"
Manitoba	Westhawk Lake	49 45' 32.0"	95 11' 28.0"
	George Lake	50 15' 49.6"	95 28' 16.2"
	Lake Athapapuskow	54 33' 01.2"	101 39' 05.4"
	Second Cranberry Lake	54 39' 08.5"	101 09' 58.2"
	Clearwater Lake	54 04' 05.5"	101 05' 33.7"
Ontario	Lake Ontario	43 45' 00"	78 00' 00"

	Lake Huron	44 30' 00"	82 15' 00"
	Lake Superior	48 00' 00"	87 00' 00"
	Lake Erie	42 15' 00"	81 00' 00"
	Cedar Lake	46 02' 46.7"	78 33' 11.9"
	Raven Lake	48 03' 30.9"	79 33' 08.5"
	Fairbank Lake	46 27' 35.0"	81 25' 37.0"
	Burchell Lake	48 35' 07.6"	90 37' 37.6"
	Lake Saganaga	48 14' 32.7"	90 56' 02.7"
	Lake 259 (ELA)	49 41' 19.9"	93 47' 8.2"
	Lake 310 (ELA)	49 39' 42.3"	93 38' 13.6"
	Lake of the Woods	49 41' 28.7"	94 48' 53.3"
	William Lake	50 04' 00"	94 04' 00"
	Horseshoe Lake	49 55' 00"	93 57' 00"
	Dicker Lake	49 57' 00"	93 55' 00"
	Passover Lake	49 32' 00"	93 14' 00"
	Trout Lake	49 45' 00"	93 29' 00"
	Burton Lake	49 41' 00"	93 47' 00"
	Squeers Lake	48 31' 00"	90 34' 00"
	Lake Nipigon	49 27' 37.0"	88 09' 57.6"
	Huston Lake	50 24' 00"	95 07' 00"
	Notellum Lake	44 40' 00"	80 54' 00"
	Lake Manitou	45 47' 00"	82 00' 00"
	Eagle Lake	49 46' 15.5"	93 36' 44.0"
	Teggau Lake	49 42' 07.7"	93 38' 53.1"
Quebec	Roddick Lake	46 14' 54.4"	75 53' 30.9"
	Lac des Iles	46 27' 36.0"	75 31' 59.2"
	Thirty-one Mile Lake	46 12' 43.1"	75 48' 46.4"
	Heney Lake	46 01' 16.4"	75 55' 29.2"
	Lake Simoneau	45 24' 44"	72 11' 22"
	Lake Memphremagog	71 27' 00"	45 30' 00"

Chapter 2: Biology, habitat and conservation of the deepwater sculpin (*Myoxocephalus thompsonii*)

Abstract

To better understand the deepwater sculpin outside of the Laurentian Great Lakes, a survey of the species was conducted from May-October, 2004. A total of 155 deepwater sculpin were collected from 20 of 35 lakes sampled, and data concerning diet, age and habitat were collected for each specimen. The most abundant prey items identified from deepwater sculpin stomachs were the burrowing amphipod *Diporeia* (0.84 frequency of occurrence), the opposum shrimp *Mysis relicta*, and chironomid larvae. *Diporeia* also appeared to be more commonly selected by smaller deepwater sculpin, while the frequency of occurrence of *Mysis* and chironomid larvae increases slightly in larger deepwater sculpin. The age of deepwater sculpin specimens was determined using sectioned otoliths. The most common age range was five to ten years. However, the maximum age of deepwater sculpin was determined to be 24 years, substantially older than the previous reported high of seven years. Habitat variables from the bottom of all lakes sampled during the survey were also measured. Both principal components analysis and canonical variates analysis of the physicochemical parameters of the lakes surveyed, including a comparison of lakes where deepwater sculpin were present and lakes where they were absent, suggested that the presence of deepwater sculpin is intimately linked to highly oligotrophic lakes and, more specifically, extremely low (<7 °C) benthic water temperatures. Even moderate amounts of eutrophication, resulting in increased benthic water temperatures, has likely caused extirpation or, at the very least, a significant reduction in population levels of deepwater sculpin. Our study suggests that the conservation of deepwater sculpin and their habitat should be a high priority within areas subject to human disturbances as deepwater sculpin are likely to be highly vulnerable to environmental perturbations such as eutrophication.

Keywords

Deepwater sculpin, *Myoxocephalus thompsonii*, glacial relict, habitat, oligotrophic, diet, age, principal components analysis, canonical variates analysis, conservation.

Introduction

The distribution of an organism is dictated by both its means of dispersal and its ability to survive and tolerate its environment (Dadswell 1974). The distribution of deepwater sculpin, *Myoxocephalus thompsonii* (Girard 1852), is the result of historical events such as glacial advancements and recessions and dispersal via proglacial lakes and their outlets, as well as present habitat conditions (Dadswell 1974). Like all Canadian fishes, the deepwater sculpin was affected by the continental ice sheets during the Pleistocene period, retreating southwards with glacial advances and colonizing northward from refuges via proglacial lakes and outlets (Hocutt and Wiley 1986; Scott and Crossman 1973; McAllister 1961). However, the deepwater sculpin also appears to have very specific habitat requirements, as it occurs only in the deepest, coldest Canadian lakes (Stewart and Watkinson 2004; Parker 1988). These aspects of its biology may be a result of the unique ancestry of the fish. The deepwater sculpin has been termed a "glacial relict" because its sister species is an Arctic marine fish (the fourhorn sculpin, *Myoxocephalus quadricornis*). The deepwater sculpin is thought to have originated when glaciers pushed a marine lineage of fourhorn sculpin inland during the Pleistocene (McAllister 1961; Ricker 1959).

The current distribution of the deepwater sculpin forms an arc from southwestern Quebec to Great Bear Lake in the Northwest Territories, with an isolated population in Upper Waterton Lake, Alberta (Parker 1988; Scott and Crossman 1973). This distribution results from dispersal pathways via proglacial lakes combined with the unique biological requirements of the species. However, distributional studies on deepwater sculpin have been limited to anecdotal reports combined with little targeted, published sampling (Dadswell 1974; Dadswell 1972). Prior to our study, deepwater sculpin had been reported from 57 lakes within Canada. However, reports from most lakes are approximately 40 years old (McAllister 1972; Delisle and Van Vliet 1968) and some of these lakes have most likely experienced biotic and abiotic changes during that time. Given the hypothesized strict habitat requirements of the deepwater sculpin, it is difficult to know for certain whether deepwater sculpin persist in lakes where they were previously reported, especially if the lakes in question have been subject to recent changes.

Although the zoogeography of deepwater sculpin has received some attention (Kontula and Vainola 2003; Scott and Crossman 1973; Dadswell 1972; McPhail and Lindsey 1970; McAllister 1961), the detailed biology and habitat requirements of this species remain poorly known. The deepwater sculpin occurs at the bottoms of some of Canada's deepest lakes (Stewart and Watkinson 2004), and is often sympatric with the glacial relict crustaceans *Mysis* and *Diporeia* (Scott and Crossman 1973; McAllister 1961). The few studies on the biology of deepwater sculpin have focused on populations within the Laurentian Great Lakes, such as Lake Michigan, Lake Superior and Lake Ontario (Geffen and Nash 1992; Selgeby 1988; Brandt 1986; Kraft and Kitchell 1986). Only a single study in Burchell Lake, northwestern Ontario has focused on the biology (parasites in particular) of deepwater sculpin from an area outside of the Laurentian Great Lakes (Black and Lankester 1981).

To gain a better understanding of deepwater sculpin biology, habitat requirements and current distribution, we carried out a range-wide survey of the species outside the Laurentian Great Lakes. The objectives of this study were: (a) to document the diet and age structure of deepwater sculpin throughout their range; and (b) to determine whether certain physicochemical parameters at the bottoms of lakes are indicative of deepwater sculpin occurrence. In conjunction with the second objective, we wanted to determine whether changing lake conditions over time, such as moderate eutrophication due to anthropogenic effects, were affecting deepwater sculpin in lakes where they were previously recorded. Age, diet and habitat requirements are all key factors that contribute to the persistence and success of populations of fish species throughout their ranges. The results of this study will substantially enhance our knowledge of deepwater sculpin throughout its range and hopefully contribute to the conservation of the species.

Methods

Field procedure

A field sampling survey targeting deepwater sculpin was conducted from May to October, 2004. Sampling efforts spanned most of the known distribution of deepwater sculpin, ranging from Alexie Lake in the Northwest Territories in the northwestern portion of its range, to Thirty-One Mile Lake in Quebec in the extreme east, and Upper Waterton Lake in Alberta in the extreme southwest (Table 2.1; Figure 2.1). The survey included lakes with previously known occurrences of deepwater sculpin, and lakes without historical records of deepwater sculpin, but with bathymetry and postglacial history that suggested the possible presence of the species.

Deepwater sculpin were captured using otter trawl tows (see Dadswell 1974; Dadswell 1972), as well as small-mesh gillnets and collapsible minnow traps set along the bottom of each lake. Minnow traps were specifically designed to lie flat along the lake bottom and were baited with dog biscuits and cyalume (glow) sticks (see chapter 1 for specific sampling protocol, fishing effort and collection details).

Deepwater sculpin were removed from fishing gear and anaesthetized in 0.5-0.6 mL/L of 2-Phenoxyethanol. Gill and fin clips were collected from each specimen and preserved for genetic analyses (Chapter 3). Specimens were subsequently preserved in a 10% buffered formalin solution and then transferred to 70% ethanol. This allowed immediate preservation of gut contents, while ensuring that otoliths remained undamaged. Where possible, one female and one male from each locality were deposited in the Royal Ontario Museum.

While sampling for deepwater sculpin, physicochemical parameters of benthic water from each lake were recorded using a YSI model 600QS logger (see Table 2.2 for a complete list of parameters and corresponding units of measurement). When lakes were too deep (>55 m) to measure bottom data directly using the logger, a horizontal water sampler was used to trap water from directly above the bottom of the lake. This water was subsequently brought to the surface where it could be measured using the YSI. This method was repeated three times in each location. To assess the accuracy of this method, measurements of water from the horizontal sampler and measurements taken directly from the bottom of the same lake with the YSI logger were compared in lakes where the logger could reach the bottom (<55 m). In all cases, the measurements were within 5% of each other for each variable. Physicochemical parameters were recorded from each locality where deepwater sculpin were captured within individual lakes. Alternatively, if deepwater sculpin were not captured within a lake, three profiles from approximately the deepest area within the lake (where attempted sampling occurred) were recorded. An average of three secchi disk values, which approximate the 15% level of percentage transmission of surface light intensity, were also recorded for each lake (Dadswell 1974).

Analysis of specimens

Length, weight, age (using sectioned otoliths), sex, stomach contents and parasite presence (not considered in this thesis) were determined for each specimen captured. These are described in more detail below.

Weight was measured using a digital scale and total length (TL) was measured as the distance from the anterior-most tip of the snout to the tip of the most extended caudal rays (Murphy and Willis 1996). Weight-length relationships for deepwater sculpin were then plotted. This curvilinear relationship was subsequently log transformed to allow linear regression analysis. Due to an unusually large specimen (235 mm TL) from Lake Ontario and only smaller individuals from Great Slave Lake (<69 mm TL), the maximum length of deepwater sculpin has been suspected to decrease with increasing latitudes (Parker 1988; Black and Lankester 1981; Scott and Crossman 1973). The length-latitude relationship was plotted to test this hypothesis.

Due to the small sample size, and small lengths of deepwater sculpin, length-frequency analysis to determine age classes was inconclusive for this set of deepwater sculpin. In a comparison of structures used for age estimates, otoliths were often more precise than scales (Lowerre-Barbieri et al. 1994). Also, because deepwater sculpin lack true scales, annular rings present on sagittal otoliths were considered ideal for age identification. The pair of sagittal otoliths was removed from the head of each individual, washed and stored dry. Buffered formalin was initially used in the field to preserve the sculpin and its stomach contents for a short period of time. However, this did not result in any decalcification of the otoliths due to the short duration of exposure.

Upon trial viewings, whole otoliths were determined to be inadequate to obtain accurate ages because annular ring formation was both unclear and appeared compressed towards the edge of the otoliths. As a result, the otoliths were mounted in Cold Cure® epoxi, dried, and cut with an Isomet® hobby rock cutter's saw machine using a diamond tipped blade. Sections were cut approximately 1 mm thick through the nucleus (kernel), identified by its central groove (sulcus acusticus) on the inner surface of the otolith (Murphy and Willis 1996). This technique ensured inclusion of early marks. Sections were then mounted on clear glass slides with Cytoseal 280® under a cover slip, and viewed under a compound microscope. Alternating hyaline (active growth marks) and opaque bands (slow growth marks) were considered as comprising one year of growth (Murphy and Willis 1996). Following Black and Lankester (1981), second and third readings were taken. All otoliths readings from within individuals were within 2 years of each other for each individual. Aging was completed at 60X magnification using both transmitted and reflected light.

Individual stomach contents were examined under a dissecting microscope. Identification and counts of prey items were recorded to determine frequency of occurrence and mean percentage by number, two methods commonly used in the quantitative description of fish diets (Murphy and Willis 1996). A chi-square test revealed no difference in dietary composition or postcapture digestion rates in deepwater sculpin captured in trawls and those captured in gillnets and traps. Although there were various degrees of digestion observed among the stomach contents, only those items that could be clearly identified were recorded. To ensure accurate identification and counts, 10% of stomachs were re-examined for a second time. In all cases, prey identification and counts were accurate within 5% of variation. A chi-square test was used to determine independence of sex and diet. Chi-square tests of independence were also used to test the null hypothesis that size (small, <65 mm TL; versus large, >65 mm TL) of deepwater sculpin and diet were independent across their range.

Analysis of habitat

For lakes where deepwater sculpin were collected, the habitat measurements from each collection locality were averaged to provide a single set of physicochemical parameters for each lake. In lakes where deepwater sculpin were absent, the three benthic measurements were averaged to provide a single set of physicochemical parameters for each lake. No deepwater sculpin were recorded (during this or previous studies) from Lake Minnewanka, Upper Kananaskis Lake and Emerald Lake. However, these lakes were deemed to lie outside the expected distributional range of the deepwater sculpin, and the absence of the species is likely due to a lack of historical connections to glacial lakes (Jackson and Little 2004). Thus, these three lakes were excluded from the habitat analysis.

All environmental variables were log transformed to improve normality (except pH, which is already on a log scale). Systat 10.0 was used to create a Pearson-product moment correlations table to examine relationships between variables. Principal components analysis (PCA) was then conducted on the 10 habitat variables (using CANOCO for Windows, v4.0) measured from 32 lakes to yield ordination axes or composite indices of the habitat variables measured (ter Braak and Smilauer 1998). PCA is an indirect, unconstrained gradient analysis method that uses a linear response model to distinguish between categories of data (ter Braak and Smilauer 1998). In our PCA, three groups, or categories, of data were used: lakes with deepwater sculpin (DWS); lakes where deepwater sculpin had previously been documented, but our sampling failed to confirm their presence (FORMER) and; lakes without historical records of deepwater sculpin and without detection during the 2004 survey (NO DWS). Ordination allows retention of a maximum amount of the trended variation in the habitat variables while representing the data on a lower dimension (Pielou 1984; Jongman et al. 1995; ter Braak and Smilauer 1998). A triplot was then completed using the first two ordination axes to visualize relationships of species (DWS, FORMER, NO DWS), environmental variables and samples to one another.

Canonical variates analysis (CVA), a version of canonical correspondence analysis (CCA), was also performed on the habitat variables from the 32 lakes surveyed for deepwater sculpin during 2004 (using CANOCO, v4.0). This technique uses canonical ordination, where axes are once again created with linear combinations of the environmental variables measured (Duff et al. 1999). In addition to the three categories used in the PCA, a passive binary group (PERTURB) was used to indicate significant human disturbance nearby the sampled lakes (following Duff et al. 1999). This designation was a subjective judgement that was included if significant housing, agricultural or other disturbances were observed. Whereas PCA is unconstrained, CVA is constrained. Multicollinearity of the environmental variables was reduced using both constrained CVAs and forward selection. First, CVAs were conducted using each environmental variable as the only variable. Monte Carlo permutation tests (999 permutations) were used to test the significance of that variable ($P<0.05$) (Duff et al. 1999). Non-significant environmental variables were excluded from further analysis. All remaining environmental variables were subject to forward selection in a second CVA, where Monte Carlo permutation tests (999 permutations) identified variables that were significant ($P<0.05$). These were the sole active variables (Duff et al. 1999) used in the derivation of the ordination diagram. All other variables were excluded from the analysis.

PCA and CVA were used to answer the following questions: (a) are there differences in the physicochemical parameters of those lakes that contained deepwater sculpin and those where deepwater sculpin were not captured?; (b) if differences are detected, are lakes where deepwater sculpin had previously been documented, but our sampling failed to confirm their presence ("FORMER" lakes) more similar to lakes with or without deepwater sculpin, in terms of their physicochemical parameters?, and; (c) which physicochemical parameters, or combination of physicochemical parameters, are most important in determining the presence or absence of deepwater sculpin from lakes?

Results

Size distribution

The minimum and maximum total lengths of deepwater sculpin captured during the survey were 47.2 mm (from Teggau Lake, ON) and 110.5 mm (from Wollaston Lake SK). Mean and median lengths from all lakes combined were 76.7 and 76.3 mm, respectively. A weight-length scatterplot and log weight-length linear regression ($r^2 = 0.919$) are shown in Figure 2.2. Although linear regression of total length and latitude shows that there appears to be a slight decrease in the size of deepwater sculpin with increasing latitude, there is no significant correlation between the two ($r^2 = 0.024$) (Figure 2.3).

Age and Growth

To our knowledge, this study represents the first investigation of the use of otolith sections to determine deepwater sculpin age (Figure 2.4). Two previous age analyses of deepwater sculpin used whole otoliths, and reported a maximum age of seven years (Selgeby 1988; Black and Lankester 1981). Upon initial analysis during our study, whole otoliths were determined to be inadequate for consistent aging of deepwater sculpin, as the markings of annular rings were extremely faint. Sectioned otoliths were found to be much more accurate and consistent (see methods for reasoning).

Our age analysis revealed that deepwater sculpin have a very different age structure than previously thought. The specimens we collected were between three (nine individuals) and 24 years (a single large male of 108.4 mm and 13.49 g from Wollaston Lake, SK) of age with a median age of seven years (Figure 2.5). Linear regression revealed that the growth of deepwater sculpin varies among individuals of the same age across their range (Figure 2.6). Furthermore, deepwater sculpin do not grow at a uniform rate throughout their lives. Our study confirms that deepwater sculpin length increments are largest during early years and smallest during the later years of their lives.

Diet

The stomach contents of 116 deepwater sculpin from 19 lakes across their range reveal that the burrowing amphipod *Diporeia* is the predominant prey item, occurring in 84% of the stomachs examined and composing 44% of the total deepwater sculpin diet by mean percentage number (Figure 2.7). Chironomid larvae and *Mysis* are also common prey items, occurring in 44% and 39% of deepwater sculpin stomachs. Other food items included Trichoptera larvae, sphaerid clams, leeches and fish eggs, each consumed by less than 5% of the deepwater sculpin examined. These four less common food items (trichopteran larvae, sphaerid clams, leeches and fish eggs) were each consumed by sculpin from single lakes. Trichopterans may be important in the diet of deepwater sculpin from Lake 259 of the Experimental Lakes Area during late May, as they were present in 83% of sculpin examined from this lake, often in large numbers (>30 individuals per stomach). This is reflected in, and inflates, the levels of Trichoptera when mean percentage numbers of the stomach contents are reported (Figure 2.7). However, from the limited data available, the diet of deepwater sculpin is shown to be relatively uniform across its range and across seasons.

Chi-square tests of frequency of occurrence suggest that sex (across age groups) does not influence diet selection. However, chi-square tests reveal that deepwater sculpin size and diet selection are not independent. Small deepwater sculpin (<65 mm TL) consume significantly more *Diporeia*, and significantly less *Mysis* and chironomid larvae, than expected throughout their range ($p<0.025$) (Figure 2.8). Furthermore, large deepwater sculpin (>65 mm) consume slightly less *Diporeia*, and slightly more *Mysis* and chironomid larvae, than expected throughout their range ($p<0.025$) (Figure 2.8).

Habitat

Lakes included in the habitat analyses were those formerly known, or suspected, to contain deepwater sculpin that were also within the overall current distribution of the species. As a result, the vast majority of the lakes sampled during 2004 (Table 2.1; Figure 2.1) were in the meso- to oligotrophic range. In general, lakes where deepwater sculpin were captured during the 2004 survey had lower benthic water temperatures (median 4.62 °C, range 3.15 - 6.93 °C) compared to lakes where sculpin were not collected (median 5.51 °C, range 4.32 - 8.31 °C) (Table 2.2). In addition, dissolved oxygen levels were relatively higher in lakes with deepwater sculpin, (median 11.48 mg/L, range 6.74 - 14.44 mg/L), compared to lakes where deepwater sculpin were not collected (median 10.85 mg/L, range 3.18 - 12.36 mg/L). This was the case even during the late stages of summer stratification. On average, maximum lake depths (median 75 m) and secchi disk visibility (SDV) levels (median 6.6 m) were also generally higher in lakes with deepwater sculpin compared to lakes where deepwater sculpin were not captured (maximum depth median 37 m, SDV median 4 m). Finally, seven of the 32 lakes included in the habitat analysis were categorized as disturbed (PERTURB), but only one of these disturbed lakes was found to contain deepwater sculpin.

Several environmental variables were significantly correlated (Table 2.3). High correlations ($r>0.93$) were found between salinity, total dissolved solids and specific conductivity. These were all highly negatively correlated with resistivity. Other significant positive correlations included maximum depth and secchi disk visibility, as well as secchi disk visibility and dissolved oxygen. Temperature was negatively correlated with maximum depth, dissolved oxygen and secchi disk visibility.

PCA revealed first and second axes of habitat data that modeled 46% and 25% of the variation in the data set, respectively (Figure 2.9). The first axis was positively associated with resistivity (r=0.968) and oxidation reduction potential (ORP) (r=0.721) and negatively associated with specific conductivity, salinity, total dissolved solids and pH (r=0.966, 0.964, 0.962, 0.551, respectively). The second PC axis was positively correlated with temperature (r=0.880) and negatively associated with visibility, maximum depth and dissolved oxygen (r=0.772, 0.760, 0.701, respectively). When lake locations were plotted on the ordination diagram, there was a small amount of overlap between lakes with deepwater sculpin and those without. These lakes could not be distinguished on the first axis. However, lakes with deepwater sculpin (DWS) were separated on the second axis from both lakes where deepwater sculpin have never been recorded (NO DWS) and lakes where deepwater sculpin were recorded prior to 2004 but not during the 2004 survey (FORMER). Lakes with deepwater sculpin were characterized by greater maximum depth, benthic dissolved oxygen levels and secchi disk visibilities, as well as extremely low temperatures, while lakes without deepwater sculpin were characterized by higher benthic temperatures.

CVA revealed the same trend as PCA (Figure 2.10). The first and second axes of habitat data captured 45% of the variation in the lake groups. Although there was again a small amount of overlap, temperature gradients separated "FORMER" lakes from "DWS" lakes. The centroids of these two groups are clearly separated by the first axis, which is highly correlated with temperature. Furthermore, lakes that showed evidence of human disturbances ("PERTURB") were closely associated with "FORMER" lakes and increasing temperature gradients.

Discussion

The biology and habitat of deepwater sculpin has remained poorly known in waterbodies outside of the Laurentian Great Lakes. Our study provides interesting findings on the age, diet and habitat requirements of these lesser known populations of deepwater sculpin. These results have important conservation implications and suggest that the preservation of deepwater sculpin throughout Canada requires special consideration.

Size and Age

Deepwater sculpin are relatively small fish, reaching an average length of 76.7 mm throughout their range. Although there does not appear to be a decreasing size trend with increasing latitude, the 2004 survey confirms that deepwater sculpin from the Laurentian Great Lakes are generally larger than deepwater sculpin from all other inland lakes sampled, including those of approximately the same latitude. For example, deepwater sculpin caught during a 1992-2004 Lake Huron index netting program averaged approximately 110 mm TL (J. Casselman, pers. comm.). However, deepwater sculpin are generally smaller than their closest relative, the Arctic marine fourhorn sculpin (*Myoxocephalus quadricornis*), which can reach 340 mm in length (McAllister 1980).

Despite the small size of the deepwater sculpin relative to its sister species, our study shows that the age structure of deepwater sculpin is more similar to the age structure of fourhorn sculpin than previously thought. Previous aging studies on deepwater sculpin using whole otoliths suggested a maximum age of seven years, with the most common ages being two to four years (Selgeby 1988; Black and Lankester 1981). This age structure was similar to that of other freshwater Nearctic sculpin species, such as mottled (*Cottus bairdii*), slimy (*Cottus cognatus*), and spoonhead sculpin (*Cottus ricei*) (Selgeby 1988; Becker 1983). The previous maximum age reported was certainly dissimilar to the fourhorn sculpin, which can reach 14 years of age (Houston 1990). Our initial examinations suggested that whole otoliths were not adequate for assessing the age of deepwater sculpin. The age structure revealed from our study using sectioned otoliths suggested that deepwater sculpin often reach ages of seven or more years, and may reach 24 years of age. Although 24 years of age was only determined for a single individual, ages up to fifteen years were common. Therefore, deepwater sculpin are much longer-lived than previously thought, and are more similar in age to their close relative, the fourhorn sculpin.

Longer-lived fish, such as deepwater sculpin, may be of particular conservation concern because of the potentially low rates of population growth. Low rates of population growth would imply that deepwater sculpin would take longer to recover after declines in population levels due to environmental disturbances. This phenomenon has occurred in Lake Ontario, where deepwater sculpin were thought to be extirpated for years (Scott and Crossman 1973). From 1953 to 1996 only a few individuals were captured and only 19 individuals have been reported since (Sheldon et al. 2006). The difficult recovery of the Lake Ontario population of deepwater sculpin would appear to support the suggestion that long-lived deepwater sculpin may have lower rates of population growth, especially under adverse environmental conditions. In light of this age structure, it is imperative that populations of deepwater sculpin be maintained before declines occur, as the population within Lake Ontario suggests that recovery from low levels is difficult.

Diet

The analyzed gut contents of deepwater sculpin throughout their range agree with previously described diets from single lakes (Geffen and Nash 1992; Selgeby 1988; Brandt 1986; Kraft and Kitchell 1986). *Diporeia*, *Mysis* and chironomid larvae compose close to the entire deepwater sculpin diet throughout their range. However, this is the first time that differences in size of deepwater sculpin have been correlated with prey type. Our study suggests that smaller deepwater sculpin eat proportionately more

Diporeia than larger individuals. Furthermore, larger deepwater sculpin eat proportionately more *Mysis* and chironomid larvae than smaller individuals.

In a previous study of deepwater sculpin, prey size was uncorrelated with predator sizes (Kraft and Kitchell 1986). This, combined with our study, suggests that the differences in proportion of *Diporeia*, *Mysis*, and chironomid larvae consumed by deepwater sculpin is not due to differences in the sizes of these prey items. It may, instead, be due to other aspects of the relationship between deepwater sculpin and their prey. Detection ability, capture efficiency and handling time (ease to swallow) are factors that affect foraging efficiency (Murphy and Willis 1996; Kraft and Kitchell 1986). A combination of these factors may allow smaller deepwater sculpin to feed more efficiently on *Diporeia* as opposed to *Mysis* and chironomid larvae. Janssen (1978) and Kraft and Kitchell (1986) reported that *Mysis* are difficult to catch and easily swallowed, whereas, *Diporeia* are difficult to swallow, but easily caught. Hoekstra and Janssen (1985) suggested that mottled sculpin rely upon mechanical stimuli when foraging for food. This would also seem logical for deepwater sculpin, which occur at depths where light penetration is extremely limited. It is possible that under-developed mechanoreception (ability to detect mechanical stimuli) limits the ability of small deepwater sculpin to consistently detect *Mysis* and chironomid larvae. It is also possible that smaller deepwater sculpin have reduced swimming abilities relative to larger individuals, and therefore may be unable to catch *Mysis* on a consistent basis.

Whatever the reason(s) for differences in the size of deepwater sculpin and the corresponding diet, our data suggest that *Diporeia* are extremely important to deepwater sculpin, especially during their early lives. Thus, the health of a deepwater sculpin population within a lake is likely to be linked to the health of the *Diporeia* population within the same lake. Any declines in *Diporeia* populations may represent a threat to deepwater sculpin populations. This is concerning, as there have been recent declines reported in *Diporeia* spp. in the lower Great Lakes (Pothoven et al. 2001). These declines have affected other fish species whose primary prey item is *Diporeia* spp. (Pothoven et al. 2001). For example, a recent decline in the *Diporeia* spp. population within Lake Michigan has been linked to deteriorating body condition and growth of lake whitefish (*Coregonus clupeaformis*) (Pothoven et al. 2001). Considering the diet of deepwater sculpin, it is likely that declining *Diporeia* spp. populations would negatively impact deepwater sculpin. In most lakes, a deteriorating deepwater sculpin population would most likely go unnoticed, as little information exists on the health or abundance of the vast majority of the species populations. When combined with habitat trends (such as gradual eutrophication) in some lakes that could be resulting in declining *Diporeia* spp. populations, there is a considerable threat to deepwater sculpin. (Lozano et al. 2001; Pothoven et al. 2001).

Habitat

All of the lakes sampled during the survey were initially known or suspected to contain deepwater sculpin. Therefore, lakes sampled were generally meso- to oligotrophic in range, and therefore nutrient-poor and slightly alkaline. The results of our survey and subsequent habitat analyses confirm that deepwater sculpin are strictly confined to oligotrophic lakes. Furthermore, it appears that the current distribution of deepwater sculpin is highly correlated with the extent of the oligotrophic nature of lakes. In particular, deepwater sculpin occurrence is strongly correlated with extremely low benthic temperature (median 4.62 °C, range 3.15 - 6.93 °C), and extremely high secchi disk visibility (median 6.6 m), dissolved oxygen level (median 11.48 mg/L, range 6.74 - 14.44 mg/L) and maximum lake depth (median 75 m) (Table 2.2). Deepwater sculpin were generally not found in a lake if it was approaching a mesotrophic state. Slightly elevated benthic temperature (median 5.51 °C), and decreased secchi disk visibility (median 4 m) and dissolved oxygen level (median 10.85 mg/L) were often indicative of the absence of deepwater sculpin from a lake (Table 2.2). This pattern was repeated consistently throughout the species range.

Furthermore, lakes where deepwater sculpin were known to occur prior to 2004, but sampling during the 2004 survey did not reveal their presence ("FORMER"), formed a distinct cluster in both PCA and CVA of habitat data, and were separated from those lakes where deepwater sculpin were found in 2004 (Figure 2.9; Figure 2.10). PCA revealed that "FORMER" lakes were asociated with higher benthic water temperature, while lakes with deepwater sculpin ("DWS") were associated with high dissolved oxygen, secchi disk visibility, and maximum depth (Figure 2.9). In addition, "FORMER" lakes were negatively associated with high dissolved oxygen, secchi disk visibility, and maximum depth, while "DWS" lakes were negatively asociated with high benthic water temperature (Figure 2.10). CVA confirmed the pattern seen in PCA, where deepwater sculpin were associated with highly oligotrophic lakes. Specifically, CVA revealed that "FORMER" lakes were correlated with high benthic water temperature while "DWS" lakes were negatively correlated with this same vector (Figure 2.10). CVA also indicated that "FORMER" lakes were closely associated with lakes that had been subject to human disturbances ("PERTURB") (Figure 2.10). Both "FORMER" lakes and "PERTURB" lakes were generally not highly oligotrophic and some were approaching a mesotrophic state. This was reflected by higher benthic temperatures, lower dissolved oxygen and lower visibility.

Taken together, our results suggests that deepwater sculpin are highly sensitive to small changes in their environments and absences from some lakes sampled in 2004 may be due to this sensitivity. Lake Heney and Lac des Iles in southwestern Quebec had records of deepwater sculpin from 40 years ago (Delisle and Van Vliet 1968). However, since the initial reports of deepwater sculpin from these lakes, they have experienced perturbations and have undergone subsequent eutrophication. Our sampling indicated that deepwater sculpin may no longer be present in these lakes. In addition, our habitat analyses suggested that these lakes may no longer be suitable for deepwater sculpin. As a best-case scenario, our data indicate that deepwater sculpin populations in these lakes are declining, and that the species may be avoiding areas within the lakes where there are elevated temperatures and lower dissolved oxygen levels. Nevertheless, our study raises conservation issues, in terms of mitigating the eutrophication of lakes where deepwater sculpin occur.

Although one-time habitat measurements, like those collected in our study, are less reliable than repeated measurements over several seasons and years (Duff et al. 1999), the logistical constraints of a survey, such as the one undertaken for deepwater sculpin in 2004, are high. In the case of sampling for deepwater sculpin in great depths within lakes that are often remote, constraints are even more substantial. There may be many additional factors (e.g. predation, competition) within individual lakes that affect population levels of deepwater sculpin (Madenjian et al. 2005; Madenjian et al. 2002 Brandt 1986). However, this study, which focused on deepwater sculpin habitat, indicates that deepwater sculpin require highly oligotrophic conditions and even small physicochemical changes to their environments may have a substantial negative impact on populations. These effects may range from stress to reduction in population levels to extirpation.

Conclusions

The findings of the age, diet and habitat analyses of our study have significant implications for the future conservation of deepwater sculpin. Our results suggest that deepwater sculpin are much longer-lived than previously thought, often reaching more than the previously reported maximum of seven years of age. The conservation of longer-lived fishes, such as deepwater sculpin, is particularly important due to the potentially low rates of population growth and the difficult recovery of these types of fishes from low population levels. The potentially slow recovery process of low deepwater sculpin population levels is demonstrated by the long, difficult and protracted recovery of deepwater sculpin populations from within Lake Ontario (Sheldon et al. 2006; Scott and Crossman 1973). Our study also indicates that deepwater sculpin rely heavily upon *Diporeia* spp. as a prey item, particularly in the early years of life. *Diporeia* spp. may also be negatively impacted by eutrophication within lakes (Lozano et al. 2001). Most significantly, our study suggests that deepwater sculpin have strict oligotrophic habitat requirements, especially low benthic temperatures. In particular, the thermal niche of deepwater sculpin appears to be quite specific (<7 °C). This, combined with the possible negative impact of eutrophication on *Diporeia*, suggests that even moderate amounts of eutrophication could have significant negative impacts on individual deepwater sculpin populations throughout their range. This may indeed be the case in both Heney Lake and Lac des Iles where deepwater sculpin were once present but not successfully collected recently. It should signal that the conservation of deepwater sculpin is of immediate concern in some areas and efforts must be taken in order to minimize human disturbances and the subsequent eutrophication of lakes where deepwater sculpin reside.

References

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, Wisconsin. 1052 pp.

Black, G.A., and M.W. Lankester. 1981. The biology and parasites of deepwater sculpin, *Myoxocephalus quadricornis thompsonii* (Girard), in Burchell Lake, Ontario. Can. J. Zool. 59:1454-1457.

Brandt, S.B. 1986. Disappearance of the deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Ontario: the keystone predator hypothesis. J. Great Lakes Res. 12(1):18-24.

Dadswell, M.J. 1972. Post-glacial dispersal of four freshwater fishes on the basis of new distribution records from eastern Ontario and western Quebec. J. Fish. Res. Bd. Can. 29:545-553.

Dadswell, M.J. 1974. Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. Nat. Mus. Nat. Sci. Pub. Zool. 11:1-110.

Delisle, C., and W. Van Vliet. 1968. First records of the sculpins *Myoxocephalus thompsonii* and *Cottus ricei* from the Ottawa Valley, southwestern Quebec. J. Fish. Res. Bd. Can. 25(12): 2733-2737.

Duff, K.E., T.E. Laing, J.P. Smol, and D.R.S. Lean. 1999. Limnological characteristics of lakes located across Arctic treeline in northern Russia. Hydrobiologia 391:205-222.

Geffen, A.J., and R.D.M. Nash. 1992. The life-history strategy of deepwater sculpin, *Myoxocephalus thompsoni* (Girard), in Lake Michigan: dispersal and settlement patterns during the first year of life. J. Fish Biol. 41 (supplement B):101-110.

Hocutt, C.H., and E.O. Wiley, eds. 1986. The zoogeography of North American freshwater fishes. John Wiley and Sons. New York.

Hoekstra, D., and J. Janssen. 1985. Non-visual feeding behavior of the mottled sculpin, *Cottus bairdii*, in Lake Michigan. Environ. Biol. Fishes 12:111-117.

Houston, J. 1990. Status of the fourhorn sculpin, *Myoxocephalus quadricornis*, in Canada. Can. Field-Nat. 104(1):7-13.

Jackson Jr., L.E., and E.C. Little. 2004. A single continental glaciation of Rocky Mountain Foothills, south-western Alberta, Canada. *In* Quaternary glaciations - extent and chronology. *Edited by* J. Ehlers and P.L. Gibbard. Elsevier B.V., Amsterdam, The Netherlands, pp. 29-38.

Janssen, J. 1978. Feeding-behavior repertoire of the alewife, *Alosa pseudoharengus*, and the ciscoes, *Coregonus hoyi* and *C. artedii*. J. Fish. Res. Bd. Can. 35:249-253.

Kontula, T., and R. Vainoloa. 2003. Relationships of Palearctic and Nearctic 'glacial relict' *Myoxocephalus* sculpins from mitochondrial DNA data. Mol. Ecol. 12:3179-3184.

Kraft, C.E., and J.F. Kitchell. 1986. Partitioning of food resources by sculpins in Lake Michigan. Environ. Biol. Fishes 16(4):309-316.

Lowerre-Barbieri, S.K., M.E. Chittenden, Jr., and C.M. Jones. 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. U.S. Nat. Mar. Fish. Serv. Fish. Bull. 92:555-568.

Lozano, S.J., J.V. Scharold, and T.F. Nalepa. 2001. Recent declines in benthic macroinvertebrate densities in Lake Ontario. Can. J. Fish. Aquat. Sci. 58:518-529.

Madenjian, C.R., G.L. Fahnenstiel, T.H. Johengen, T.F. Nalepa, H.A. Vanderploeg, G.W. Fleischer, P.J. Schneeberger, D.M. Benjamin, E.B. Smith, J.R. Bence, E.S. Rutherford, D.S. Lavis, D.M. Robertson, D.J. Jude, and M.P. Ebener. 2002. Dynamics of the Lake Michigan food web, 1970-2000. Can. J. Fish. Aquat. Sci. 59:736-753.

Madenjian, C.R., D.W. Hondorp, T.J. Desorcie, and J.D. Holuszko. 2005. Sculpin community dynamics in Lake Michigan. J. Great Lakes Res. 31:267-276.

McAllister, D.E. 1961. The origin and status of the deepwater sculpin, *Myoxocephalus thompsonii*, a Nearctic glacial relict. Nat. Mus. Can. Bull. 172:44-65.

McAllister, D.E., and J.C. Ward. 1972. The deepwater sculpin, *Myoxocephalus quadricornis thompsoni*, new to Alberta, Canada. J. Fish. Res. Bd. Can. 29:344-345.

McAllister, D.E. 1980. *Myoxocephalus quadricornis* (Linnaeus) fourhorn sculpin. *In* Atlas of North American freshwater fishes. *Edited by* D.S. Lee, C.R. Gilbert, G.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. North Car. State Mus. Nat. Hist. Biol. Surv. Pub. 1980-12. p. 826

McPhail, J.D., and C.C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Bd. Can. Bull. 173. 966 pp.

Murphy, B.R. and D.W. Willis. 1996. Fisheries techniques 2nd edition. Amer. Fish. Soc. Bethesda, Maryland, USA.

Parker, B.J. 1988. Status of the deepwater sculpin, *Myoxocephalus thompsoni*, in Canada. Can. Field-Nat. 102(1):126-131.

Pielou, E.C. 1984. The interpretation of ecological data. A primer on classification and ordination. Wiley, New York. 263 pp.

Ricker, K. E. 1959. The origin of two glacial relict crustaceans in North America, as related to Pleistocene glaciation. Can. J. Zool. 37: 871-893.

Scott, W.B., and E.J. Crossman. 1973. The freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184. 966 pp.

Selgeby, J.H. 1988. Comparative biology of the sculpins of Lake Superior. J. Great Lakes Res. 14:44-51.

Sheldon, T.A., N.E. Mandrak, J.M. Casselman, C.C. Wilson, and N.R. Lovejoy. 2006. Update COSEWIC status report on the deepwater sculpin *Myoxocephalus thompsonii* in Canada, *In* COSEWIC assessment and update status report on the deepwater sculpin *Myoxocephalus thompsonii* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 43 pp.

Stewart, K.W., and D.A. Watkinson. 2004. The freshwater fishes of Manitoba. The University of Manitoba Press, Winnipeg, Manitoba. 276 pp.

ter Braak, C.J.F. and P. Smilauer. 1998. CANOCO reference manual and user's guide to CANOCO for windows: software for canonical community ordination, ver 4. Microcomputer Power, Ithaca, N.Y.

Tables and Table Captions

Table 2.1. Results of 2004 survey for deepwater sculpin from inland lakes across their range. Historical presence constitutes a minimum of one record for deepwater sculpin prior to 2004 survey. "No." corresponds to numbers on Figure 2.1. "N" indicates number of deepwater sculpin captured in 2004. *Indicates new distribution record of deepwater sculpin.

No.	Lake	Region	Latitude (N)	Longitude (W)	Historical presence	2004 survey presence	N
1	Roddick Lake	Quebec	46 14' 54.4"	75 53' 30.9"	Yes	Yes	8
2	Lac des Iles		46 27' 36.0"	75 31' 59.2"	Yes	No	0
3	Thirty-One mile		46 12' 43.1"	75 48' 46.4"	Yes	Yes	6
4	Heney Lake		46 01' 16.4"	75 55' 29.2"	Yes	No	0
5	Lake 259 (ELA)	Ontario	49 41' 19.9"	93 47' 08.2"	Yes	Yes	6
6	Teggau (ELA)*		49 42' 07.7"	93 38' 53.1"	No	Yes	2
7	Lake 310 (ELA)		49 39' 42.3"	93 38' 13.6"	Yes	No	0
8	Lake 258 (ELA)		49 41' 41.6"	93 48' 02.9"	No	No	0
9	Eagle Lake*		49 46' 15.5"	93 36' 44.0"	No	Yes	11
10	Burchell Lake		48 35' 07.6"	90 37' 37.6"	Yes	Yes	17
11	Fairbank Lake		46 27' 35.0"	81 25' 37.0"	Yes	Yes	6
12	Cedar Lake		46 02' 46.7"	78 33' 11.9"	Yes	No	0
13	Saganaga Lake		48 14' 32.7"	90 56' 02.7"	Yes	Yes	10
14	Lake Nipigon		49 27' 37.0"	88 09' 57.6"	Yes	Yes	2
15	High Lake	Manitoba/Ontario	49 42' 05.2"	95 08' 01.2"	No	No	0
16	Westhawk Lake	Manitoba	49 45' 32.0"	95 11' 28.0"	Yes	Yes	6
17	George Lake		50 15' 49.6"	95 28' 16.2"	Yes	Yes	1
18	Lake of the Woods		49 41' 28.7"	94 48' 53.3"	Yes	No	0
19	Clearwater Lake*		54 04' 05.5"	101 05' 33.7"	No	Yes	5
20	Second Cranberry Lake*		54 39' 08.5"	101 09' 58.2"	No	Yes	18
21	Lake Athapapuskow		54 33' 01.2"	101 39' 05.4"	Yes	Yes	9
22	Mirond Lake	Saskatchewan	55 07' 20.3"	102 48' 07.6"	Yes	No	0
23	Lac La Ronge		55 12' 06.9"	105 03' 59.2"	Yes	No	0
24	Reindeer Lake		56 23' 34.7"	102 58' 22.2"	Yes	Yes	4
25	Wollaston Lake		58 14' 59.3"	103 29' 44.4"	Yes	Yes	4

26	Lac La Plonge		55 08' 16.8"	107 15' 43.2"	Yes	Yes	2
27	Chitty Lake	Northwest Territories	62 43' 42.0"	114 07 57.2"	No	No	0
28	Alexie Lake		62 40' 36.0"	114 06' 08.0"	Yes	Yes	1
29	Great Slave Lake		62 29' 15.0"	110 52' 44.0"	Yes	Yes	9
30	Cold Lake	Alberta	54 31' 23.0"	110 06' 30.8"	No	No	0
31	Peerless Lake		56 40' 23.0"	114 41' 04.0"	No	No	0
32	Upper Waterton Lake		49 00' 17.9"	113 54' 16.8"	Yes	Yes	28
33	Upper Kananaskis		50 36' 41.4"	115 09' 55.9"	No	No	0
34	Lake Minnewanka		51 16' 02.2"	115 25' 57.4"	No	No	0
35	Emerald Lake	British Columbia	51 26' 25.1"	116 31' 39.8"	No	No	0

Table 2.2. Ranges and median values of environmental variables taken from the benthic water of lakes sampled for deepwater sculpin. Measurements were taken from inland lakes within Canada where deepwater sculpin (*Myoxocephalus thompsonii*) were captured during the 2004 survey (n=20), as well as lakes where sampling for deepwater sculpin was conducted without detection of presence (n=12).

	DWS present			DWS absent		
Variable	Minimum	Median	Maximum	Minimum	Median	Maximum
Latitude (N)	46º12.72’	49º45.60’	62º29.28’	46º01.26’	49º41.88’	62º43.68’
Longitude (W)	75º48.78’	95º20.04’	113º54.30’	75º31.98’	94º58.50’	114º41.04’
pH (surface)	7.24	8.3	9.04	6.92	8.05	8.97
pH (bottom)	7.21	8.45	8.9	6.76	8.11	9.04
Sp. Cond. (mS/cm)	0.019	0.116	0.383	0.019	0.116	0.27
Secchi depth (m)	3.5	6.6	13.5	1.9	4	6
Temperature (ºC)	3.15	4.62	6.93	4.32	5.51	8.31
Oxygen (mg/L)	6.74	11.48	14.44	3.18	10.85	12.36
Salinity (ppm)	10	55	180	10	50	130
Resistivity (Kohm*cm)	4.25	12.6	85.3	6.1	13.8	87.6
TDS (mg/L)	12	84.000	249	12	76.000	175
ORP (mV)	207	306	407	184	249	401
Max. depth (m)	24	75	614	20	37	100

Table 2.3. Pearson product-moment correlations of selected environmental variables taken from the benthic water of 32 inland lakes sampled for deepwater sculpin.

Variables	TEMP	MD	SDV	DO	SC	pH	SAL	RES	TDS
TEMP									
MD	**-0.590**								
SDV	**-0.509**	**0.543**							
DO	**-0.595**	0.241	**0.406**						
SC	0.082	0.034	0.150	-0.020					
pH	-0.024	0.313	0.234	**-0.456**	**0.461**				
SAL	0.028	0.050	0.162	0.043	**0.980**	**0.393**			
RES	-0.065	-0.069	-0.100	-0.002	**-0.940**	**-0.386**	**-0.962**		
TDS	0.023	0.089	0.116	0.030	**0.931**	**0.377**	**0.957**	**-0.999**	
ORP	-0.263	0.093	-0.005	0.171	**-0.603**	**-0.438**	**-0.589**	**0.619**	**-0.606**

Values in bold; $p<0.05$.

TEMP, temperature; MD, maximum depth; SDV, secchi disk visibility depth; DO, dissolved oxygen; SC, specific conductivity; SAL, salinity; RES, resistivity; TDS, total dissolved solids; ORP, oxidation reduction potential. Measurement units in Table 2.2.

Figure Captions

Figure 2.1. Results of 2004 survey for deepwater sculpin compared to historical records.

Figure 2.2. Relationship between total length and weigth for deepwater sculpin from 19 lakes across the species range (a) Weight-length scatterplot; (b) Regression of weight by length.

Figure 2.3. Regression of length by latitude for deepwater sculpin from 19 lakes across their range.

Figure 2.4. Sectioned otolith of a deepwater sculpin, age 15, from Reindeer Lake, SK. Image is via transmitted light at 30X magnification. Aging was completed at approximately 60X magnification.

Figure 2.5. Ages obtained from sectioned otoliths for deepwater sculpin from 19 lakes across their distribution.

Figure 2.6. Relationship between age and length of deepwater sculpin for 19 lakes from across the species range (a) Age-length scatterplot; (b) Regression of length by age.

Figure 2.7. Stomach contents of deepwater sculpin from 19 lakes across the species range (a) Frequency of occurrence of prey items; (b) mean percentage number of prey items.

Figure 2.8. The frequency of the three most common prey items of deepwater sculpin by size. Sample size of 19 lakes.

Figure 2.9. Plot of the first two principal components based on habitat data of 32 lakes surveyed for deepwater sculpin during 2004. Numbers in parentheses following axis labels indicate the percent of total variation explained by the axis. DWS- lakes with deepwater sculpin; FORMER- lakes where deepwater sculpin had previously been documented, but our sampling failed to confirm their presence; NO DWS- lakes without historical records of deepwater sculpin and without detection during the 2004 survey.

Figure 2.10. Plot of the first two canonical variates based on habitat data from 32 lakes surveyed for deepwater sculpin during 2004. Eigenvalues for each axis follow the axis label. The centroid for each lake classification is denoted by an open triangle, including disturbed (PERTURB) lakes. DWS, FORMER, NO DWS- as defined for Figure 2.9.

Figures

Figure 2.1.

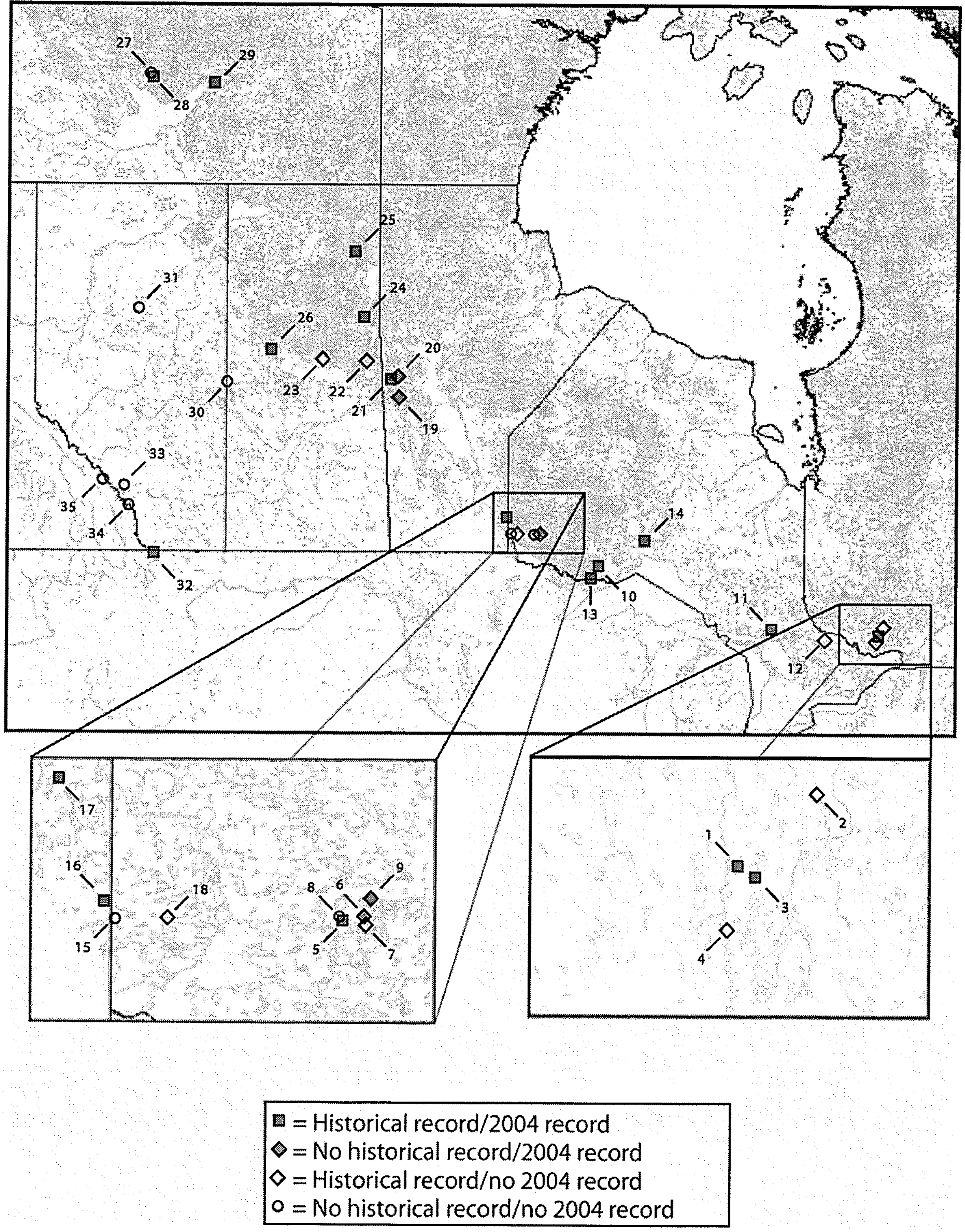
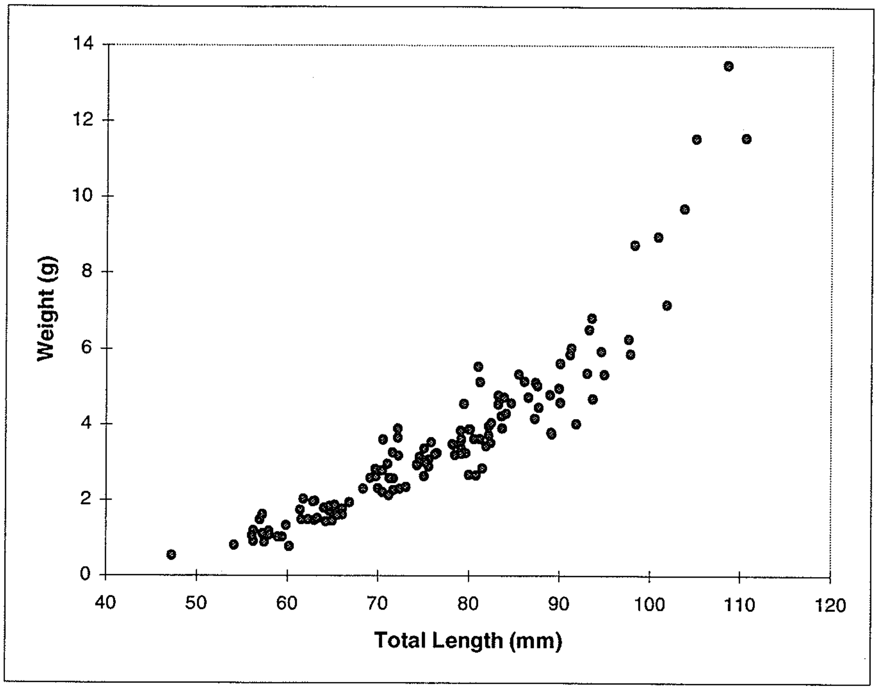


Figure 2.2.

A.


14
12
10
8
6
4
2
0
Weight (g)
40
50
60
70
80
90
100
110
120
Total Length (mm)

B.

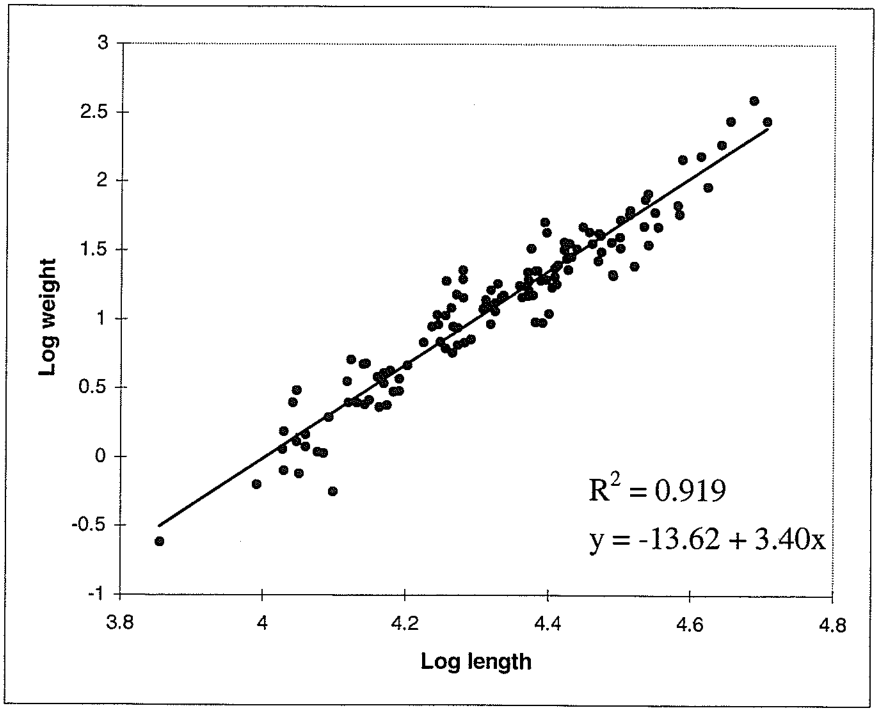

3
2.5
2
1.5
1
0.5
0
-0.5
-1
Log weight
3.8
4
4.2
4.4
4.6
4.8
Log length
$R^2 = 0.919$
y = -13.62 + 3.40x

Figure 2.3.

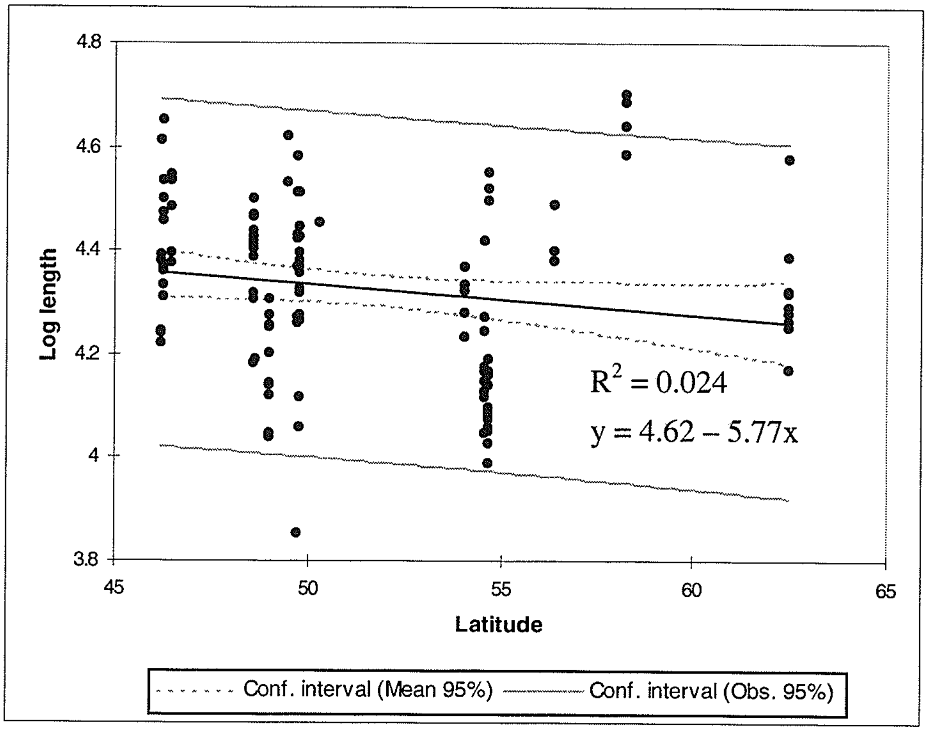

4.8
4.6
4.4
4.2
4
3.8
Log length
45
50
55
60
65
Latitude
$R^2 = 0.024$
$y = 4.62 - 5.77x$
Conf. interval (Mean 95%)
Conf. interval (Obs. 95%)

Figure 2.4.

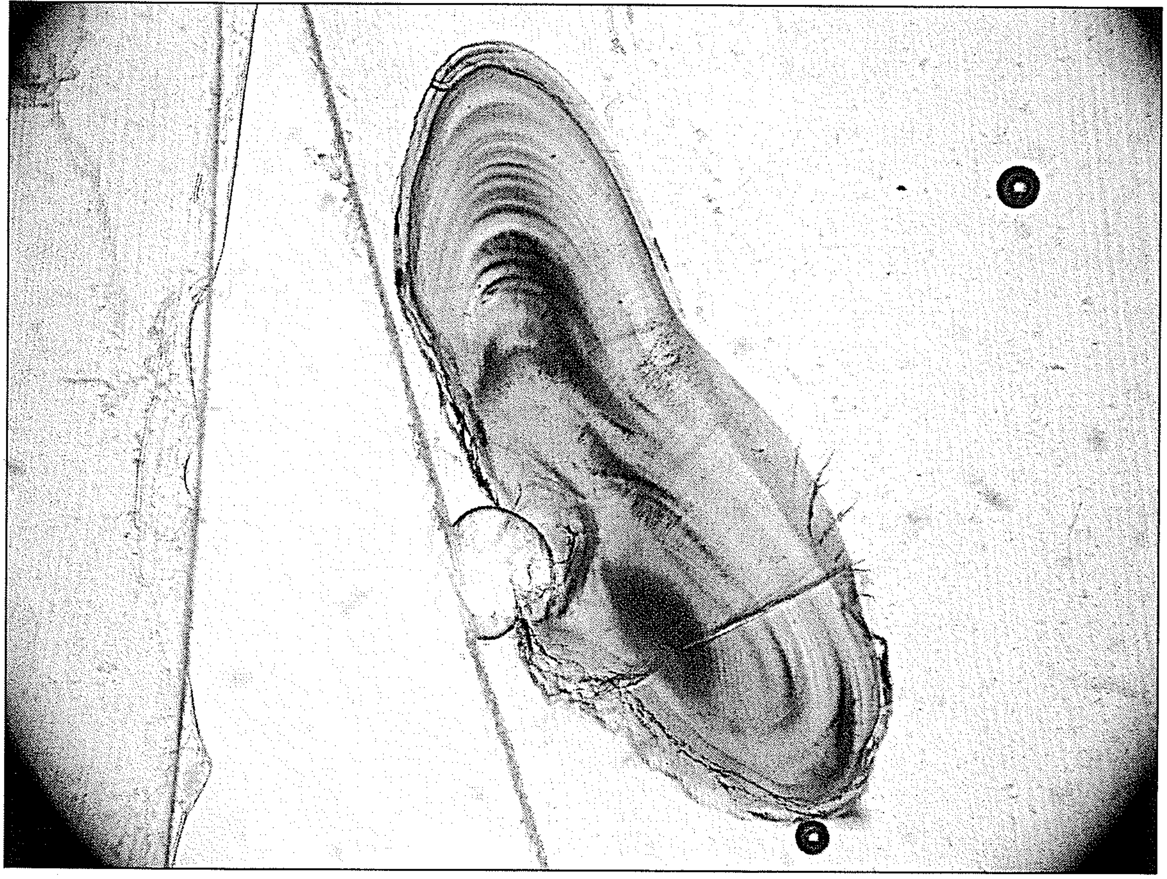


Figure 2.5.

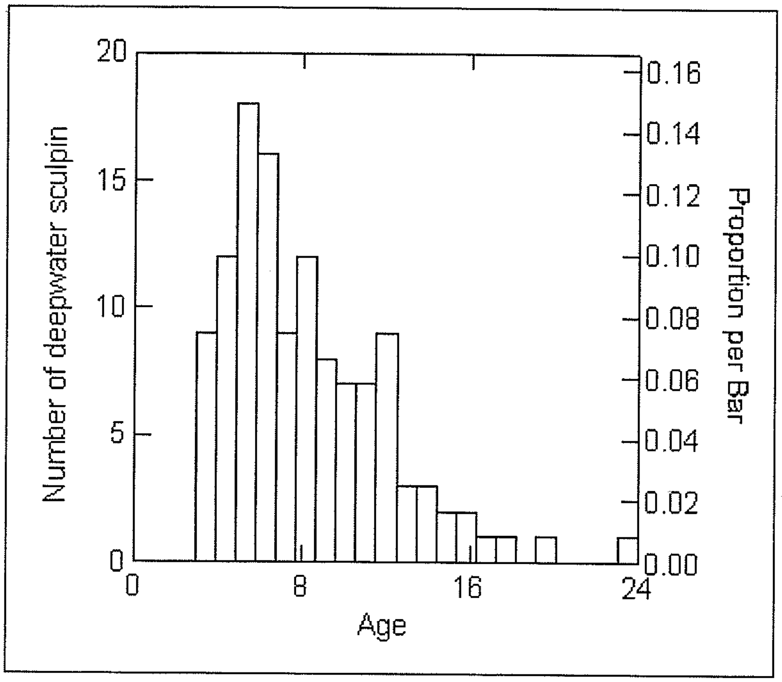
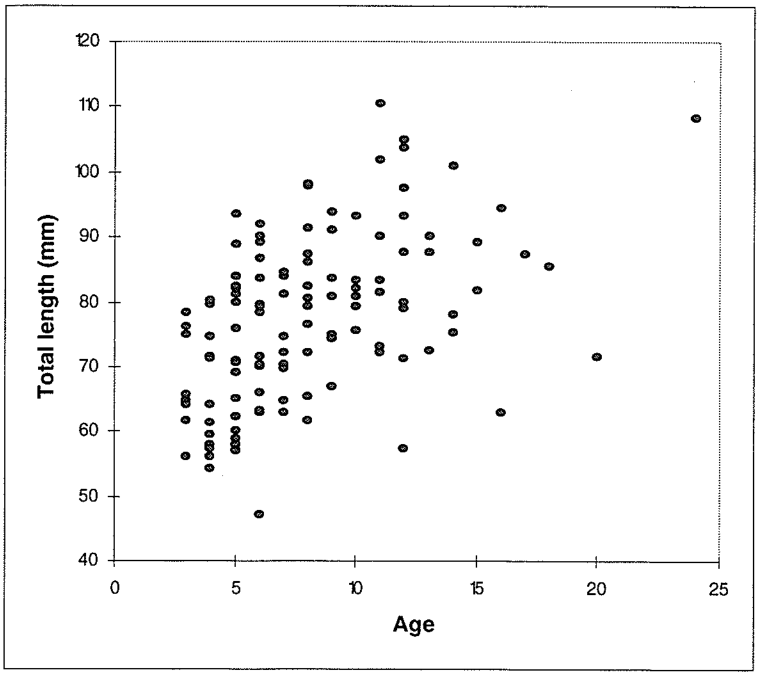

Number of deepwater sculpin
20
15
10
5
0
Proportion per Bar
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
8
16
24
Age

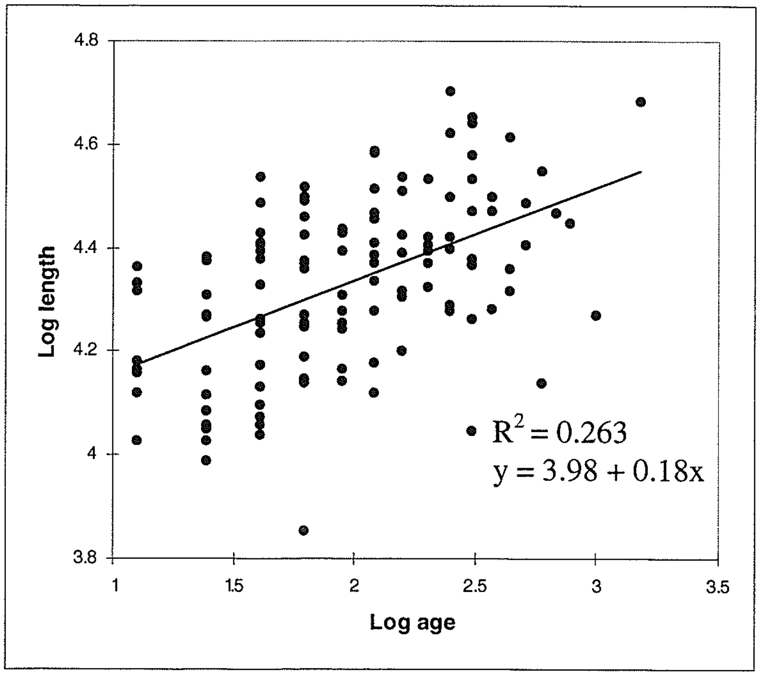
Figure 2.6.

A.



120
110
100
90
80
70
60
50
40
Total length (mm)
0
5
10
15
20
25
Age

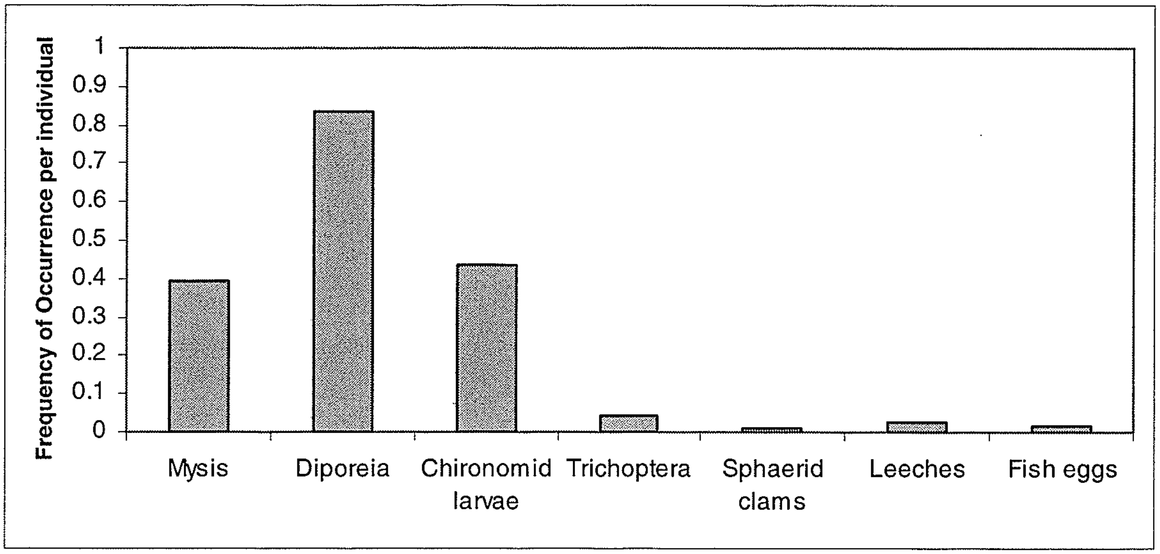
B.



4.8
4.6
4.4
4.2
4
3.8
Log length
1
1.5
2
2.5
3
3.5
Log age
R² = 0.263
y = 3.98 + 0.18x

Figure 2.7.

A.


Frequency of Occurrence per individual
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mysis
Diporeia
Chironomid larvae
Trichoptera
Sphaerid clams
Leeches
Fish eggs

B.

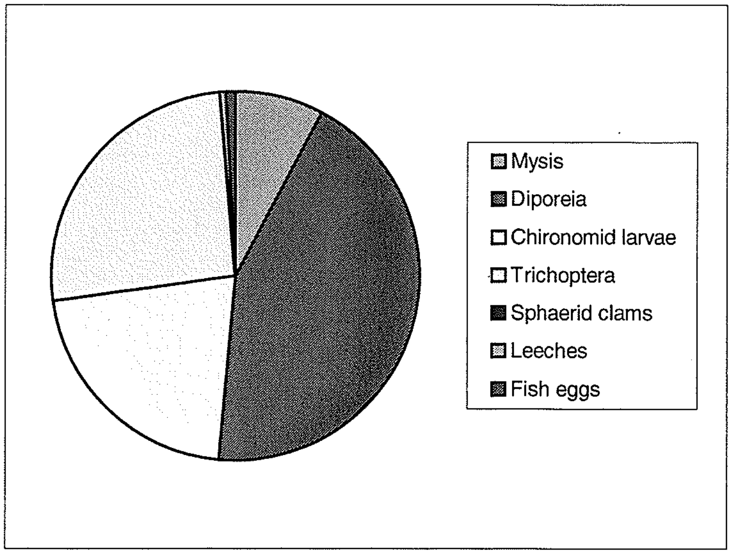

Mysis
Diporeia
Chironomid larvae
Trichoptera
Sphaerid clams
Leeches
Fish eggs

Figure 2.8.

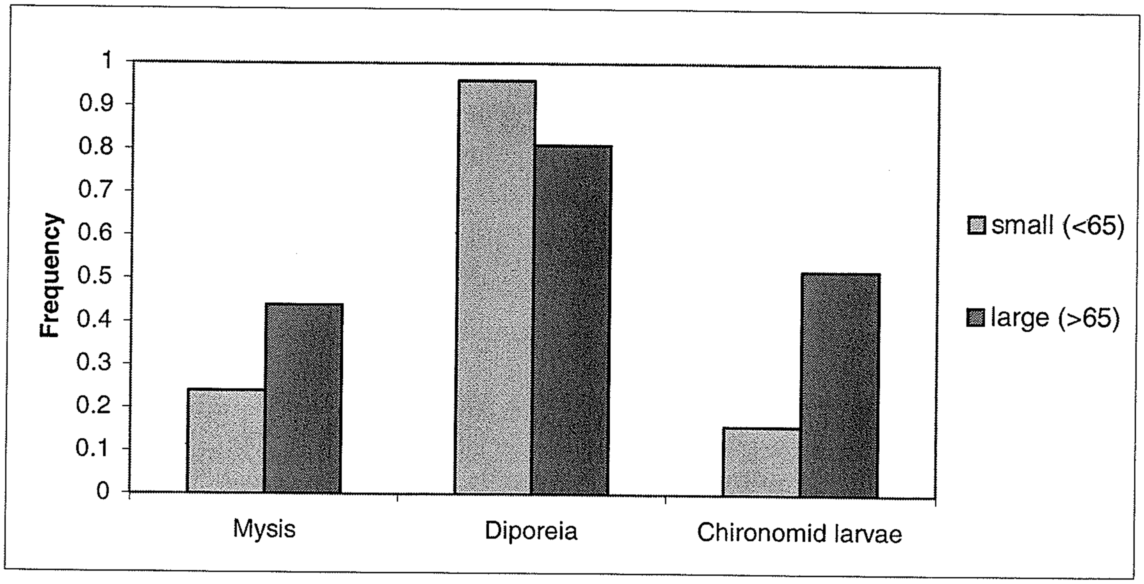

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency
Mysis
Diporeia
Chironomid larvae
small (<65)
large (>65)

Figure 2.9.

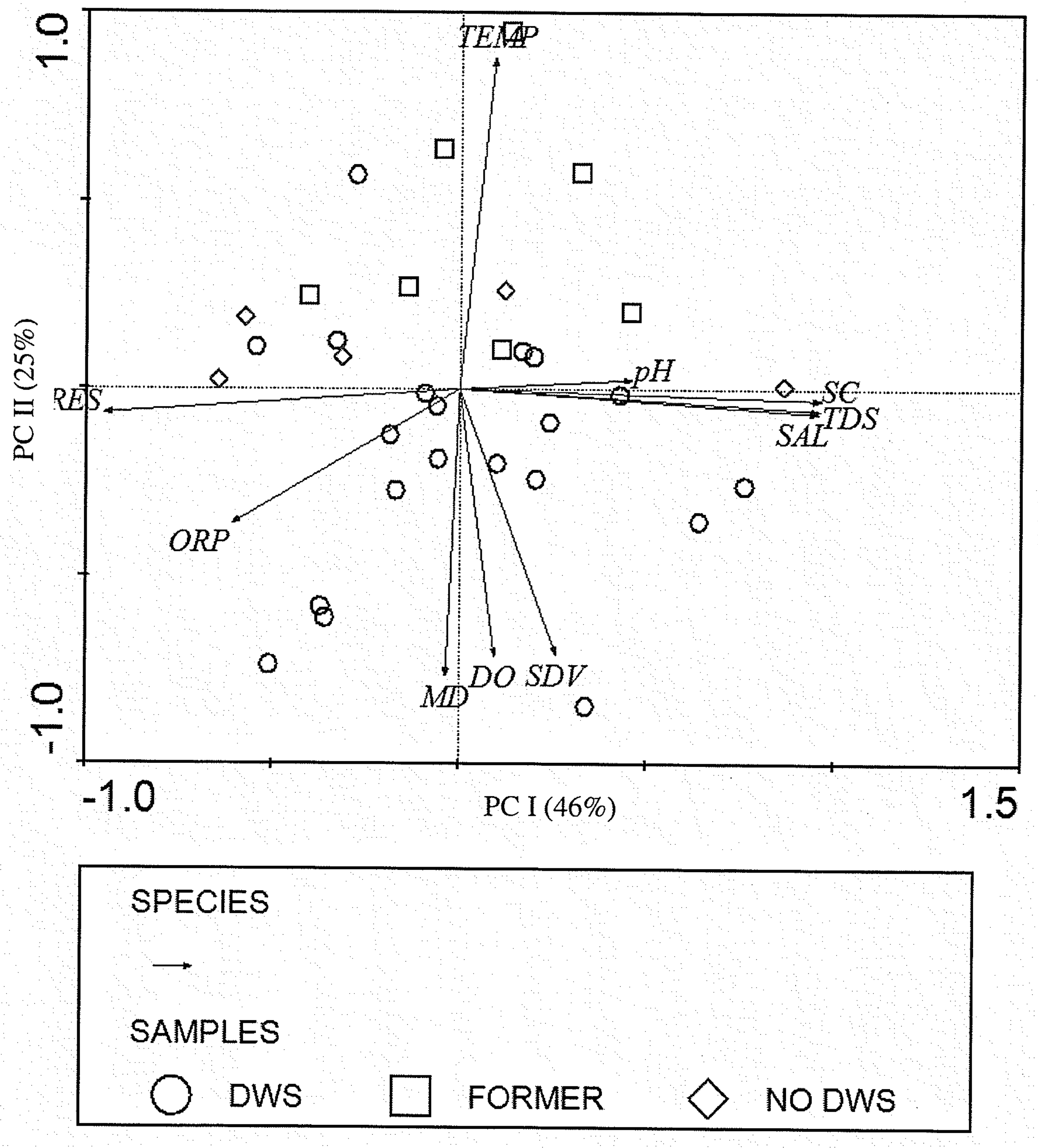
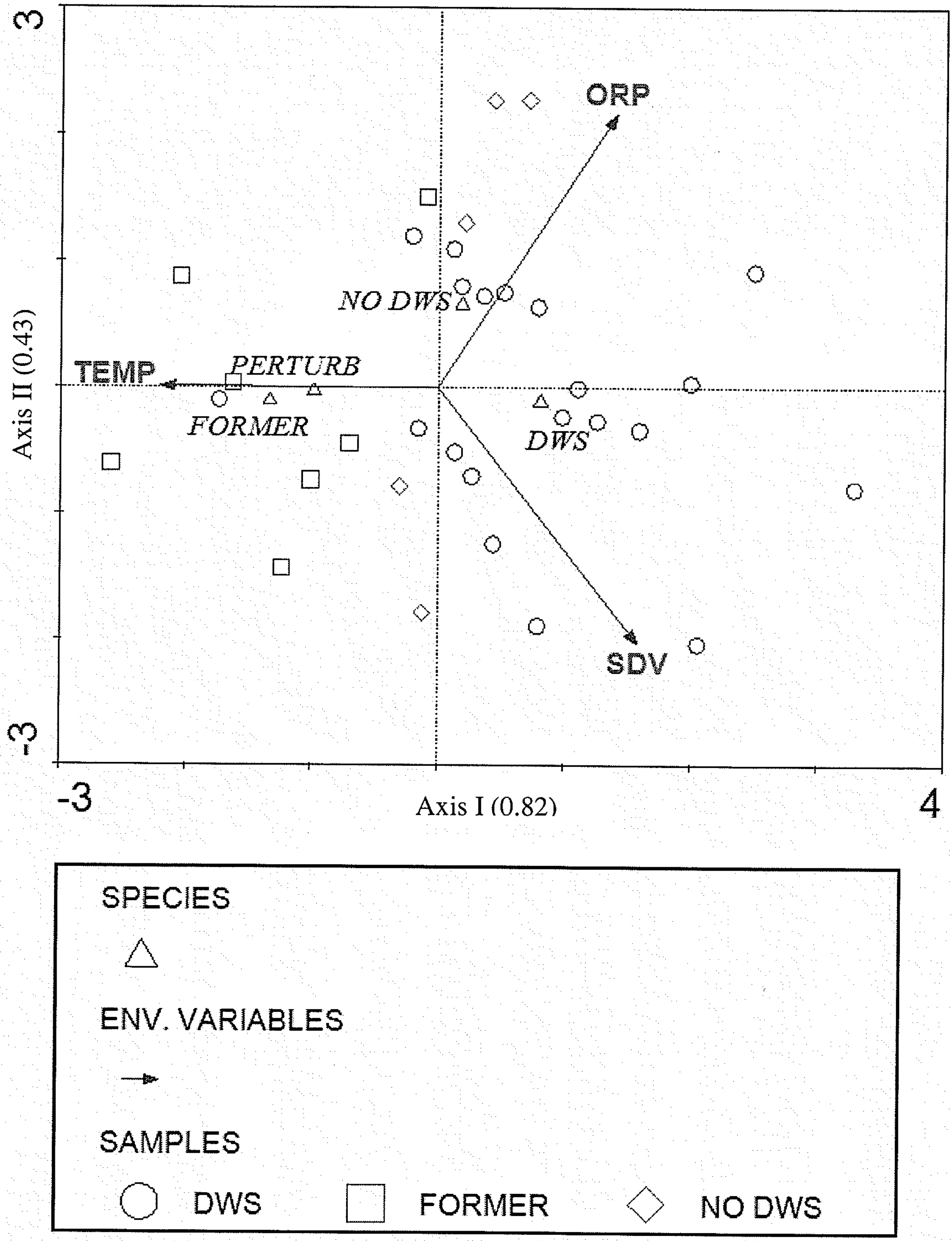

1.0
-1.0
PC II (25%)
-1.0
1.5
PC I (46%)
TEMP
pH
SC
TDS
SAL
RES
ORP
MD
DO
SDV
SPECIES
SAMPLES
DWS
FORMER
NO DWS

Figure 2.10.



3
-3
ORP
NO DWS
TEMP
PERTURB
FORMER
DWS
SDV
Axis II (0.43)
-3
Axis I (0.82)
4
SPECIES
ENV. VARIABLES
SAMPLES
DWS
FORMER
NO DWS

Chapter 3: Evolution and phylogeographic history of the deepwater sculpin (*Myoxocephalus thompsonii*)

Abstract

The deepwater sculpin, *Myoxocephalus thompsonii*, is a North American lake-dwelling sculpin whose taxonomic and genetic structure is poorly understood. Phylogenetic analyses of the mitochondrial DNA control region and ATPase6,8 sequences of deepwater sculpin from 23 inland lakes across Canada, as well as marine and freshwater fourhorn sculpin (*M. quadricornis*) from 11 locations across the arctic, support the distinction of deepwater sculpin and fourhorn sculpin as two species. MtDNA-based divergence estimates suggest that the deepwater sculpin originated during the mid- Pleistocene. Within the deepwater sculpin, three well-defined mitochondrial haplotype-lineages were identified. Phylogeographic analyses reveal that one lineage occurs throughout the species range, while the remaining two lineages are locally distributed in Upper Waterton Lake, Alberta and Fairbank Lake, Ontario, respectively. This pattern is inferred to have resulted from the isolation of deepwater sculpin in Mississippian, Southwestern and Atlantic glacial refugia, followed by dispersal via proglacial lakes and their outlets. Similar patterns have been observed in several other North American fish species. Our findings reinforce the intimate link between the distribution of deepwater sculpin and dispersal via proglacial lake avenues.

Keywords

Deepwater sculpin, *Myoxocephalus thompsonii*, Fourhorn sculpin, *M. quadricornis*, mtDNA, Cottidae, Pleistocene, glaciation, evolution, phylogeography

Introduction

Within North America, Pleistocene glaciation significantly impacted both the physical and biological characteristics of inland lakes, and the evolution and biogeography of the Canadian aquatic fauna (Pielou 1991). The distributions of more than one hundred species of freshwater fishes were shaped by repeated glacial advances and recessions during this period (Mandrak and Crossman 1992). Although these glaciations resulted in levels of intraspecific genetic diversity that were much lower than in non-glaciated areas (Bernatchez and Wilson 1998), the vast proglacial lakes formed due to meltwater also provided tremendous avenues of dispersal across the continent (Wilson and Hebert 1996).

The evaluation of Pleistocene glacial effects and subsequent dispersal routes have been traditionally inferred using species distributions and information derived from fossils, parasites and morphological variation in relation to geography (Riffel and Schreiber 1998; Burns 1991; Black 1983). Over the past 15 years, however, genetics, including allozymes (Riffel and Schreiber 1995), microsatellites (Hanfling et al. 2002) and especially mitochondrial DNA (mtDNA) has emerged as an additional tool, providing further independent data for understanding Pleistocene refugial origins and dispersal routes (Turgeon and Bernatchez 2001). MtDNA phylogeographic studies of groups such as lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), burbot (*Lota lota*), and lake cisco (*Coregonus artedii*), have been conducted to study glacial impacts on genetic structure (Turgeon and Bernatchez 2001; Taylor et al. 1999; Wilson and Hebert 1998; Wilson et al. 1996; Bodaly et al. 1992; Bernatchez and Dodson 1991; Bernatchez and Dodson 1990a,b). In many cases, mtDNA studies have complemented or even substantially altered previous views on the origins of North American freshwater fishes (Taylor et al. 1999; Wilson and Hebert 1998; Bernatchez and Dodson 1991).

While the current distribution of most Canadian fish species were affected by advancing and retreating glaciers, the deepwater sculpin, *Myoxocephalus thompsonii*, is thought to owe both its origin and current distribution to glacial effects (Kontula and Vainola 2003; Scott and Crossman 1973; McAllister 1961; Hubbs and Lagler 1958). The sister species of the deepwater sculpin is the Arctic marine fourhorn sculpin, *Myoxocephalus quadricornis* (McAllister 1961; Linnaeus 1758). This curious relationship led researchers to call the deepwater sculpin a "glacial relict," a species derived from an Arctic marine lineage that was driven south into freshwater habitats by early Pleistocene glacial advances (Kontula and Vainola 2003; Dadswell 1972; McAllister 1961). This origin is thought to be shared with other freshwater glacial relicts, such as the crustaceans *Mysis* and *Diporeia*, that similarly have close relationships with Arctic marine taxa (Audzijonyte 2005; Martin and Chapman 1965; Ricker 1959).

Once established in freshwater, the deepwater sculpin would have been subjected to any subsequent glacial advances, presumably surviving in glacial refugia along with other North American fish species. Dispersal from these refugia into formerly glaciated regions was facilitated by extensive melt-water lakes that formed along the margins of the retreating glaciers. Thus, investigation of deepwater sculpin phylogeography, in relation to glacial events, promises to illuminate both the origin of the species, and its current distribution in lakes across Canada.

The *Myoxocephalus* sculpin complex (McAllister and Aniskowicz 1976) has a circumpolar distribution and consists of two closely related, yet distinct taxa, the deepwater sculpin and the fourhorn sculpin. The deepwater sculpin, *M. thompsonii* (Girard 1852), is a benthic species that is widely, but sporadically, distributed throughout landlocked, deep, freshwater lakes of northern North America. It occurs from southwestern Quebec eastward through the Laurentian Great Lakes, Manitoba, northern Saskatchewan to Great Bear Lake in the Northwest Territories (Parker 1988; Scott and Crossman 1973). An additional isolated population occurs in Upper Waterton Lake in southwestern Alberta (Parker 1988; McAllister and Ward 1972). The fourhorn sculpin, *M. quadricornis* (Linnaeus 1758), is benthic, but rarely descends to depths greater than 20 m (McAllister 1980; Khan 1971). It occurs in arctic marine coastal areas of the Nearctic and Palearctic, inhabiting estuaries and brackish waters. Freshwater populations of fourhorn sculpin occur in several lakes on Canadian arctic islands, as well as in some European lakes (COSEWIC 2003; Kontula and Vainola 2003).

The close relationship between the deepwater sculpin and fourhorn sculpin is well-accepted (Kontula and Vainola 2003; McAllister and Anizkowicz 1976; McAllister 1961). The existence of possibly "intermediate" forms, however, such as the freshwater fourhorn sculpin populations in arctic lakes, has prompted disagreement over the taxonomic status of the two fishes. The freshwater fourhorn populations exhibit intermediate morphological characters between the marine fourhorn sculpin and deepwater sculpin (McPhail and Lindsey 1970). Although gene flow has not been discussed, the idea that there could be a continuum between deepwater sculpin and fourhorn sculpin has led many to consider the deepwater sculpin to be a subspecies (*M. quadricornis thompsonii*) of the fourhorn sculpin (Kontula and Vainola 2003; McAllister and Aniskowicz 1976; Scott and Crossman 1973; McPhail and Lindsey 1970; Hubbs and Lagler 1958). Alternatively, a study that compared the distribution, ecology, and morphology of marine fourhorn and deepwater sculpin, concluded that they were closely related, but distinct species (McAllister 1961).

Aside from taxonomic uncertainty, there are other interesting questions concerning the zoogeography of the deepwater sculpin. First, it is not known when deepwater sculpin entered freshwater or how many colonizations of freshwater took place. The timing of the divergence(s) between fourhorn and deepwater sculpin is not well understood. Second, it is not known whether deepwater sculpin were isolated within glacial refugia during later Pleistocene glaciations. The specific role of glacial refugia and subsequent reinvasion of deepwater sculpin to their present distribution is also not well understood. Dispersal avenues via proglacial lakes and their outlets are not known.

Thus, the deepwater and fourhorn sculpin system could clearly be profitably examined with a detailed phylogeographic study.

In a single genetic study on the *Myoxocephalus* sculpin complex thus far, Kontula and Vainola (2003) used mtDNA sequence data from eight deepwater sculpin from two locations, four fourhorn sculpin from two locations in the Arctic, and five fourhorn sculpin from five locations in northern Europe. They suggested an early to mid Pleistocene origin for deepwater sculpin and a more recent origin for the European freshwater fourhorn sculpin populations. They also suggested subspecific designation of deepwater sculpin. Finally, they suggested that North America harbored a single deepwater sculpin lineage, based on a common haplotype observed in both Upper Waterton Lake, Alberta and Lake Michigan. However, both sample sizes and geographic coverage of the dataset severely limits their phylogeographic conclusions (Kontula and Vainola 2003). The power of such an mtDNA study would be better substantiated by surveys across the full ranges of the species involved (Turgeon and Bernatchez 2003). To date, due to the isolation of deepwater sculpin at great depths within lakes and the associated difficulty with detection and capture, acquisition of suitable sample sizes has hindered taxonomic and phylogeographical study.

Using a significantly higher number of individuals from a range-wide survey, our study more fully describes the taxonomy of fourhorn (both marine and freshwater) and deepwater sculpin, as well as the population structure of deepwater sculpin. We use mitochondrial DNA sequence data to evaluate (1) the glacial origins of deepwater sculpin (timing and number of colonizations); (2) the current distribution of deepwater sculpin in relation to Pleistocene glaciations, glacial refugia, and proglacial lakes and; (3) the taxonomic status of deepwater sculpin and freshwater fourhorn sculpin. A phylogeographical description of deepwater sculpin throughout their range is a superb model for understanding the effects of Pleistocene glaciations on the Canadian aquatic fauna.

Methods

Specimens

Fourhorn sculpin from marine waters across the Canadian arctic and tissues from a population of fourhorn sculpin from Garrow Lake on Little Cornwallis Island were gathered by colleagues (Table 3.1). Deepwater sculpin were collected from the Laurentian Great Lakes by colleagues and from inland lakes by ourselves (Table 3.1) using gill nets, deepwater trawls and rectangular minnow traps designed to lie flat on the lake bottom (see Chapter 1). The traps were baited with cyalume sticks and dog biscuits. Inland collections of deepwater sculpin were made during May-October 2004. Despite the difficulty and effort required to catch deepwater sculpin in remote lakes and at great depths, this sample-set is the largest and most widespread that we are aware of. Deepwater sculpin were collected from 23 lakes across the species range, and geographic coverage was judged to be sufficient for determination of phylogeographic structure.

Gill tissue was removed and frozen immediately in liquid nitrogen, while fin clips were preserved in a solution of 20% DMSO, 0.25 M EDTA at pH 8, saturated with NaCl (Seutin et al. 1991). Gill tissues were subsequently frozen at –80 °C, while fin clips were stored at –30 °C. Specimens were preserved in a 10% buffered formalin solution and then transferred to 70% ethanol. Biological data collection and complete necropsies were conducted on the deepwater sculpin resulting in the destruction of most of the individuals, but whenever possible, two individuals (male and female) from each locality were deposited in the Royal Ontario Museum. Catalogue numbers are listed with the respective sequences in GenBank.

Data collection

Based on a preliminary study of numerous mitochondrial genes for a subset of samples, two regions of the mitochondrial DNA were selected to investigate the genetic structure of deepwater sculpin and its relationship to the fourhorn sculpin. The coding ATPase6,8 genes and the non-coding control region showed rates of molecular evolution that were useful at both interspecific and intraspecific levels. These regions have also been shown to be useful in previous species and population level studies of taxa affected by glaciation (Kontula and Vainola 2001; Kontula et al. 2003; Turgeon and Bernatchez 2001; Volckaert et al. 2002)

Total genomic DNA was extracted using the procedures outlined in the Qiagen spin-column tissue kit and eluted in 200 µL of ddH_2O. The majority of the ATPase6,8 genes, which are known to have a 10 bp region of overlap, were amplified as a single 861 base pair fragment in 25 µL reactions containing 1 µL of DNA, 3 mM $MgCl_2$, 20 mM Tris HCl pH 8.4, 50 mM KCl, 200 µM dNTPs, 0.4 µM of each primer (Table 3.2) and 0.25 units of Gibco Taq polymerase. Polymerase chain reaction (PCR) amplification was performed under the following conditions: 3 minute denaturation at 95° C to begin, followed by 50 cycles of 94° C denaturation for 45 seconds, 52° C annealing for 30 seconds and 72° C elongation for 90 seconds. An 842 base pair fragment representing most of the control region was also amplified in 25 µL reactions containing 1 µL of DNA, 2 mM $MgCl_2$, 20 mM Tris HCl pH 8.4, 50 mM KCl, 200 µM dNTPs, 0.4 µM of each primer (Table 2.2) and 0.2 units of Gibco Taq polymerase. Control region PCR amplification was performed using a 2 minute denaturation to start, followed by 45 cycles of 94° C denaturation for 45 seconds, 50° C annealing for 60 seconds, and 72° C extension for 60 seconds, followed by a final 72° C extension for 6 minutes.

PCR product was cleaned using Qiagen pre-sequencing purification kits. An initial subset of samples were cycle sequenced with the amplification primers (Table 3.2) using BigDye™ (Applied Biosystems, Inc., Foster City, CA) terminator cycle sequencing kits and resolved on an ABI 377 automated sequencer according to manufacturer specifications (Applied Biosystems, Inc., Foster City, CA) or at the University of Calgary Core DNA and Protein Services (also with an ABI 377 automated sequencer). Based on these sequences, internal sequencing primers (Table 3.2) were designed and used to sequence all remaining samples. All sequences are deposited in GenBank.

Both ATPase6,8 and the control region were initially aligned using Sequencher software (Genecodes, Ann Arbor, MI). Alignment of ATPase was trivial, with no indels detected. Alignment of control region sequences was refined by eye- the minimal number of indels obviated the need for more sophisticated multiple alignment methods. However, the control region has a multiple random repeat region towards the "proline" end that was omitted during phylogenetic analysis. In total, 265 deepwater sculpin, 36 marine fourhorn sculpin from North America, and seven freshwater fourhorn sculpin from Garrow Lake on Little Cornwallis Island were sequenced using either ATPase6,8, the control region, or both, resulting in a dataset of over 300 individuals.

Phylogenetic analysis

Three data matrices were assembled for phylogenetic analyses. Separate ATPase6,8 and control region matrices were created, in addition to a combined matrix which included all individuals where both ATPase6,8 and control region sequences were available. Combined analyses are valid because the mitochondrial genes represent a single, non-recombinant unit. Both deepwater and fourhorn sculpin sequences from Kontula and Vainola (2003), including North European fourhorn sculpin population, were included in the ATPase analyses.

Each matrix was analyzed using the maximum parsimony criterion with the heuristic search algorithm in PAUP* (version 4.0b10, Swofford, 2002) using tree bisection-reconnection (TBR) branch swapping and 100 replicates of random taxon addition. All characters were equally weighted, and for both the control region and combined matrices, gaps were treated as a fifth character. For the ATPase matrix, a sequence from the sculpin *Myoxocephalus octodecemspinosus* (Kontula and Vainola 2003) was included and used as an outgroup. From this analysis, it was determined that fourhorn sculpin were an appropriate outgroup to use as a root for the control region and combined datasets when assessing the phylogeographical structure of deepwater sculpin.

To determine support for individual nodes, bootstrap proportions were calculated with PAUP* using 200 replicates with 100 random taxon additions per replicate. Estimates of sequence divergence were calculated as in Lovejoy and Collette (2001) as means of pairwise patristic distances between terminals using the ACCTRAN optimization protocol.

Results

Phylogeny

Compiled sequence data for the ATPase6,8 matrix consisted of 861 bp from 162 deepwater sculpins, 38 marine fourhorn sculpins from North America, four North European freshwater fourhorn sculpin, eight freshwater fourhorn sculpins, and one individual of *M. octodecemspinosus* (Table 3.1). 772 characters were found to be constant and 89 characters were variable, of which 24 were parsimony informative. The ATPase6,8 matrix collapsed into 57 haplotypes plus the outgroup (*M.*

octodecemspinosus). Phylogenetic analysis of the ATPase matrix produced most parsimonious trees of 110 steps with retention indices (RI) of 0.93 and consistency indices (CI) of 0.86. A strict consensus of these trees is shown in Figure 3.1. The tree is well resolved, with deepwater sculpins forming a monophyletic clade with 88% bootstrap support. Within *M. thompsonii*, three distinct clades are resolved (labeled Clades A, B, and C). Clade A is relatively diverse and represented by 31 haplotypes, while Clades B and C have only two haplotypes each. The ATPase analysis does not support the monophyly of *M. quadricornis* relative to *M. thompsonii*. Also, the freshwater North American *M. quadricornis* haplotypes do not make up a monophyletic group. Although North European freshwater fourhorn sculpins form a distinct group, they are well-nested within North American marine fourhorn lineages. The sequence divergence estimate between fourhorn and deepwater sculpin is 1.30% for the ATPase6,8 region. Nucleotide sequence divergences among the three mtDNA lineages of deepwater sculpin ranged from 0.53-0.62% for ATPase6,8.

Sequence data from the control region consisted of 842 bp from 191 deepwater sculpin and two fourhorn sculpin, subsequently collapsing into 56 deepwater sculpin haplotypes and two fourhorn sculpin haplotypes. There were a total of 13 insertion and deletion events, and, in all cases, these were single repeats. 788 characters were found to be constant and 54 characters were variable, of which 25 were parsimony informative. Phylogenetic analysis of the control region matrix produced most parsimonious trees of 83 steps with a retention index of 0.76 and a consistency index of 0.69. A strict consensus of these trees is shown in Figure 3.2. Nucleotide sequence divergences among the mtDNA lineages of deepwater sculpin ranged from 1.35-1.85%.

Compiled sequence data for the combined dataset consisted of 1701 bp from 85 deepwater sculpins and two marine fourhorn sculpins from North America, resulting in a total of 45 non-redundant haplotypes. 1631 characters were found to be constant and 70 characters were variable, of which 31 were parsimony informative. Phylogenetic analysis of the combined matrix produced most parsimonious trees of 88 steps with a retention index of 0.84 and a consistency index of 0.80. A strict consensus of these trees is shown in Figure 3.3. The tree is well resolved, with deepwater sculpins forming a monophyletic clade with 97% bootstrap support and deepwater sculpin lineages forming monophyletic clades with 79-96% bootstrap support.

Distribution of haplotypes

Nearly all data for the fourhorn sculpins consists of ATPase6,8 sequences. Although there are many North American fourhorn sculpin haplotypes, there is no indication of genetic structure associated with geography. Multiple North American marine fourhorn sculpin haplotypes share geographical locations and multiple geographical locations share common haplotypes (e.g. Phillips Bay and Tolken Point individuals are found throughout the fourhorn sculpin clade in the ATPase6,8 consensus tree). North European fourhorn sculpin haplotypes within the ATPase6,8 consensus tree are nested within North American marine fourhorn sculpin taxa. North European fourhorn sculpin taxa are separated from each other by a maximum of one character in the dataset. The freshwater fourhorn sculpin haplotypes from Garrow Lake (on Little Cornwallis Island) and Melville Island do not comprise a monophyletic group. These haplotypes are nested within marine fourhorn sculpin haplotypes. Furthermore, freshwater fourhorn sculpins from both Garrow Lake and Melville Island share haplotypes with North American marine fourhorn sculpin.

The geographic distribution of deepwater sculpin haplotypes can be inferred from the ATPase6,8, control region, and combined datasets. All three analyses indicate that genetic structure is associated with geography. For ATPase6,8, Clade A haplotypes occur throughout the entire species range, in every lake surveyed except Fairbank Lake, near Sudbury, ON (Figure 3.4). The other two lineages (Clades B and C) are locally distributed. One of these (Clade B) is the only lineage present in Fairbank Lake, ON, while the other (Clade C) is present only in Upper Waterton Lake, AB, where it co-occurs with Clade A. For the most part, the control region analysis agrees with ATPase6,8. In the control region analysis, however, the haplotypes from Fairbank Lake are nested within the large lineage (Clade A from ATPase6,8) that is found throughout the species range. As in the ATPase6,8 analysis, there is a locally distributed lineage (co-occurring with the widespread lineage) in Upper Waterton Lake. Most importantly, the combined analysis agrees with the ATPase6,8 dataset, indicating the presence of the same three mtDNA lineages of deepwater sculpin (Clades A, B, and C), each well supported by bootstrap proportions.

In the ATPase6,8 consensus tree, rare haplotypes within Clade A were most often separated from the most common haplotype by a single mutation. Several of these rare haplotypes were common within single lakes, such as Lake 259, ON, where all individuals were one mutation different than the most common Clade A haplotype for ATPase6,8. In addition, some rare haplotypes were shared between individuals from distant locations, such as a rare haplotype in individuals from Wollaston, Roddick, and Upper Waterton Lakes. Generally, there was more variation within each deepwater sculpin lineage in the control region when compared to ATPase6,8.

Discussion

Origin of deepwater sculpin

Our analysis of the ATPase6,8 region shows a major genetic split between deepwater sculpin and fourhorn sculpin (Figure 3.1). Sequence divergence estimates between these lineages suggest that their separation is reasonably ancient. Based on divergence estimates of approximately 1% per million years (Smith 1992; Wilson and Hebert 1998), deepwater sculpin originated approximately 1.3 million years before present (mybp), in the mid- Pleistocene. Our data definitely indicate that the origin of deepwater sculpin is older than the Wisconsinan (approximately 10 000 years b.p.). The monophyly of deepwater sculpin is well-supported by bootstrap proportions, indicating a single continental invasion of deepwater sculpin into freshwater. These results are in accord with the results of Kontula and Vainola (2003), who proposed a monophyletic origin of deepwater sculpin in the early to mid- Pleistocene.

A previous hypothesis for the origin of deepwater sculpin, based on continental fossils in the Glenns Ferry Formation of southwest Idaho, suggests that the species first entered freshwater during the Pliocene (Smith 1975). A second hypothesis suggested a much more recent Wisconsinan origin whereby ancestors of the deepwater sculpin entered freshwater either on their own or were pushed inland in waters ahead of advancing glaciers (McAllister 1961). Finally, McPhail and Lindsey (1970) suggested that deepwater sculpin may have been the product of multiple colonization events. However, sequence divergence estimates refute the timing premises of each of these scenarios. Rather, our data are relatively consistent with the hypothesis for deepwater sculpin origins proposed by Kontula and Vainola (2003).

Several other species have been proposed to be glacial relicts in both North America and Europe. Most of these species are crustaceans, such as *Mysis* and *Diporeia* (Audzijonyte 2005; Segerstrale 1962; McAllister 1961). Available molecular analyses suggest these relicts were not all affected by the same glacial events. There are commonalities among these species, such as a primary split between lineages from continental North American and from the Canadian Arctic and Eurasia (Audzijonyte 2005). However, sequence divergences between the marine and freshwater lineages are often different, suggesting not only that the Pleistocene glaciations were extremely dynamic, but that different species were affected to various degrees and in various manners during this period.

Phylogeography and refugial origins of deepwater sculpin

After the origins of the deepwater sculpin in continental North America, estimated at 1.3 mybp, the deepwater sculpin was presumably affected by subsequent glacial events, much as other North American fishes were. During the Pleistocene, there were four major glacial advances and recessions, as well as numerous minor glacial fluctuations (McPhail and Lindsey 1970). With each ice advancement, fishes were pushed southward and isolated in glacial refugia causing genetic divergence at a within-species level

Sequence divergence estimates of deepwater sculpin mtDNA lineages (0.53-0.62% for ATPase6,8 and 1.35-1.85% for the control region) suggest that the clades originated as a result of isolation in glacial refugia during the mid-Pleistocenene glaciations between 500,000 and 1.3 mybp. This is based on mtDNA divergence estimates of approximately 1% per million years (Smith 1992; Wilson and Hebert 1998). Deepwater sculpin mtDNA divergence estimates are relatively similar to the range of intraspecific divergence estimates of other North American freshwater fish species that were affected by Pleistocene glaciations, including lake trout, lake cisco, lake whitefish, and burbot (Van Houdt et al. 2005; Van Houdt et al. 2003; Turgeon and Bernatchez 2001; Wilson and Hebert 1998; Wilson and Hebert 1996; Bernatchez and Wilson 1998;

Wilson et al. 1996; Bernatchez and Dodson 1994; Bernatchez and Dodson 1991; Billington and Hebert 1991). The principal phylogeographical split within the *Myoxocephalus* sculpins is also in accord with those seen in other northern circumpolar fish complexes, such as burbot (Van Houdt et al. 2005; Van Houdt et al. 2003) and lake whitefish (Bernatchez and Dodson 1994). Small differences in intraspecific divergence estimates and distributions for these species is likely a result of differential response to displacement and habitat loss, which in turn is caused by variation in ecological and physiological characteristics of each species. For example, deepwater sculpin have a narrow thermal niche and are found on the bottom of our deepest lakes at temperatures consistently below 7 °C (Chapter 2). It is altogether possible that water temperatures in this range were more common during the much cooler Pleistocene period in which the glaciations occurred. The cold thermal tolerance of deepwater sculpin might have made it easier for them to disperse via cold proglacial lakes, perhaps explaining the extremely wide distribution of Clade A across the whole range of the species.

Thus far, hypotheses of the refugial origins of deepwater sculpin based on the current species distribution are unresolved or speculative (Mandrak and Crossman 1992; Kontula and Vainola 2003). Deepwater sculpin occur from the Gatineau region of southwestern Quebec through the Laurentian Great Lakes and northward to Great Bear Lake, with an additional population in southwestern Alberta (Parker 1998; Scott and Crossman 1973). The distribution covers both the Hudson Bay and Mackenzie drainages. However, deepwater sculpin are not found in any former glacial refugial areas. Its distribution is adjacent to an Atlantic refuge to the southeast, a Beringian refuge to the northwest, a Mississippian refuge to the south, and a southwestern refuge to the extreme southwest, but it does not presently occur in any of these areas. Furthermore, there is no evidence of deepwater sculpin fossils from these refugia. Thus, genetic data from our study is an essential tool to infer the refugial origins of deepwater sculpin.

When using genetic or morphological characters, it is generally accepted that refugia are located in close proximity to the areas of increased clade diversity (Turgeon and Bernatchez 2001; Cann et al. 1987). It is also accepted that a high frequency of occurrence of a lineage within an area generally indicates that its origin is highly correlated to a nearby refuge (Turgeon and Bernatchez 2001). These ideas were demonstrated in a phylogeographic study of lake trout, where hypothesized refugial areas were highly correlated with increased frequency of occurrence of specific mtDNA lineages in nearby areas (Wilson and Hebert 1996; Wilson and Hebert 1998).

In our study, the widespread Clade A does not show a center of highest diversity (Figure 3.4). This may be an artifact of relatively low sample sizes from each location. Clade A, however, is either the sole lineage or the lineage with the highest frequency of occurrence near all former refugial areas where it is found, including the Mackenzie and Hudson Bay drainages, as well as southwestern Alberta. This would seem to indicate its plausible dispersal from one of Mississippian, Beringian, or Southwestern refugia, or a combination of these. Dispersal from a Beringian refuge throughout the entire distribution of deepwater sculpin, including southwestern Quebec and Upper Waterton Lake, however, seems highly unlikely. First, the distribution of deepwater sculpin is not similar to other species that have dispersed from a Beringian refuge (Hocutt and Wiley 1986). There are no known coastal populations of deepwater sculpin near eastern Hudson and James Bay, indicating that dispersal along the arctic coast towards eastern Canada did not occur. In addition, a waterfall on the lower Mackenzie River that existed for over 5000 years during the proglacial lake period would have prevented inland dispersal of a Beringian refugial lineage of deepwater sculpin (Hocutt and Wiley 1986; Wilson and Hebert 1998). Dispersal from a Southwestern refuge also seems unlikely as routes of dispersal towards the northwest were extremely limited and characterized by poorly suited habitat for cold-water fishes, such as lake trout and deepwater sculpin (Wilson and Hebert 1998; Dyke and Prest 1987). Lake trout, which are much better suited to active dispersal than deepwater sculpin, were unable to disperse in significant numbers from a southwestern refugium (Wilson and Hebert 1998).

Therefore, the Mississippian seems to be the most logical refuge where Clade A persisted during glacial maxima (Figure 3.5). The widespread distribution of Clade A is suggestive of a Mississippian origin, as fishes residing within a Mississippian refugium had the most extensive postglacial avenues to the proglacial lake system (Mandrak and Crossman 1992). The dispersal of Clade A and its subsequent distribution also agrees with other North American freshwater fishes affected by glaciations that dispersed from a Mississippian refugium (Turgeon and Bernatchez 2001; Bernatchez and Dodson 1991; Wilson and Hebert 1998). Finally, deepwater sculpin from a Mississippian refuge would have had access to both Lake McConnell via the Clearwater River (9500 ya) and southwestern Quebec via the Fossmill-Petawawa outlet (10 500 to 9500 ya), providing routes for their dispersal to distant regions where the lineage is currently found (Dyke and Prest 1987; Eschman and Karrow 1985).

Clade C has a highly restricted distribution, occurring in only a single lake. The localized distribution of Clade C in Upper Waterton Lake, AB suggests it originated in a southwestern refugium in western Montana during the Pleistocene glaciations (Figure 3.5). A southwestern refugium has also been proposed to explain the presence of several endemic cold-water fish in this area, including lake trout and fossils of arctic grayling (*Thymallus arcticus*) (Crossman and McAllister 1986; Wilson and Hebert 1998; Burns 1991). It is highly unlikely that Clade C deepwater sculpin resided in a Missourian refuge, as this would have resulted in dispersal via Lake Agassiz and a much more significant distribution of the clade (Wilson and Hebert 1998).

The presence of both Clades A and C in Upper Waterton Lake (Figure 3.4 and 3.5) suggests two separate colonization events into the area. The first colonization most likely occurred during the early to mid-Pleistocene, subsequent to the isolation of Clade C in a southwestern refugium. The second colonization would have occurred subsequent to the isolation of Clade C deepwater sculpin. Most likely, the second colonization of Upper Waterton Lake, this time by Mississippian deepwater sculpin, occurred subsequent to the Wisconsinan glaciations. Sequence divergence estimates between the two lineages (0.62% for ATPase6,8 and 1.85% for the control region) support this hypothesis. Geological evidence of the first advance of deepwater sculpin into the area was most likely erased by the subsequent advancement of glaciers throughout the area (Fulton

1989). However, there is evidence of two separate glacial advances of the continental ice sheet to within at least 10 km of Upper Waterton Lake during the last glacial maximum (Jackson Jr. and Little 2004). Within Upper Waterton Lake itself, there are glaciolacustrine sediments that were deposited in glacial lakes by continental ice sheet margins during the last glaciation (Jackson Jr. and Little 2004). There are also scattered Canadian Shield clasts within the Waterton area (Jackson Jr. and Little 2004). These suggest that dispersal of a Mississippian lineage of deepwater sculpin into Upper Waterton Lake was possible subsequent to the last continental ice sheet advance.

Explanations of the origin of Clade B are much less straightforward, as this clade occurs in a single lake (Fairbank Lake, ON) that is completely surrounded, geographically, by the much more widespread Clade A (Figure 3.4). Furthermore, Fairbank Lake is extremely close to Lake Huron, which is part of the Clade A distribution. As a result, it is difficult to infer a glacial refugium for Clade B. Its close proximity to a Mississippian refugium would seem to indicate its persistence within this refuge. However, if Clade B persisted in a Mississippian refugium, its presence would be expected within the Great Lakes. Its distribution would also be expected to be much more widespread (similar to Clade A), as fish with Mississippian refugial origins had the most extensive postglacial dispersal routes to the proglacial lake system (Mandrak and Crossman 1992). Although a Mississippian refugium is thus an unlikely source for Clade B, there is definitely a signal of a distinct evolutionary pathway for the deepwater sculpin within Fairbank Lake. There are clearly several haplotypes representing the clade without any overlap in distribution with the other lineages (Figure 3.3). However, because of the lack of a clear-cut dispersal route for Clade B, the geological and glacial lake history of the area must be closely scrutinized to hypothesize an origin.

Fairbank Lake is located within the Sudbury area that was deglaciated between 10 500 and 10 000 years ago (Barnett and Bajc 2002; Bajc and Barnett 1999; Eschman and Karrow 1985). Within this area, Fairbank Lake occurs at the elevated southwestern rim of the Sudbury basin. It is therefore at higher elevations than the surrounding topography (Barnett and Bajc 2002). Fairbank Lake is also in an area that was covered by glacial Lake Algonquin, which fronted the receding glacier (Dyke and Prest 1987; Barnett and Bajc 2002). Several ancestral phases of glacial Lake Algonquin occurred within the Sudbury region, including the Cedar Point, Payette, Sheguiandah, and Korah phases (Barnett and Bajc 2002; Barnett and Bajc 1999). The outlets within the North Bay area controlled the water levels of the phases of glacial Lake Algonquin during glacial recession of the Sudbury area (Barnett and Bajc 2002; Barnett and Bajc 1999). The phases of glacial lakes, such as those from Lake Algonquin were often very dynamic and short-lived. These characteristics provided opportunities for separate temporal and/or geographical routes of dispersal into the same region (Barnett and Bajc 2002; Turgeon and Bernatchez 2001; Dadswell 1974).

The Quaternary geology of the Sudbury area has been studied extensively (Barnett and Bajc 2002; Bajc and Barnett 1999; Eschman and Karrow 1985). A delta, which forms where meltwater enters a lake, is known from the south end of Fairbank Lake (Barnett and Bajc 2002). This delta formed at approximately 335 m above sea level (asl) during the highest level of glacial Lake Algonquin, commonly known as the Cedar Point phase (Barnett and Bajc 2002). It is thought that the southern rim of the Sudbury basin formed a wall upon which the retreating glacier was momentarily stabilized. At this point, glacial Lake Algonquin would have covered the southern tip of what is now Fairbank Lake. This would have provided the earliest point of entry for deepwater sculpin into Fairbank Lake, approximately 10 500 years ago (Barnett and Bajc 2002). Theoretically, Fairbank Lake would have remained accessible until recession of the ice began from the Cartier I moraine shortly thereafter. Once the glacier had receded 5 km from the Cartier I moraine, the water level of glacial Lake Algonquin dropped to the Suez delta level, approximately 304 m asl (Barnett and Bajc 2002; Bajc and Barnett 1999). Once water levels began to drop, access to Fairbank Lake via glacial Lake Algonquin or any other dispersal route would have been impossible. However, at the Suez delta level of glacial Lake Algonquin, all surrounding locations where deepwater sculpin are known, including the Laurentian Great Lakes and southwestern Quebec populations would have remained accessible.

Dispersal of deepwater sculpin into Fairbank Lake, therefore, had to occur between the Cedar Point phase and Suez delta level of glacial Lake Algonquin. Furthermore, dispersal of deepwater sculpin from the widespread Mississippian clade into surrounding areas, including populations in southwestern Quebec most likely occurred once access to Fairbank Lake was cut off, subsequent to Clade B's dispersal into Fairbank Lake. The remaining populations of Clade B deepwater sculpin that were outside Fairbank Lake and still accessible via glacial lakes or their outlets could have been subsequently replaced by a much larger, more widespread, and ultimately more successfully dispersing, source of deepwater sculpin from a Mississippian refuge.

We, therefore, suggest that the lineage within Fairbank Lake may be associated with an Atlantic refugium that had early access to the Sudbury region during deglaciation of the area (Figure 3.5). This could be the result of sequential dispersal from an Atlantic refuge via the Hudson River into glacial Lake Vermont, to glacial Lake Iroquois and through the Kirkfield outlet (Eschman and Karrow 1985). The Kirkfield outlet connected the Georgian Bay basin of glacial Lake Algonquin to glacial Lake Iroquois approximately 12 000 to 11 800 ya (Mandrak and Crossman 1992; Eschman and Karrow 1985). Alternatively, there may have been a previously undescribed connection from the Champlain Sea directly to the northern part of glacial Lake Algonquin along the receding ice front approximately 11 000 ya, as the two were in extremely close proximity to one another (Dyke and Prest 1987). The preference that deepwater sculpin have for permanently cold water temperatures (Chapter 2) would have made small, standing waterbodies along the very edge of the receding glaciers possible dispersal routes.

These routes would have allowed dispersal of deepwater sculpin from an Atlantic refugium into Fairbank Lake immediately upon its deglaciation during the Cedar Point phase (highest level) of Lake Algonquin. Lowering of Lake Algonquin levels would have then cut off access to Fairbank Lake, isolating the Atlantic refugial source of deepwater sculpin within Fairbank Lake. Subsequent dispersal of the Mississippian lineage into the northern region of glacial Lake Algonquin would have allowed the replacement of remaining Atlantic refugial deepwater sculpin at lower elevations by the Mississippian form. However, access to Fairbank Lake would have been impossible due to the lowered glacial lake level. Dispersal routes in all directions from Lake Algonquin where the Mississippian lineage of deepwater sculpin is currently found would have remained open at this time. In particular, access to southwestern Quebec would have been possible via the Fossmill-Petawawa outlet, to the Ottawa River, and subsequently into glacial Lake Barlow-Ojibway, which inundated the area (Turgeon and Bernatchez 2002; Mandrak and Crossman 1992). In addition, access to McConnell Lake via the discharge of the Clearwater River from glacial Lake Agassiz was available from 9900 to 9500 ya (Dyke and Prest 1987; Turgeon and Bernatchez 2002; Smith 1994).

An alternative, but seemingly less likely possibility, is that Clade B deepwater sculpin came from a previously undescribed refuge to the south, in the Michigan highlands or Iron Hills area. A refuge in this area would have been separate from a Mississippian refuge. Subsequent to the colonization of Fairbank Lake by Clade B, the Mississippian lineage would have once again replaced all remaining Clade B populations at lower elevations in the same manner as described above. Despite the high topography in northern Michigan, however, a glacial refuge from that area has not been described. Thus, we believe that dispersal from an Atlantic refuge is the most likely explanation for the Clade B lineage in Fairbank Lake.

Deepwater and fourhorn sculpin taxonomy

Historically, the presence of freshwater forms of the fourhorn sculpin seemed to provide an intermediate form or continuum between deepwater and fourhorn sculpin (COSEWIC 2003). This has caused the separate species designations of the two taxa to be contentious. Further confusing matters is the fact that reproductive isolation, as a species distinctiveness criterion, cannot be used for allopatric taxa (McAllister 1961). Based, for the most part, on morphological and ecological data that allowed the complete separation of the two species, McAllister (1961) suggested full specific taxon designations for both deepwater and fourhorn sculpin. However, his study did not include freshwater forms of the fourhorn sculpin. McPhail and Lindsey (1970) reasoned that the freshwater forms of the fourhorn sculpin allowed the joining of the extreme forms (marine fourhorn and deepwater sculpin) in a continuum. Therefore, they proposed subspecific designation of deepwater sculpin as *M. quadricornis thompsoni* (McPhail and Linsey 1970).

Our genetic data can be used to provide additional evidence of the species-level separation of fourhorn and deepwater sculpin. Freshwater fourhorn sculpin haplotypes from Garrow Lake on Little Cornwallis Island and Melville Island are clearly nested within marine fourhorn sculpin haplotypes. This confirms that, despite geographic isolation, there is no differentiation in the mitochondrial regions we examined in freshwater and marine fourhorn sculpin. Although these fourhorn sculpin are from two arctic lakes, results suggest that other fourhorn sculpin populations isolated in arctic lakes will most likely be genetically undifferentiated from marine fourhorn sculpin. Our results also indicate that freshwater fourhorn sculpin populations in arctic lakes and northern Europe have originated in complete independence of the continental invasion of deepwater sculpin into Canada. In addition, it appears that freshwater evolution of fourhorn sculpin has occurred multiple times. Most likely, fourhorn sculpin in arctic lakes have originated due to isostatic rebound of the arctic islands and subsequent isolation in gradually freshening waterbodies that have been cut off from the Arctic ocean (COSEWIC 2003; Stewart and Platford 1986; McAllister and Aniskowicz 1976).

Although fourhorn sculpin from arctic lakes appear to have intermediate morphological characters of marine fourhorn sculpin and deepwater sculpin, they have always been historically grouped with marine fourhorn sculpin (McAllister and Aniskowicz 1976). Our results indicate that there is no genetic intermediacy of fourhorn sculpin from arctic islands. They are clearly nested within marine fourhorn sculpin in a clade that is well supported (Figure 3.1). Therefore, our results reduce the ambiguity of the taxonomic relationship between fourhorn and deepwater sculpin. In the mtDNA regions we examined, fourhorn sculpin from lakes on arctic islands seem to be genetically identical to marine fourhorn sculpin and both are now clearly distinct from deepwater sculpin. There are no genetically intermediate forms. As a result of this study, there is now evidence of clear genetic separation between fourhorn and deepwater sculpin, in addition to the habitat and morphological differentiation previously shown by McAllister (1961).

Based on the total weight of evidence from morphological data, evolutionary distinctness, ecological differences, and now genetic separation, full specific taxon designation of deepwater sculpin is well supported. This is further substantiated with evidence of adaptive distinctiveness criteria as applied by Crandall et al. (2000) in their evaluation of evolutionary processes in conservation biology. When comparing taxon distinctiveness based on the genetic and ecological exchangeability of populations for both recent and historical times, there is evidence of significant population differentiation between deepwater and fourhorn sculpin (Crandall et al. 2000). This is based on the rejection of the null hypotheses of genetic and ecological exchangeability for both historical (> 1 mya) and recent (< 1 mya) time frames. As a result, the recommended management action would be to treat deepwater sculpin and fourhorn sculpin as distinct species (Crandall et al. 2000). We, therefore, propose the retention of full species recognition for both *M. quadricornis* and *M. thompsonii.*

Conclusions

Results from our study indicate that the *Myoxocephalus* sculpin complex is yet another useful model to study the effects of the Pleistocene glaciations on the Nearctic aquatic fauna. Our study confirms a single continental invasion of deepwater sculpin dating from the mid-Pleistocene and suggests the separation of fourhorn and deepwater sculpin as closely related, but distinct, species.

Our phylogeographic results indicate that subsequent to the initial continental invasions, three distinct evolutionary lineages of deepwater sculpin evolved, each of which is associated with a separate glacial refuge. The lineage associated with a Mississippian refuge was clearly able to take advantage of widespread dispersal routes and is therefore found throughout the entire species range. In contrast, the remaining two lineages have limited distributions. This may have been due to both the inability of these lineages to exploit particular glacial lake dispersal avenues, and the replacement of haplotypes in certain lakes by the widespread Mississippian lineage. The lineage restricted to Upper Waterton Lake, AB was clearly associated with a southwestern refuge and was unable to exploit dispersal routes to the northeast, a biogeographic pattern exhibited by other cold-water adapted freshwater fish from this refuge. The lineage isolated within Fairbank Lake is the most contentious. However, the geological and glacial lake history as well as the topography of the area suggests that the lineage within Fairbank Lake is most likely derived from an Atlantic refuge and accessed the area during the highest phase of glacial lake Algonquin (Cedar Point phase).

Due to the extreme difficulties of collecting deepwater sculpin from an adequate number of locations throughout the species range, our study provides the first comprehensive genetic analyses and interpretation of phylogeographical patterns of the deepwater sculpin, a species that has been of interest to the zoogeographic community for many years.

References

Audzijonyte, A., J. Damgaard, S. Varvio, J.K. Vainio and R. Vainola. 2005. Phylogeny of *Mysis* (Crustacea, Mysida): history of continental invasions inferred from molecular and morphological data. Cladistics. 21:575-596.

Bajc, A.F., and Barnett, P.J. 1999. Quaternary geology and geomorphology of the Sudbury region; Geological Association of Canada- Mineralogical Association of Canada, Joint Annual Meeting, Sudbury, Ontario. Field Trip A5 Guidebook, 68pp.

Barnett, P.J., and A.F. Bajc. 2002. Quaternary Geology. *In* Physical Environment of the city of Greater Sudbury, Ontario. Ontario Geological Survey, Special Volume 6, pp. 56-85.

Bernatchez, L., and R.G. Danzmann. 1993. Congruence in control region sequence and restriction site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis Mitchill*). Mol. Biol. Evol. 10:1002-1014.

Bernatchez, L. and J.J. Dodson. 1990a. Mitochondrial DNA variation among anadromous populations of cisco (*Coregonus artedii*) as revealed by restriction analysis. Can. J. Fish. Aquat. Sci. 47:533-543.

Bernatchez, L. and J.J. Dodson. 1990b. Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial DNA restriction analysis. Evol. 44:1263-1271.

Bernatchez, L., and J.J. Dodson. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) and its relations to Pleistocene glaciations. Evol. 45:1016-1035.

Bernatchez, L., and C.C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. Mol. Ecol. 7(4):431-452.

Billington, N., and P.D.N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45:643-654.

Black, G.A. 1983. *Cystidicola farionis* (Nematoda) as an indicator of lake trout (*Salvelinus namaycush*) of Bering ancestry. Can. J. Fish. Aquat. Sci. 40:2034-2040.

Bodaly, R.A., J. Vuorinen, R.D. Ward, M. Luczynski, and J.D. Reist. 1991. Genetic comparisons of New and Old World coregonid fishes. J. Fish Biol. 38:37-51.

Burns, J.A. 1991. Mid-Wisconsinan vertebrates and the environment from January Cave, Alberta, Canada. Quat. Res. 35:130-143.

Cann, R.L., M. Stoneking, A.C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 325:31-36.

COSEWIC. 2003. COSEWIC assessment and update status report on the fourhorn sculpin *Myoxocephalus quadricornis* (freshwater form) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 24 pp. (www.sararegistry.gc.ca/status/status_e.cfm).

Crandall, K.A., O.R.P. Bininda-Edmonds, G.M. Mace, and R.K. Wayne. 2000. Considering evolutionary processes in conservation biology. TREE 15(7):290-295.

Crossman, E.J., and D.E. McAllister. 1986. Zoogeography of freshwater fishes of the Hudson Bay drainage, Ungava Bay and the Arctic Archipelago. *In* The

Zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. Jon Wiley and Sons, New York, pp. 53-104.

Dadswell, M.J. 1974. Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. Nat. Mus. Nat. Sci. Pub. Zool. 11:1-110.

Dyke, A.S., and V.K. Prest. 1987. Late Wisconsinan and Holocene history of the Laurentide Ice Sheet. Geogr. Phys. Quat. 41:237-263.

Eschman, D.F. and P.F. Karrow. 1985. Huron basin glacial lakes: a review. In Quaternary evolution of the Great Lakes. Geol. Assoc. Can., Special Paper 30. pp. 79-93.

Fulton, R.J. eds. 1989. Quaternary geology of Canada and Greenland. Geol. Surv. Can., Geol. Can., no. 1.

Girard, G. 1852. Contributions to the natural history of the freshwater fishes of North America. I. A monograph of the cottids. Smiths. Contrib. Knowl. 3 (article 3). 80 pp.

Hanfling, B., B. Hellemans, F.A.M. Volckaert, and G.R. Carvalho. 2002. Late glacial history of the cold-adapted freshwater fish *Cottus gobio*, revealed by microsatellites. Mol. Ecol. 11:1717-1729.

Hocutt, C.H., and E.O. Wiley, eds. 1986. The zoogeography of North American Freshwater Fishes. John Wiley and Sons. New York.

Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. North Carolina State Museum of Natural History Biological Survey Publication 1980 -12. pp. 827-830.

Houston, J. 1990. Status of the Fourhorn Sculpin, *Myoxocephalus quadricornis*, in Canada. Can. Field-Nat. 104(1):7-13.

Hubbs, C.L., and K.F. Lagler. 1958. Fishes of the Great Lakes region. Bull. Cranbrook Inst. Sci. 26:1-213 rev. ed.

Jackson Jr., L.E., and E.C. Little. 2004. A single continental glaciation of Rocky Mountain Foothills, south-wester Alberta, Canada. *In* Quaternary glaciations - extent and chronology. *Edited by* J. Ehlers and P.L. Gibbard. Elsevier B.V., Amsterdam, The Netherlands, pp. 29-38.

Khan, N.Y. 1971. Comparative morphology and ecology of the pelagic larvae on nine Cottidae (Pisces) of the northwest Atlantic and St. Lawrence drainages. M.Sc. thesis, University of Ottawa, Ottawa, ON. 234 pp.

Kontula, T., S.V. Kirilchik, and R. Vainola. 2003. Endemic diversification of the monophyletic cottoid fish species flock in Lake Baikal explored with mtDNA sequencing. Mol. Phylogenet. Evol. 27:143-155.

Kontula, T., and R. Vainoloa. 2003. Relationships of Palearctic and Nearctic ‘glacial relict’ *Myoxocephalus* sculpins from mitochondrial DNA data. Mol. Ecol. 12:3179-3184.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis, Laurentii Salvii, Holmiae, 12th Edition, Volume 1.

Lovejoy, N.R., M. Iranpour, and B.B. Collette. 2004. Phylogeny and jaw ontogeny of beloniform fishes. Integr. Comp. Biol. 44:366-377.

Mandrak, N.E., and E.J. Crossman. 1992. Postglacial dispersal of freshwater fishes into Ontario. Can. J. Zool. 70:2247-2259.

Martin, N.V., and L.J. Chapman. 1965. Distribution of certain crustaceans and fishes in the region of Algonquin Park, Ontario. J. Fish. Res. Bd. Can. 22(4):969-976.

McAllister, D.E. 1980. *Myoxocephalus quadricornis* (Linnaeus) fourhorn sculpin. *In* Atlas of North American freshwater fishes. *Edited by* D.S. Lee, C.R. Gilbert, G.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. North Car. State Mus. Nat. Hist. Biol. Surv. Pub. 1980-12. p. 826

McAllister, D.E. 1961. The origin and status of the deepwater sculpin, *Myoxocephalus thompsonii*, a Nearctic glacial relict. National Museum of Canada Bulletin 172:44-65.

McAllister, D.E., and J. Aniskowicz. 1976. Vetebral number in North American sculpins of the *Myoxocephalus quadricornis*-complex. J. Fish. Res. Bd. Canada. 33:2792-2799.

McAllister, D.E., and J.C. Ward. 1972. The deepwater sculpin, *Myoxocephalus quadricornis thompsoni*, new to Alberta, Canada. J. Fish. Res. Bd. Canada 29:344-345.

McPhail, J.D., and C.C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Bd. Can. Bull. 173. pp. 319-323.

Parker, B.J. 1988. Status of the deepwater sculpin, *Myoxocephalus thompsoni*, in Canada. Can. Field-Nat. 102(1):126-131.

Pielou, E.C. 1991. After the Ice Age: the Return of Life to Glaciated North America. University of Chicago Press, Chicago, Illinois.

Ricker, K. E. 1959. The origin of two glacial relict crustaceans in North America, as related to Pleistocene glaciation. Can. J. Zool. 37: 871-893.

Riffel, M., and A. Schreiber. 1995. Coarse-grained population structure in Central European sculpin (*Cottus gobio* L.): secondary contact or on-going genetic drift? J. Zool. Syst. Evol. Res. 33:173-184.

Riffel, M., and A. Schreiber. 1998. Morphometric differentiation in populations of the Central European sculpin *Cottus gobio* L., a fish with deeply divergent genetic lineages. Can. J. Zool. 76:876-885.

Roseman, E.F., D.J. Jude, M.K. Raths, T.G. Coon, and W.W. Taylor. 1998. Occurrence of the deepwater sculpin (*Myoxocephalus thompsoni*) in western Lake Erie. J. Great Lakes Res. 24(2):479-483.

Scott, W.B., and E.J. Crossman. 1973. The freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184. 966 pp.

Segerstrale, S.G. 1962. The immigration and prehistory of the glacial relicts of Eurasia and North America. A survey and discussion of modern view. Int. Revue der Gesam. Hydrobiol. 47:1-25.

Seutin, G., B.N. White and P.T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69:82-90.

Smith, D.G. 1994. Glacial Lake McConnell: paleogeography, age, duration, and associated river deltas, MacKenzie River Basin, Western Canada. Quat. Sci. Rev. 13:829-844.

Smith, G.R. 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41:41-57.

Smith, G.R. 1975. Fishes of the Pliocene Glenns Ferry Formation, southwest Idaho. University of Michigan Museum of Paleontology, Papers on Paleontology. 14:1-68.

Stewart, K.M. and R.F. Platford. 1986. Hypersaline gradients in two Canadian high arctic lakes. Can. J. Fish. Aquat. Sci. 43:1795-1803.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taylor, E., S. Pollard, and D. Louie. 1999. Mitochodrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation. Mol. Ecol. 8:1155-1170.

Turgeon, J. and L. Bernatchez. 2001. Mitochondrial DNA phylogeography of lake cisco (*Coregonus artedi*): evidence supporting secondary contacts between two glacial races. Mol. Ecol. 10:987-1001.

Van Houdt, J.K., B. Hellemans and F.A.M. Volckaert. 2003. Phylogenetic relationships among Palearctic and Nearctic burbot (*Lota lota*): Pleistocene extinctions and recolonizations. Mol. Phylogenet. Evol. 29:599-612.

Van Houdt, J.K.J., L. De Cleyn, A. Perretti and F.A.M. Volckaert. 2005. A mitogenic view on the evolutionary history of the holarctic freshwater gadoid, burbot (*Lota lota*). Mol. Ecol. 14:2445-2457.

Volckaert, F.A.M., B. Hanfling, B. Hellemans, and G.R. Carvalho. 2002. Timing of the population dynamics of bullhead *Cottus gobio* (Teleostei: Cottidae) during the Pleistocene. J. Evol. Biol. 15: 930-944.

Wilson, C.C., and P.D.N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America. Can. J. Fish. Aquat. Sci. 55:1010-1024.

Wilson, C.C., P.D.N. Hebert, J.D. Reist. 1996. Phylogeographic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. 53:2764-2775.

Wilson, C.C., P.D.N. Hebert, J.D. Reist, and J.B. Dempson. 1996. Phylogeography and postglacial dispersal of arctic charr *Salvelinus alpinus* in North America. Mol. Ecol. 5:187-197.

Tables and Table Captions

Table 3.1. Collection sites for deepwater (DWS) and fourhorn sculpin (FHS-marine, FHS-arctic lakes) across their ranges. Geographical locations and numbers of individuals sequenced for both ATPase6,8 (N-ATP) and the control region (N-CR).

No.	Taxon	Waterbody	Region	Latitude (N)	Longitude (W)	N-ATP	N-CR
1	DWS	Roddick Lake	Quebec	46 14' 54.4"	75 53' 30.9"	8	8
2		Thirty-One mile		46 12' 43.1"	75 48' 46.4"	6	3
3		Lake 259 (ELA)	Ontario	49 41' 19.9"	93 47' 8.2"	6	4
4		Teggau (ELA)		49 42' 07.7"	93 38' 53.1"	2	2
5		Eagle Lake		49 46' 15.5"	93 36' 44.0"	11	2
6		Burchell Lake		48 35' 07.6"	90 37' 37.6"	17	16
7		Fairbank Lake		46 27' 35.0"	81 25' 37.0"	6	6
8		Saganaga Lake		48 14' 32.7"	90 56' 02.7"	12	2
9		Lake Nipigon		49 27' 37.0"	88 09' 57.6"	3	4
10		Lake Ontario		Unknown	Unknown	3	1
11		Lake Huron		Various	Various	3	21
12		Lake Superior		Various	Various	11	69
13		Lake Michigan	U.S.A.	Various	Various	6 + (2*)	17
14		Westhawk Lake	Manitoba	49 45' 32.0"	95 11' 28.0"	6	4
15		George Lake		50 15' 49.6"	95 28' 16.2"	1	1
16		Clearwater Lake		54 04' 05.5"	101 05' 33.7"	5	5
17		Second Cranberry Lake		54 39' 08.5"	101 09' 58.2"	18	4
18		Lake Athapapuskow		54 33' 01.2"	101 39' 05.4"	9	2
19		Reindeer Lake	Saskatchewan	56 23' 34.7"	102 58' 22.2"	3	0
20		Wollaston Lake		58 14' 59.3"	103 29' 44.4"	4	4
21		Alexie Lake	Northwest Territories	62 40' 36.0"	114 06' 08.0"	1	1
22		Great Slave Lake		62 29' 15.0"	110 52' 44.0"	9	7
23		Upper Waterton Lake	Alberta	49 00' 17.9"	113 54' 16.8"	9 + (2*)	8
24	FHS-marine	Shingle Point	Northwest Territories	68 95'	137 22'	9	0
25		Phillips Bay	Northwest Territories	69 17'	138 30'	5	1
26		Tolken Point	Northwest Territories	Not given	Not given	5	1

27		Dundas harbour	Nunavut Territory	74 33'	82 30'	17	0
*28		Frederik E. Hyde Fjord	Greenland	Not given	Not given	1	0
*29		Bothnian Bay, Baltic Sea	Finland	Not given	Not given	1	0
*30		Lake Kallavesi	Finland	Not given	Not given	1	0
*31		Lake Suvasvesi	Finland	Not given	Not given	1	0
*32		Lake Munozero	Russia	Not given	Not given	1	0
33	FHS-arctic lakes	Garrow Lake	Nunavut Territory	75 23'	96 48'	7	0
*34		Melville Island (pool)	Northwest Territories	Not given	Not given	1	0
*35	M. octodecemsp.	Hudson Canyon	U.S.A.	Not given	Not given	1	0

* Sequences were taken from Kontula and Vainola (2003)

Table 3.2. PCR amplification and cycle sequencing primers used in the study.

	Name	Sequence	Length (bp)	Function	Reference
ATPase6,8	L8933	TTA GCC TTT TAA GCT AAA GAT TGG TG	26	Amplification	Kontula et al. 2003
	H9795	RTG TGC TTG GTG KGC CAT TA	20	Amplification	Kontula et al. 2003
	ISPAF	CAT GTG TTG GTT GAT TTC GC	20	Sequencing	*
	ISPAR	AAT AAA CCA CCC TTG GAG GG	20	Sequencing	*
Control region	HN-20	GTG TTA TGC TTT AGT TAA GC	20	Amplification and Sequencing	Bernatchez and Danzmann (1993)
	Tpro-2a	ACC CYT RRC TCC CAA AGC CAG	21	Amplification	*
	ISPDF	AAA AGA TAA ACC CTG GCT CG	20	Sequencing	*

* Primers designed in the course of this study.

Figure Captions

Figure 3.1. ATPase6,8 strict consensus phylogram of fourhorn and deepwater sculpin (outgroup is *M. octodecemspinosus*). Branch lengths correspond to character changes. Numbers indicate bootstrap proportions. *Refers to freshwater fourhorn haplotypes.

Figure 3.2. Control region strict consensus phylogram of fourhorn and deepwater sculpin. Branch lengths correspond to character changes. Computational limitations prevented the calculation of bootstrap proportions.

Figure 3.3. Combined (ATPase6,8 and control region) strict consensus phylogram of divergences among deepwater sculpin haplotypes. Branch lengths correspond to character changes. Numbers indicate percent bootstrap support.

Figure 3.4. Distribution of fourhorn and deepwater sculpin haplotype clades. Locations of some taxa included in the study from Kontula and Vainola (2003) are not shown.

Figure 3.5. Distribution of fourhorn and deepwater sculpin haplotype clades, and hypothesized refugial origins and dispersal routes for deepwater sculpin populations.

Figures

Figure 3.1.

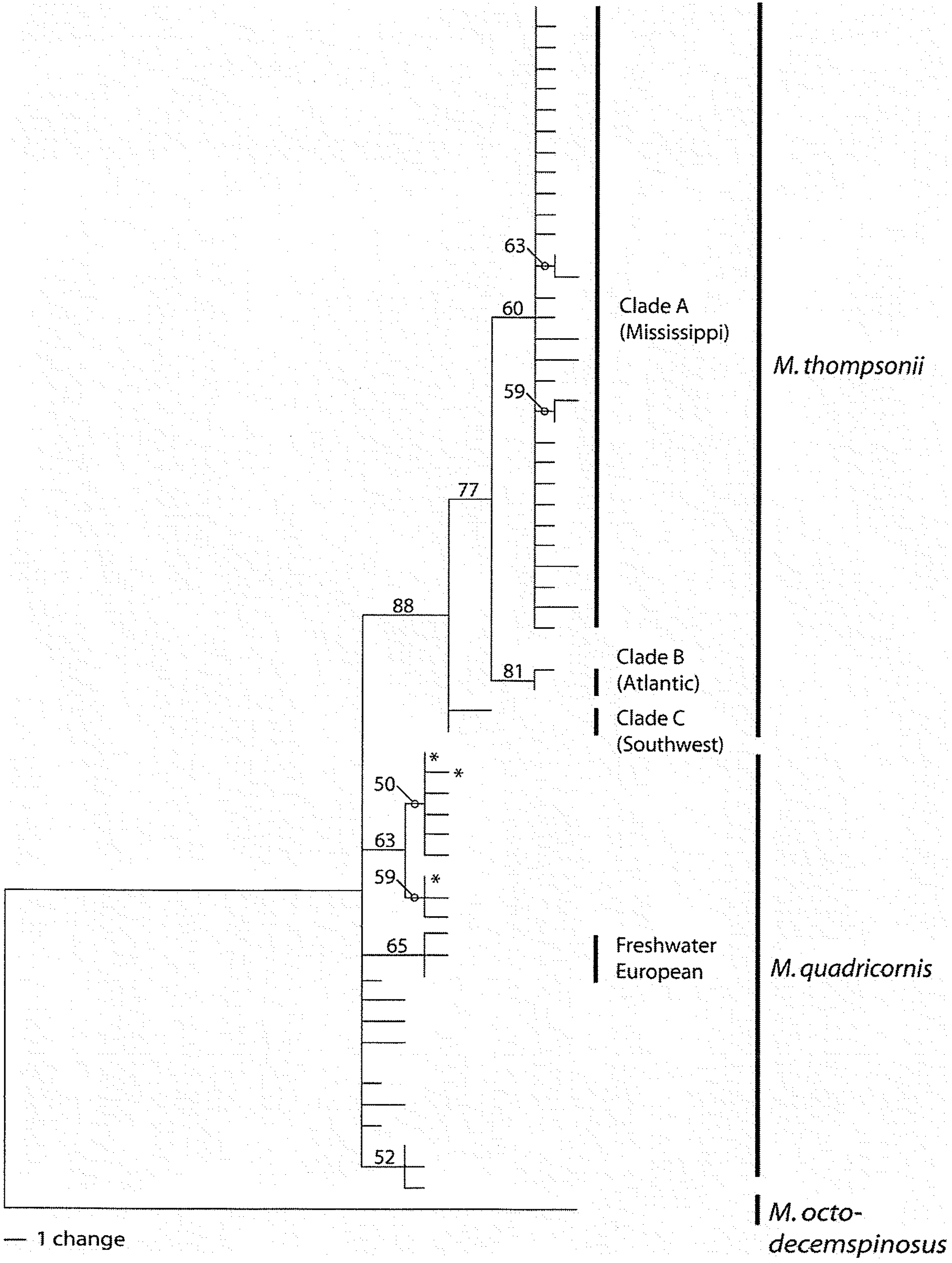


Figure 3.2.

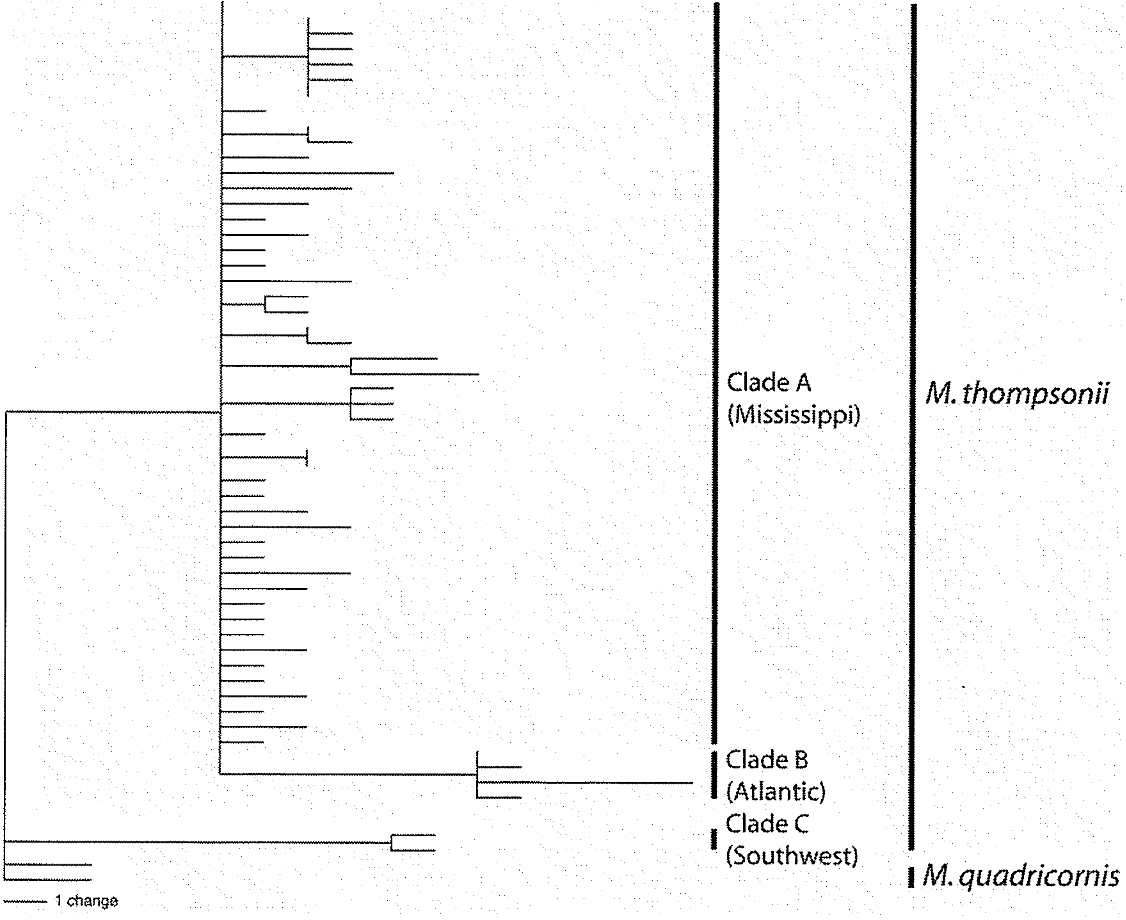

Clade A
(Mississippi)
Clade B
(Atlantic)
Clade C
(Southwest)
M. thompsonii
M. quadricornis
1 change

Figure 3.3.

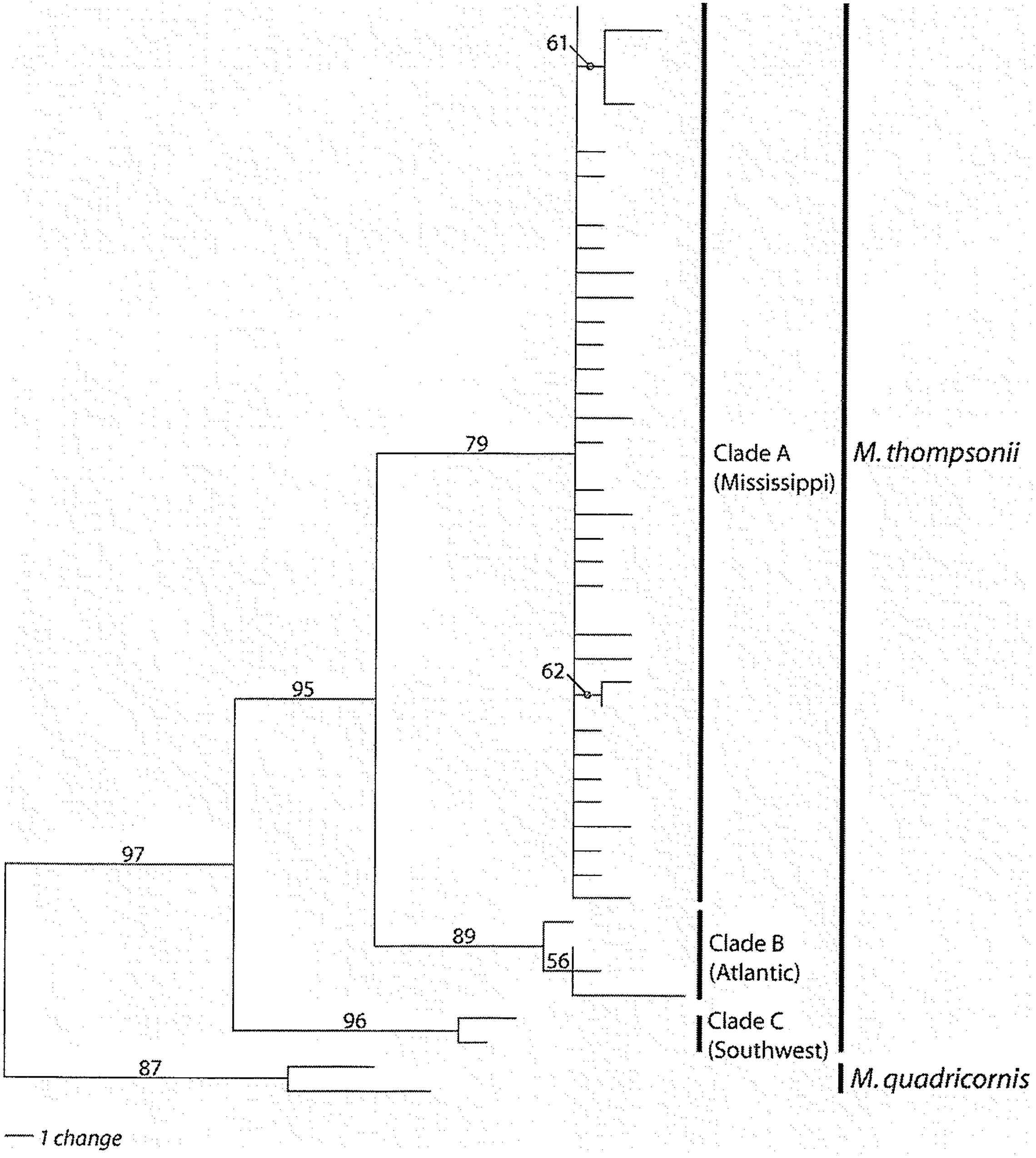

61
62
79
95
97
89
56
96
87
Clade A (Mississippi)
Clade B (Atlantic)
Clade C (Southwest)
M. thompsonii
M. quadricornis
1 change

Figure 3.4.

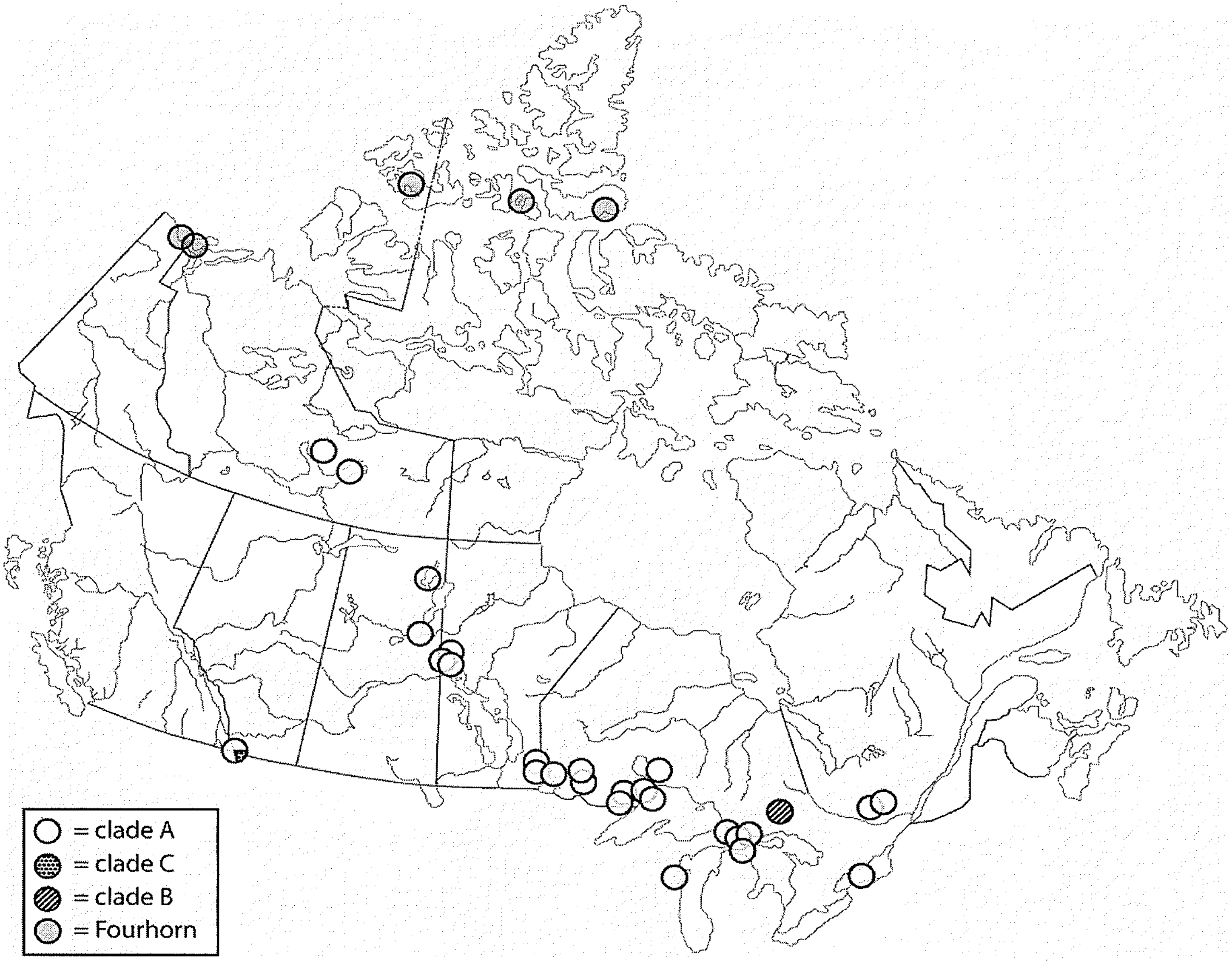
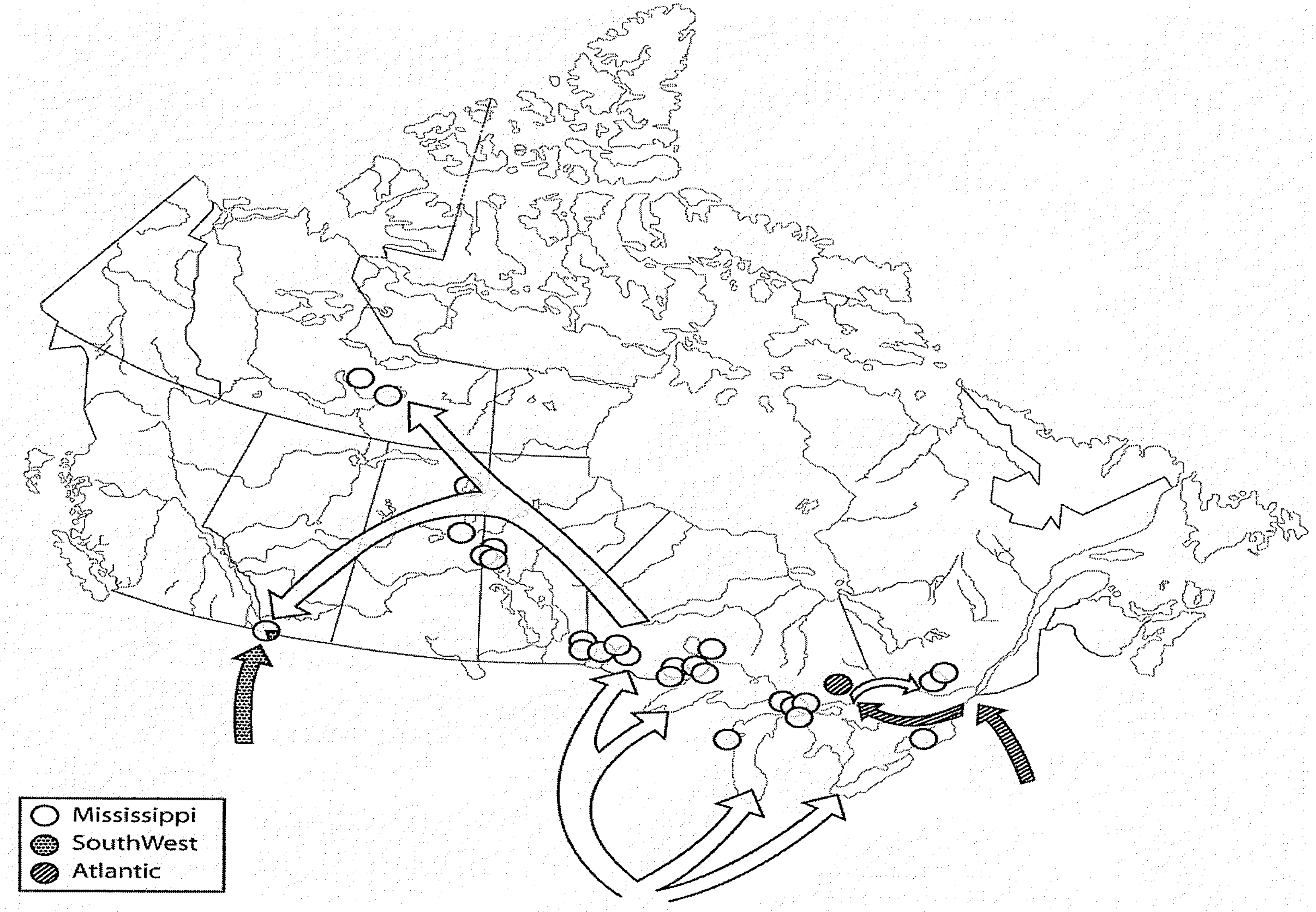


Figure 3.5.



Thesis Discussion

Despite the significant role of mid-level consumers within lacustrine systems, it is often the top-level fishes of direct economic importance that are studied extensively. For example, most phylogeographical studies of Canadian freshwater fishes have focused on large economically important species such as walleye, lake trout, and arctic char (Wilson et al. 1996; Wilson and Hebert 1998; Bodaly et al. 1992). Ecological studies show a similar pattern, with biological and habitat studies of mid-level fish fauna limited relative to studies of fishes that are of direct economic importance. However, the smaller under-studied fishes are often vital for sustaining the economically important fishes within our lakes.

Historically, the deepwater sculpin, a mid-level trophic consumer, has received little attention. However, the species is suspected to be a critical part of the deepwater benthic community in lakes where it occurs. In studies focusing on burbot and lake trout, the deepwater sculpin has been shown to be an integral part of their diets (Day 1983). It was not until the virtual extirpation of deepwater sculpin from Lake Ontario, however, that the species received attention (Brandt 1986; Kraft and Kitchell 1986). In addition, it seems that the decline of deepwater sculpin within Lake Ontario only received attention because it occurred simultaneously with a dramatic decline in lake trout populations (Scott and Crossman 1973; Brandt 1986). Although the deepwater sculpin began to receive some attention within the Laurentian Great Lakes, populations throughout the remainder of the species range were still largely ignored or overlooked.

This thesis represents the first in depth research of the deepwater sculpin throughout its entire range. My findings provide basic biological information that will be important for the conservation of this valuable taxon.

The new survey of the species range throughout Canada has identified four new lacustrine populations of deepwater sculpin. However, the survey also revealed the possible disappearance of deepwater sculpin from some lakes within their range. Finally, the survey confirmed that dispersal of deepwater sculpin has most likely not occurred since the late stages of the proglacial lake phase, nor is it expected to occur in the future.

The findings of the age, diet, and habitat analyses are also significant for the future conservation of deepwater sculpin. Deepwater sculpin appear to be much longer-lived than previously thought. The conservation of longer-lived fish, such as deepwater sculpin, is particularly important due to the potentially low rates of population growth and the difficult recovery of these types of fish from low population levels. Analyses also indicate that deepwater sculpin rely heavily upon *Diporeia* spp. as a prey item, particularly in the early years of life. *Diporeia* spp. may be negatively impacted by eutrophication within lakes (Lozano et al. 2001). My findings also suggest that deepwater sculpin have strict oligotrophic habitat requirements, especially low benthic temperatures. In particular, the thermal niche of deepwater sculpin appears to be quite specific (<7 °C). This, combined with the possible negative impact of eutrophication on *Diporeia* spp., suggests that even moderate amounts of eutrophication can have long-term negative impacts on individual deepwater sculpin populations throughout their range. This may indeed be the case in both Heney Lake and Lac des Iles where deepwater sculpin were not successfully collected.

Lastly, results from our study indicate that the *Myoxocephalus* sculpin complex is a useful model for studying the effects of the Pleistocene glaciations on aquatic fauna. Analyses reveal clear separation of fourhorn and deepwater sculpin as closely related, but distinct, species, and confirm a single continental invasion of deepwater sculpin dating from the early to mid Pleistocene. Our phylogeographic results also indicate the presence of three distinct evolutionary lineages of deepwater sculpin, each of which is presumably associated with a separate refuge: the Mississippian, Southwestern, and Atlantic.

Due to the extreme difficulties of collecting deepwater sculpins from an adequate number of locations throughout their range, our study provides the first in depth ecological and genetic analysis of the species. Phylogenetic results suggest that three lineages of deepwater sculpin are potentially susceptible to environmental perturbations. However, the distinct evolutionary lineages that are restricted to Upper Waterton Lake (Southwestern lineage) and Fairbank Lake (Atlantic lineage) are perhaps the most susceptible to disturbances due to their extremely limited geographic range. The conservation of the Southwestern and Atlantic mitochondrial lineages is important for maintaining full genetic diversity of the species. Results from this study should also signal that the conservation of deepwater sculpin is of immediate concern in other areas and efforts must be taken in order to minimize human disturbances and the subsequent eutrophication of lakes where deepwater sculpin reside. As the deepwater sculpin is almost entirely endemic to Canada, efforts should be made to preserve the genetic diversity of the species and ensure the species persistence throughout its entire range.

References

Bodaly, R.A., J. Vuorinen, R.D. Ward, M. Luczynski, and J.D. Reist. 1991. Genetic comparisons of New and Old World coregonid fishes. J. Fish Biol. 38:37-51.

Brandt, S.B. 1986. Disappearance of the deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Ontario: the keystone predator hypothesis. J. Great Lakes Res. 12(1):18-24.

Day, S.C. 1983. Biological population characteristics, and interactions between an unexploited Burbot (*Lota lota*) population and an exploited lake trout (*Salvelinus namaycush*) population from Lake Athapapuskow, Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Kraft, C.E., and J.F. Kitchell. 1986. Partitioning of food resources by sculpins in Lake Michigan. Environ. Biol. Fishes 16(4):309-316.

Lozano, S.J., J.V. Scharold, and T.F. Nalepa. 2001. Recent declines in benthic macroinvertebrate densities in Lake Ontario. Can. J. Fish. Aquat. Sci. 58:518-529.

Scott, W.B., and E.J. Crossman. 1973. The freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184. 966 pp.

Wilson, C.C., and P.D.N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America. Can. J. Fish. Aquat. Sci. 55:1010-1024.

Wilson, C.C., P.D.N. Hebert, J.D. Reist. 1996. Phylogeographic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. 53:2764-2775.