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Living on the Edge: Conservation of Fish Species at Risk in Canada

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Living on the Edge: Conservation of Fish Species at Risk in Canada

By

William R. Glass

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(November 8, 2012)

DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this dissertation incorporates material that is result of joint research, as follows: All data chapters and the general introduction and discussion were written with the supervision of my co-advisors Dr. Nicholas Mandrak and Dr. Lynda Corkum. In all cases Drs. Corkum and Mandrak contributed valuable feedback and editorial input. In addition, chapter three was prepared as a manuscript and submitted for publication in Environmental Biology of Fishes, while chapter four was prepared as a manuscript and published in Transactions of the American Fisheries Society. Chapters five and six were the result of collaborative research that in addition to myself, included Dr. Nicholas Mandrak, Dr. Lynda Corkum, Dr, Daniel Heath and Dr. Ryan Walter. Chapter six has been prepared as a manuscript and has been submitted to Conservation Genetics. Dr. Walter assisted with the analysis of sequence data and creation of phylogenetic trees. Drs. Walter, Heath, Corkum and Mandrak also provided editorial input. Dr. Walter assisted with the creation of figure 5.1 in chapter five and provided valuable guidance on data interpretation for this chapter.

With the above caveats, in all cases the primary contribution, data analysis, interpretation and key ideas were my own.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my dissertation, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this dissertation, and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

This dissertation includes three original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status*
Chapter 3	Glass, W.R., Corkum, L.D. and Mandrak, N.E. 2011. Pectoral fin ray aging: an evaluation of a non-lethal method for aging gars and its application to a population of the Threatened Spotted Gar. Environmental Biology of Fishes 90:235 – 242	*published*
Chapter 4	Glass, W.R., Corkum, L.D. and Mandrak, N.E. 2012. Spring and summer distribution and habitat use by adult Threatened Spotted Gar in Rondeau Bay, Ontario, using radiotelemetry. Transactions of the American Fisheries Society 141:1026 – 1035	*published*

Chapter 6	Glass, W.R., Walter, R.P., Heath, D.D., Mandrak, N.E. and Corkum, L.D. DNA sequences reveal presence of Threatened Spotted Gar (*Lepisosteus oculatus*) in Canadian commercial fish market. Conservation Genetics	*submitted*

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ABSTRACT

Many species in North America range northward and barely into southern Canada. Some of these species are classified as species at risk and afforded legal protection in Canada, yet the decision to protect these populations at the edge of their range is controversial. To determine if edge populations are more likely to be listed as at risk, fish species were grouped based on whether they are listed as at risk in Canada then assigned values for several life history and ecological traits and a discriminant function analysis was conducted. Conservation status was correctly predicted 93% of the time. Traits that predicted conservation status were endemic distribution, recognized distinct populations, edge distributions and long-lived.

Northern edge populations of Spotted Gar (*Lepisosteus oculatus*) were investigated for the presence of local adaptations. Adaptations in the form of delayed age at maturity and lower body condition were seen in the Rondeau Bay population of Spotted Gar. Differences in habitat selection and offshore distance were also seen in the Rondeau Bay population when compared to southern core populations of the species. Microsatellite analyses showed that northern edge populations were divergent from southern core populations and the Rondeau Bay population carried the entirety of the genetic diversity found in the north.

A phylogeny based on mitochondrial gene sequences was created and used to identify five commercially obtained gar samples. Four individuals obtained at a pet shop in Kitchener, Ontario, labeled as Spotted Gar, were identified as Florida Gar (*Lepisosteus*

platyrhincus). A specimen obtained at a commercial fish market in Toronto, Ontario was identified as a Spotted Gar and likely originated from Long Point Bay, Lake Erie.

The presence of local adaptation affirms the need to protect edge populations to conserve the overall diversity within the Spotted Gar and other species in Canada.

DEDICATION

Dedicated to my father Bill Glass Sr. and my grandfather Melvin Ascott. Fishing with you as a boy fostered my love of fishes. And to my grandmother Audrey Ascott, you always said I would be a doctor one day. I miss all of you greatly.

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CHAPTER 1

GENERAL INTRODUCTION

Introduction

The distributions of many species in North America extend northward into southern Canada (Page and Burr 2011). Many of these species are common in the core of their range but are considered rare in Canada. Some of these species, such as the Lake Chubsucker (*Erimyzon sucetta*), Grass Pickerel (*Esox americanus*) and Spotted Gar (*Lepisosteus oculatus*) are listed under the federal *Species at Risk Act* in Canada and are afforded legal protection based on their rarity and limited distribution in this country. There is ongoing debate in whether or not edge species should be protected based on political boundaries alone, when populations are abundant in other jurisdictions (e.g. Arponen 2012, Rodrigues and Gaston 2002). The American Fisheries Society Endangered Species Committee (Jelks et al. 2008) has published a list of the freshwater and diadromous fishes in North America that they consider to be at risk. The list of Jelks et al. (2008) includes species, subspecies and populations considered to be biologically distinct, but does not consider political boundaries as a criterion for listing. For Canadian fishes, this list is quite different from the species at risk listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and afforded protection under the Species at Risk Act (*Species at Risk Act* 2002 s.2(1)), which list more species than Jelks et al.

COSEWIC lists only taxa that are native to Canada, depend on Canadian habitat and now have, or historically had, regular occurrence in Canada (COSEWIC 2010). Classification units lower than the sub-species level (termed "designatable units") will be considered if there is evidence that the units are genetically distinct, are separated by a major range disjunction, or are biogeographically distinct. Further criteria required for a

species to be listed are that it meets one of the following: exhibits a large reduction in population size; has a small distribution and a decline or fluctuation in distribution; has a small total population size and is in decline; or, has a very small population or restricted distribution (COSEWIC 2010). COSEWIC also considers the "rescue effect" when listing a species at risk. The rescue effect occurs when immigration from high density source populations to low density population areas decreases the probability of local extinction in the sink population (Hanski and Gilpin 1991). The rescue effect can maintain populations in marginal habitat areas if there are enough migrants from the source population to offset decreased population growth rates in the sink area (Hanski and Gilpin 1991). If there are extra-regional populations from which propagules are likely to arrive to the region and there is no evidence of local adaptation, the status of the species may be downgraded.

In Canada, freshwater fish species are disproportionately highly represented (second only to vascular plants) in the species listed under the *Species at Risk Act*, with 19% of all listings being fish species (Hutchings and Festa-Bianchet 2009). The freshwater species diversity and number of species at risk in Canada is highest in the southern portion of the country, with southern Ontario having the highest diversity and number of listed freshwater fish species (Dextrase and Mandrak 2006), many of which are at the northern edge of their range. The major threats to the freshwater fish species at risk in Canada are habitat loss and degradation, with invasive species and pollution also posing significant threats (Dextrase and Mandrak 2006).

Arguments for the protection of edge populations

There are several arguments for conserving species at the edge of their range in Canada. Edge species may be locally abundant and thus play an important function in local ecosystem processes. The aquatic plant *Potamogeton polygonifolius*, for example, is abundant in marginal habitats at the edge of its range (Zalewska-Galosz et al. 2012), presumably filling the same niche that the species occupies elsewhere. Additionally, due to differences in population density, edge populations may exhibit higher growth rates compared to core populations of the species (Angert 2006), potentially providing migrants that will maintain other populations. The Carolinian forest ecosystem in southwestern Ontario provides many examples of species at the northern edge of their range that can be locally abundant and persist provided that enough habitat area is protected (Klinkenberg 2002).

These edge species, although rare in Canada, may have populations that have evolved life history, ecological, behavioural, and/or genetic adaptations due to their isolation, which allow persistence of the population away from the core of the species' range. Isolated populations at the edge of a species' range often have lower amounts of gene flow and increased genetic drift compared to populations at the centre of their range (Bunnell et al. 2004). Vucetich and Waite (2003) have estimated that the effective population size for edge populations are 2 to 30 times smaller than the population size for core populations, consequently leading to a proportional increase in the rate of genetic drift. Isolation, combined with small population sizes and increased gentic drift make edge populations evolve at a much faster rate compared to core populations (Lesica and Allendorf 1995). Because environmental conditions at the edge of a species' range may

differ, and are often harsh compared to conditions at the core of a species' range, edge populations are often subjected to increased selective pressure (Case and Taper 2000). This increased selection pressure can lead to an increase in genetic variability at the edge of a species' range compared to the core, as seen in the Eastern Spadefoot Toad, *Pelobates syriacus*, a species that is critically endangered in Israel where the edge populations are found (Munwes et al. 2010). Local adaptation has also been demonstrated in an over-exploited edge population of Eurasian Perch (*Perca fluviatilis*), where populations at the Asian edge of the species' natural range showed distinct genetic differences, attributed to isolation and natural and anthropogenic stressors (Yang et al. 2012).

The evolved variation in ecology, life history and genetic structure of edge populations compared to the core populations of a species, along with the ecosystem function that edge populations may provide, is important to conserve in the face of environmental change. With the spectre of climate change looming, populations at the northern edge of a species' range may prove to be even more important for conservation. Species that are adapted to the northern limits of the species' temperature tolerance may be more likely to colonize new habitats that open up as climate changes. Additionally, populations that persist in marginal environments, such as those found at the edge of a species' range, can develop increased phenotypic plasticity (Chevin and Lande 2011) making them more likely to invade new habitats. Populations at the edge of a species' range also tend to contain more dispersive morphs than populations at the core (Phillips et al. 2007), facilitating rapid expansion into newly available habitats. Melles et al. (2011) showed the northward advance of a threatened species, the Hooded Warbler

(*Wilsonia citrina*), due to warming climate. This colonization of new habitats in response to climate change has been shown on a large scale by a poleward shift in species' range distributions (Parmesan and Yohe 2003). Conservation of the populations near to their northern edge will be important to ensure that future expansion is possible.

It has been shown that when species decline in distribution and abundance, their ranges tend to shift to the periphery of their original range rather than to the centre (Channell and Lomolino 2000). This tendency for species to disappear from the core of their historical range while still persisting at the edge makes protecting edge populations of species (preferably before it goes into decline) even more important to ensure the survival of the species as a whole.

A final argument for the protection of edge populations is an ethical one. It is important for species to be protected where locally threatened because each jurisdiction should be responsible for protecting the species within its borders, rather than relying on others to do so (Arponen 2012). This is particularly important where the jurisdiction that encompasses edge populations has more conservation funding and ability than its neighbour (Arponen 2012).

Arguments against protecting edge populations

Opponents of protecting species at the edge of their range argue that low population levels are due to normal population dynamics, where edge populations are often low and where extinctions and re-colonizations are common (Hanski 1982, Hanski and Gyllenberg 1993). The "abundant centre hypothesis" (Sagarin and Gaines 2002) states that populations tend to be larger at the centre of a species' range, and decrease in

size towards the edges of the range. It follows that protecting the habitat of core populations will protect a larger number of individuals, compared to protecting the smaller edge populations. Empirical evidence for the abundant centre hypothesis, however, is lacking, owing to insufficient sampling throughout the distributional range of a species (Sagarin and Gaines 2002).

Peripheral habitats may also be population "sinks", areas of marginal habitat quality that require constant influx of new individuals from higher density areas to maintain viable populations, leading to the edge populations not diverging from the core populations of the species (Gaggiotti and Smouse 1996). A reciprocal transplant study conducted on an annual flowering plant, *Lasthenia fremontii*, showed that individuals raised at the edge of the species' range performed poorly compared to those raised at the centre (Emery et al. 2011). In this case, selection applied to populations at the core of the range would have a disproportionate effect on the evolution of the species, compared to selection applied to populations at the edge of the range due to regional population dynamics (Emery et al. 2011).

Alternatively, there may be no real or perceived threats to Canadian populations at the edge of their range such that resources used to protect such populations could be used more effectively elsewhere. The Warmouth (*Lepomis gulosus*), for example, is at the edge of its range in Canada (COSEWIC 2005a). This species has a limited distribution in Canada and is listed as a species of special concern. Two of the main areas that the Warmouth inhabits in Canada are protected by a provincial (Rondeau Provincial Park) and national park (Point Pelee National Park), thus the species' persistence in Canada is likely not in jeopardy (COSEWIC 2005a). In this case,

allocating research or conservation funds to this species may not be the most efficient use of limited funds. Finally, determining conservation priority based on political boundaries can also lead to species receiving more protection at the edge of its range than at the centre and generally result in less efficient use of conservation funds than when there is collaboration between political jurisdictions (Rodrigues and Gaston 2002).

My thesis seeks to further inform the debate on protecting edge populations of species at risk in Canada. Greenwald et al. (2012) argued that peer-reviewed science is not necessarily taken into account when making decisions regarding the protection of species and their habitats. The intent of my thesis is to empirically examine whether fish species at risk in Canada at the edge of their range, are deserving of legal protection. The results of this study will help policy managers make informed decisions on the conservation of Canada's aquatic resources.

Thesis Contents

The objective of my thesis is to investigate the validity of protecting species at risk that are at the edge of their range in Canada. In chapter two, I review the processes that affect populations at the edge of a species' range and how these processes, such as differential selection pressure, lack of gene flow, and small population size along with genetic drift can lead to differentiation of edge populations compared to populations at the core of the species' range. All freshwater fish species, recognized sub-species and distinct populations in Canada were grouped *a priori* based on their conservation status and whether the species was at the edge of its range in Canada. To determine if edge populations are more likely to be listed as at risk, 136 distributional, ecological and life

history traits were summarized for each species and a discriminant function analysis was conducted by conservation status. Conservation status (i.e. at risk vs. not at risk) was correctly predicted at a rate of 93%, with 9 of 54 species that are listed as species at risk in Canada predicted to not be at risk and 6 of 154 species that are not listed predicted to be at risk. The traits that predicted conservation status included endemic distribution, edge distribution, recognized distinct population and long lived.

In subsequent chapters, the Spotted Gar was used to test the validity of protecting species with limited distribution and at the edge of their range in Canada. The Spotted Gar is an ideal species for this study because of its limited distribution in Canada, inhabiting only three coastal wetlands of Lake Erie (Point Pelee marsh, Long Point Bay and Rondeau Bay) (COSEWIC 2005b). These populations are isolated from each other as well as from other populations in its native range, thus, dispersal among them is limited. Point Pelee, in particular, has no contemporary connection with the western basin of Lake Erie and migration in and out of this site is not possible. The Spotted Gar ranges as far south as the Gulf of Mexico (Page and Burr 2011) and is not considered to be at risk outside of the Great Lakes portion of its range (COSEWIC 2005b) (Figure 3.1). The species is designated as Threatened under the Canadian *Species at Risk Act*, Threatened in Ontario, Endangered in Ohio and a species of Special Conservation Concern in Michigan. The Spotted Gar was listed by COSEWIC as Threatened due to its limited distribution and the threats caused by pollution and habitat loss (COSEWIC 2005b).

In chapter three, I investigated the life history differences between a Canadian population of Spotted Gar, found in Rondeau Bay, and a population in the southern core

of the species' range in Lake Pontchartrain Louisiana (Love 2004). To determine the age of Spotted Gar specimens collected in Rondeau Bay, I used pectoral fin ray sections, a novel technique for aging gars in a non-lethal manner. Growth rate and life expectancy did not differ among the populations, however, Spotted Gar in Rondeau Bay were found to reach sexual maturity at a later age than those in the southern population. Additionally, the Spotted Gar in Rondeau Bay had a lower body condition than the accepted standard for weight at a specific length. The delayed maturity and reduced body condition may lead to reduced lifetime reproductive output for the species in Canada, thus contributing to its rarity supporting its designation as a species at risk in Canada.

In chapter four, I investigate the behavioural adaptations of Spotted Gar in Canada, compared to Spotted Gar from the core of the species' range. I used radiotelemetry to track the movement and habitat use of 37 individual Spotted Gar in Rondeau Bay. I mapped the movements of each individual using ArcGIS software and calculated home range size and preference for specific habitat variables. I then compared the home range and habitat use by the Spotted Gar in Rondeau Bay to a population in the Atchafalaya River, Louisiana in the south of the species range (Snedden et al. 1999). I found that the home range of individual Spotted Gar in Rondeau Bay tended to be much farther offshore than the home ranges of Spotted Gar in the Atchafalaya River basin. I also found that the Spotted Gar of Rondeau Bay were often associated with macrophytes as cover, whereas, the individuals in Louisiana waterbodies tended to associate with flooded timber, demonstrating local ecological adaptation in the northern edge population of Spotted Gar.

Chapter five investigates the genetic differentiation of northern edge populations of Spotted Gar compared to populations in the southern core of the species' range. I used microsatellite DNA sequences to describe the population genetic structure of Spotted Gar from eight locations throughout the species' range; four populations in the southern range and four populations from the northern edge of the range. The analysis showed that there were distinct population clusters, with all of the southern populations grouping together. Within the northern population cluster, the populations from Michigan and Point Pelee (Ontario) were distinct from each other; the population from Rondeau Bay contained genotypes from both Michigan and Point Pelee. Results show that there is a genetic difference between populations at the core of the species' range and those found at the northern edge of the range. To preserve the overall genetic diversity within the species, it will be important to conserve populations at the northern edge of the range, particularly the population found at Rondeau Bay.

The objective of chapter six was to create a phylogeny for the gar family (Lepisosteidae) based on mitochondrial sequences. I created the phylogeny using combined Cytochrome Oxidase I and Cytochrome b gene sequences. The phylogeny that I created supported the molecular-based phylogeny produced by Wright et al. (2012) which calls into the question Grande's (2010) placement of the Shortnose Gar (*Lepisosteus platostomus*) basal to the other *Lepisosteus* on the basis of skeletal anatomy (Grande 2010). The phylogeny that I created based on mitochondrial sequences was also used to determine the identity and origin of gar specimens that were found in commercial trade in Ontario. Using the phylogeny, I identified gar specimens sold as Spotted Gar in an Ontario pet shop were actually Florida Gar (*Lepisosteus platyrhincus*), and a specimen

purchased at a live food fish market in Toronto (Ontario) was a Spotted Gar. This chapter highlights the need to educate commercial fishers about the potential presence of species at risk in their catch.

Overall, my research demonstrates that edge populations are more likely to be listed as species at risk in Canada and that populations of Spotted Gar at the northern edge of its range possess local adaptations. These adaptations have been demonstrated through life history, ecological, and genetic differences in the Spotted Gar population of Rondeau Bay compared to southern core populations. These differences support the continued conservation of Spotted Gar in Canada. Conservation of edge populations is important to protect the entirety of the diversity of a species.

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CHAPTER 2

TRAITS OF FRESHWATER FISH SPECIES AT RISK IN CANADA

Introduction

In North America, the native ranges of many species reach northward into southern Canada. These species at the edge of their range often have limited distributions and are considered rare in Canada. Several aquatic species whose range extends into southern Canada, and whose main population is found further south, are listed by the Committee on the Status of Endangered Wildlife in Canada and subsequently, have been afforded protection under the federal Species at Risk *Act* (SARA). Of the 54 species listed as at risk in Canada, 24 are at the northern edge of their range. There is an ongoing debate as to whether populations at the edge of the species' range deserve protection. In many cases conservation designations are based on political boundaries, protecting species that are rare within a jurisdiction even if the species is more abundant elsewhere in its range. Jelks et al. (2008) published a list of the freshwater and diadromous fishes at risk in North America. This list includes biologically distinct populations, but did not consider political boundaries (Jelks et al. 2008) and differs markedly from the listing of Canadian freshwater fishes under the federal *Species at Risk Act* (SARA). In Canada, the SARA considers species, subspecies, and distinct populations for protection based solely on their Canadian distributions (*Species at Risk Act* 2002, s. 2(2)).

The objective of this paper is to examine the processes that affect species at the edge of their range and to contribute to the debate on the protection of range-edge populations. By comparing traits of freshwater fish species in Canada that are at risk with those that are not listed, we will determine if species at the edge of their range are more likely to be listed as at risk.

Life on the edge

MacArthur and Wilson's (1967) theory of island biogeography, and the subsequent meta-population model (Levins 1969) describe the distribution of species and populations in natural environments. Species do not exist as one homogenous population, but rather as a series of smaller populations in habitat patches, separated from each other geographically and with varying amounts of gene flow between them (Levins 1969). Populations in habitat patches may go extinct, and vacant patches may be colonized by dispersing individuals (Levins 1969). Freshwater fishes are well suited to this model, with single waterbodies representing a habitat patch or island (Keddy 1976). These habitat patches are connected to varying degrees or separated by uninhabitable areas (dry land). Hanksi (1982) showed that as the number of local patches that are occupied by a species increases, so too does the population size for the patches. Thus, the species in an area can be broadly grouped in one of two categories: core species, that are locally abundant and common in the region; and, satellite species that are rare on both local and regional scales (Hanski 1982). The abundance of a species tends to be highest in the centre of the species' geographic range, where many patches are occupied and decreases towards the edge of the species' geographic range (Brown 1984). Because of this, populations at the edge of a species' range tend to be smaller and more isolated than populations at the core of the range.

Due to the nature of edge populations, such as isolation and low density, there are several evolutionary processes that differ for edge populations than for those at the core of the range. Firstly, small population size leads to an increased risk of extinction of the local population (Pimm et al. 1988). Vacant habitat patches may then be recolonized by dispersing individuals from nearby patches. Recolonization by a small number of individuals may lead to a founder effect, where the newly established population has a greatly reduced genetic and phenotypic diversity compared to other larger populations of the species (Mayr 1954). Small population size also leads to an increase in homozygosity through inbreeding effects (Hendrick and Kalinowski 2000). Increased homozygosity leads to an increased expression of recessive traits, including those that may be deleterious (Barrett and Charlesworth 1991). Genetic drift also plays an important role in small populations. The effective population size for edge populations are 2 to 30 times smaller than those of core populations, which leads to proportional increases in the rate of genetic drift (Vucetich and Waite 2003). Small population size, isolation, and increased levels of genetic drift lead edge populations to be more evolutionarily dynamic than core populations (Lesica and Allendorf 1995). The pattern of edge populations as less diverse and differentiated from core populations has been observed over a wide variety of taxa (Eckert et al. 2008).

In some cases, the extinction of the edge population may be prevented by individuals dispersing from larger core populations to the smaller populations at the edge, a phenomena known as the rescue effect (Brown and Kodric-Brown 1977). The rescue effect provides gene flow and reduces differentiation of edge populations (Gagiotti and Smouse 1996). However, as the distance from the center of the species distribution

increases, gene flow typically decreases facilitating genetic divergence and loss of diversity by genetic drift (Garcia-Ramos and Kirkpatrick 1997). In cases of local adaptation in edge populations, reduced gene flow and increased inbreeding can be beneficial to preserve this local adaptation (Arnaud-Haond et al. 2006).

Other processes that affect edge populations can be related to the physical environment. Because the natural environment is not homogenous, populations in nature exist along an environmental gradient. Populations densities are highest where environmental conditions are most favorable, the core of the species' range, and decline in areas where the environmental conditions are less favorable, termed the "abundant centre distribution" (Sagarin and Gaines 2002). Because edge populations are often found in less than optimal environmental conditions, the level of selection on these populations may be higher than on populations in the core of the range in the form of abiotic stress and interspecific competition (Case and Taper 2000). Increased selection pressure on edge populations can lead to increased genetic diversity, as seen in the Eastern Spadefoot Toad, *Pelobates syriacus*, (Munwes et al. 2010).

Another consequence of life at the edge of a species range is an increase in the number of dispersive morphs in the edge population relative to the overall population (Phillips et al. 2007). This increase in dispersive morphs is due to these individuals' increased ability to reach the distant habitats. Edge populations also display increased phenotypic plasticity (Chevin and Lande 2011), thus increasing their ability to colonize varied habitats. An interesting implication of protecting edge populations of a species is the possibility that the species as a whole declines in abundance and becomes endangered. When species become endangered, it is common for them to disappear from

the core of its range while still surviving at the edges of its historic range (Lomolino and Channell 1995). In cases where there is a reasonable prospect of the species becoming endangered throughout a significant portion of its range, edge populations become more important for the long-term survival of this species. In situations where a species collapses to the periphery of its range, there may be asymmetrical gene flow out of the peripheral populations due to dispersal from the edge populations to other areas. Asymmetrical gene flow can also result from environmental conditions. For example, downstream dispersal may be easier than upstream dispersal. Hernandez-Martich and Smith (1997) demonstrated that the gene flow in Eastern Mosquitofish (*Gambusia holbrooki*) was predominantly in the downstream direction. The asymmetrical dispersal and gene flow leads to the population at the upstream edge of the range having a greater influence on the genetic diversity of the species (Pringle et al. 2011). Whenever this asymmetrical pattern of dispersal from the edge population exists it becomes particularly important to protect the edge population.

Canada's freshwater fishes

There are 183 native freshwater and diadromous fishes listed by Scott and Crossman (1998) comprising 25 different families. There are an additional 24 sub-species or distinct populations that are recognized and listed in the SARA registry and one additional subspecies (Banff Longnose Dace, *Rhinichthys cataractae smithii*) that is considered extinct. Of the 208 total species, subspecies and distinct populations, there are 53 listed under schedule 1 of the Species at Risk Act (sararegistry.gc.ca). Of the listed species three are Extirpated from Canada, 23 are listed as Endangered, 10 as Threatened, and 17 as Special Concern (sararegistry.gc.ca). All but nine of the families

have at least one listed species. Dextrase and Mandrak (2006) described the distribution of fish species (listed and not listed as at risk) in Canada, based on the national freshwater biogegraphic regions designated by COSEWIC. The southern regions of the country have the highest fish species diversity, particularly the Great Lakes – Upper St. Lawrence region, the Saskatchewan – Nelson River region and the Pacific region (Dextrase and Mandrak 2006). In general, the distributions of species at risk follows the same pattern (Dextrase and Mandrak 2006), as does the distribution of edge species (Figure 2.1). The Pacific Islands region, however, has a very high number of species at risk relative to the number of species present (Dextrase and Mandrak 2006) although none of the species are at the edge of their range. This high number of listed species in the Pacific Islands is driven by endemic species and recognized distinct populations (sararegistry.gc.ca).

I hypothesize that with a discriminant function analysis, the freshwater fish species listed as at risk in Canada will be distinguished from species that are not listed as at risk based on their distribution. I predict that species at the edge of their range will be more likely to be listed as at risk which will be evidenced by edge distribution as a significant predictor of at risk status. Additionally, I hypothesize that species at the edge of their range in Canada will differ from species that are not at the edge of their range based on ecological and life history characteristics.

Methods

The freshwater and diadromous fishes of Canada, including recognized sub-species were assigned to a category as either a species at the edge of its range in Canada or not and edge species. Distinct populations listed under SARA were not included in the

analysis to avoid confounding because all recognized distinct populations are listed. We defined an edge species as one that extends less than 100 km into Canada or is found in only a single watershed in Canada, while also having a distribution outside of Canada based on the distribution maps in Scott and Crossman (1998). For sub-species and distinct populations not listed in Scott and Crossman (1998), the distribution as described in the species at risk registry (sararegistry.gc.ca) was used. Fishes were also classified by whether or not they were found in a single watershed, were endemic, or were a distinct population of a species in the analysis.

For each of the fishes in the analysis, we also assigned categorical values for conservation status based on SARA Schedule 1 listing, and several life history and ecological traits based on information from Scott and Crossman (1998). Life history parameters were chosen for their potential to influence listing status. Categorical parameters included riverine or lacustrine, stream or lake spawners, anadromous, had benthic juvenile stages, benthic adult stages, juvenile and adult feeding guilds, whether the species are nest builders and whether the species experiences human exploitation in Canada. Human exploitation included both commercial and sport fishing harvest as well as commercial harvest for the bait industry. Additionally, continuous variables were assigned for maximum age, maximum length, age at maturity, and length at maturity (Appendix A). For cases where specific information was lacking in Scott and Crossman (1998), the species at risk registry (sararegistry.gc.ca) and Coker et al. (2001) were used to fill in the gaps. The ecological and life history traits were chosen for their potential to influence conservation status. Benthic life stages, for example, were chosen as possible

predictors because siltation caused by runoff and erosion has the potential to affect benthic species.

Once all available data had been compiled for each species, a discriminant function analysis was conducted with edge species and non-edge species as the two *a priori* groups. This analysis was used to determine if edge species differed from non-edge species based on life history and ecological traits. A second analysis was conducted with listed and non-listed species as the assigned groups to determine predictors of at risk status. Discriminant function analysis was chosen because it has been shown to be equally as successful in predicting outcome as classification and regression trees for ecological datasets, while better resolving difficult cases (Karels et al. 2004).

Results

We classed 43 Canada's freshwater fish species as having edge distributions in Canada. Of these edge species, 24 are listed under SARA. There were also three endemic species and sub-species and 24 distinct populations, all of which are listed. A large proportion of the listed species were benthic and riverine species (Table 2.1). A majority of the edge species were also riverine (Table 2.2). Exploitation by humans was present for six of the listed taxa in our analysis and 65 of the 154 non-listed species (Table 2.1).

The only significant factor delineating *a priori* groups in the discriminant function analysis conducted with listed and not listed species and predicting a species to be listed was the taxon having an edge distribution (Wilks' lambda 0.889, p=0.000000) (Table

2.5). The analysis was able to correctly assign cases to their *a priori* group for 146 (88%) of the species (Table 2.3).

When the analysis was conducted with edge species and non-edge species as the *a priori* groups, the significant factors delineating groups and predicting a species that has an edge distribution were: conservation listing status (Wilks' lambda 0.819, p=0.000) and no human exploitation (Wilks' lambda 0.723, p=0.005) (Table 2.5). The analysis correctly assigned 142 (86%) of the cases (Table 2.4).

Discussion

Our analysis showed that having an edge distribution was the significant predictor of a species being listed as at risk in Canada. Additionally, the discriminant function analysis to classify species with edge distributions and widespread species showed no differences in ecological or life history traits between edge and widespread taxa. The listing status of taxa and a lack of human exploitation were the only predictors for edge taxa. This indicates that species at the edge of their range and widespread taxa are not different ecologically. There were several misclassifications in the analysis. In the analysis of listed and non-listed taxa, there were 18 misclassified cases. Many of these cases resulted when the species in question has an edge distribution in Canada but the species is not listed as a species at risk. In the classification of species with edge distributions and widespread taxa, the model failed to correctly classify 23 cases. These species tended to be either species with edge distributions that were not listed as species at risk or they were species listed as at risk that did not have edge distributions in Canada.

In all cases the recognized distinct populations and endemic taxa were also listed under SARA and protected. The protection of endemic taxa is often seen as a conservation priority (Myers et al. 2000, Wilson et al. 2006) and the conservation of distinct populations is also a recognized way to protect genetic diversity of species (Jelks et al. 2008). The protection of edge populations, however, is more controversial. Hunter and Hutchinson, (1994) argued that protecting populations based on geographic boundaries rather than ecological ones leads to an inappropriate allocation of conservation funds, where wealthy nations are more likely to fund protection of their own species rather than focus on areas where conservation dollars can have the greatest effect. Collaboration among jurisdictions to protect species at the core of their range, rather than focusing on the edge populations can be a more effective use of conservation funds (Rodrigues and Gaston 2002).

To conserve or not to conserve?

The results of our analysis indicate that there are no ecological or life-history differences for taxa with edge distributions in Canada or for those that are widespread, though these species comprise a disproportionate number of the listed species at risk. Protecting edge populations of otherwise widespread species may not always be sound conservation practice. Due to normal metapopulation dynamics, these populations may be small and prone to natural extinctions (Pimm et al. 1988). Habitats at the edge of a species' range may also be population sinks, having higher mortality than the number of individuals the habitat can produce (Pulliam 1988). In this case, populations are only maintained through immigration from more productive sites (Pulliam 1988).

In some cases, a species, despite having no real or perceived threats, may receive protected status and the conservation dollars that follow with that designation. The Warmouth (*Lepomis gulosus*), for example, is a species at the northern edge of its range in Canada and is listed as Special Concern under SARA (COSEWIC 2005). Because the Warmouth is found in southern Ontario where its habitat falls within a national park (Point Pelee) and two provincial parks (Rondeau Provincial Park, Long Point Provincial Park), the threat of habitat loss and extirpation of this species would be minimal. The Warmouth, however, was predicted to be listed as a species at risk based on the discriminant function analysis.

There are also several arguments for conserving populations at the northern edge of their range. Although rare overall in Canada, these species may be locally abundant and play important ecosystem functions, such as predator or prey species, where they are found. Edge populations, where locally abundant, may also be highly valued by the human population (Hunter and Hutchinson 1994). In some cases these species may be utilized in either commercial or aboriginal fisheries. The Blueback Herring (*Alosa aestivalis*), for example, is an edge species in Canada that is regularly taken in a commercial fishery in the maritime region of Canada (Scott and Crossman 1998).

The preservation of genetic diversity within a species is another reason for the protection of edge populations of a species. These edge populations are often genetically distinct from populations at the core of the species' range. Differences in gene frequency may result from various processes such as isolation and inbreeding, genetic drift (Garcia-Ramos and Kirkpatrick 1997), directional selection (van Heerwaarden et al. 2009), or a combination of several factors. When edge populations persist in harsh or variable

environments compared to the core range, they may become adapted to these local conditions. Edge populations also exhibit increased phenotypic plasticity compared to core populations (Chevin and Lande 2011). This genetic diversity and phenotypic plasticity provides insurance for the survival of the species should the local environment change through stochastic events.

Climate change, in particular, has the potential to drastically alter ecosystems and species assemblages. Due to climate warming, species' ranges are shifting poleward at average rates of 6.1 km per decade (Parmesan and Yohe 2003). Perry et al. (2005) described the northward distributional shift in many fish species in the North Sea, in response to climate change. Chu et al. (2005) predicted that freshwater fishes in the southern region of Canada, many of which are populations with edge distributions, will expand northward due to the effects of climate change. Populations at the edge of a range tend to have more dispersive morphs (Phillips et al. 2007) and thus would be better able to track the changing environment and subsequent range shifts. Additionally, these edge populations are pre-adapted to living at the climatic extreme for the species and may be better able to survive in the newly suitable habitat. Maintaining these range edge populations will be critical to ensure that potential colonizers of newly suitable habitat are available.

Our analysis clearly shows that in Canada, species are likely to be afforded protection when the Canadian population is at the edge of the species' range. There is evidence to support the conservation of edge populations, though in reality conservation dollars are often limited and difficult decisions as to which species to protect must be made by resource managers. Allendorf et al. (1997) have outlined criteria to prioritize

populations for conservation. Some of the population attributes that they have outlined that make a population more valuable for conservation when compared to other populations of the same species are: high genetic divergence from other populations, living in unusual habitat, having unusual life history traits, geographic isolation, and existence at the edge of a the species range (Allendorf et al. 1997). Bunnell et al. (2004) also suggested that disjunct edge populations should get conservation priority due to their increased levels of divergence from core populations. Edge populations that exist where asymmetrical gene flow towards core populations is likely should also receive consideration for protection. Additionally, when a reasonable expectation that the species will decline to become endangered throughout most of its range, with a subsequent retraction to the edges exists, edge populations should receive conservation priority. These features of edge populations, isolation, local adaptation and genetic divergence, along with the ubiquitous concern of climate change induced range shifts support the continuation of affording protection to edge populations.

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Table 2.1 Number of listed and non-listed species possessing various life history and ecological characters.

Status	Total Species	Edge Species	Riverine	Diadromous	Stream Spawner	Benthic Juvenile	Benthic Adult	Nest Building	Exploited
Listed	34	23	24	3	24	16	16	12	6
Non-Listed	154	19	88	29	97	63	56	57	65

Table 2.2 Number of edge and widespread species possessing various life history and ecological characters.

Status	Total Species	Listed Species	Riverine	Diadromous	Stream Spawners	Benthic Juvenile	Benthic Adult	Nest Building	Exploited
Edge	42	23	33	1	34	21	20	15	2
Endemic	3	3	1	1	1	2	1	2	1
Widespread	138	8	78	30	86	57	52	52	68

Table 2.3 Classification of cases for discriminant function analysis of listed vs. non-listed species

A Priori Group	Percent Correct	Model Predicted Not Listed	Model Predicted Listed
Not Listed	92.5	135	11
Listed	57.9	8	11

Table 2.4 Classification of cases for discriminant function analysis of edge vs. non-edge species

A Priori Group	Percent Correct	Model Predicted Non-edge	Model Predicted Edge
Non- edge	97.8	131	3
Edge	35.5	20	11

Table 2.5 Discriminant function analysis variables and loadings. Significant variables are highlighted in bold.

	Listed vs. Not Listed		Edge vs. Widespread	
Variable	Wilks' lambda	P value	Wilks' lambda	P value
Status	-	-	**0.818**	**0.000**
Length at Maturity	0.744	0.556	0.690	0.319
Age at Maturity	0.743	0.747	0.687	0.538
Max Length	0.745	0.472	0.695	0.143
Maximum Age	0.746	0.441	0.687	0.559
Riverine	0.748	0.318	0.687	0.539
Anadromous	0.745	0.508	0.699	0.092
Stream Spawning	0.751	0.187	0.688	0.482
Benthic Juvenile	0.743	0.784	0.688	0.501
Benthic Adult	0.745	0.450	0.686	0.691
Juvenile Feeding Guild	0.743	0.668	0.686	0.697
Adult Feeding Guild	0.746	0.381	0.693	0.207
Nest Building	0.746	0.381	0.687	0.591
Human Exploitation	0.743	0.713	**0.723**	**0.005**
Edge distribution	**0.889**	**0.000**	-	-
Single Watershed	0.745	0.463	-	-

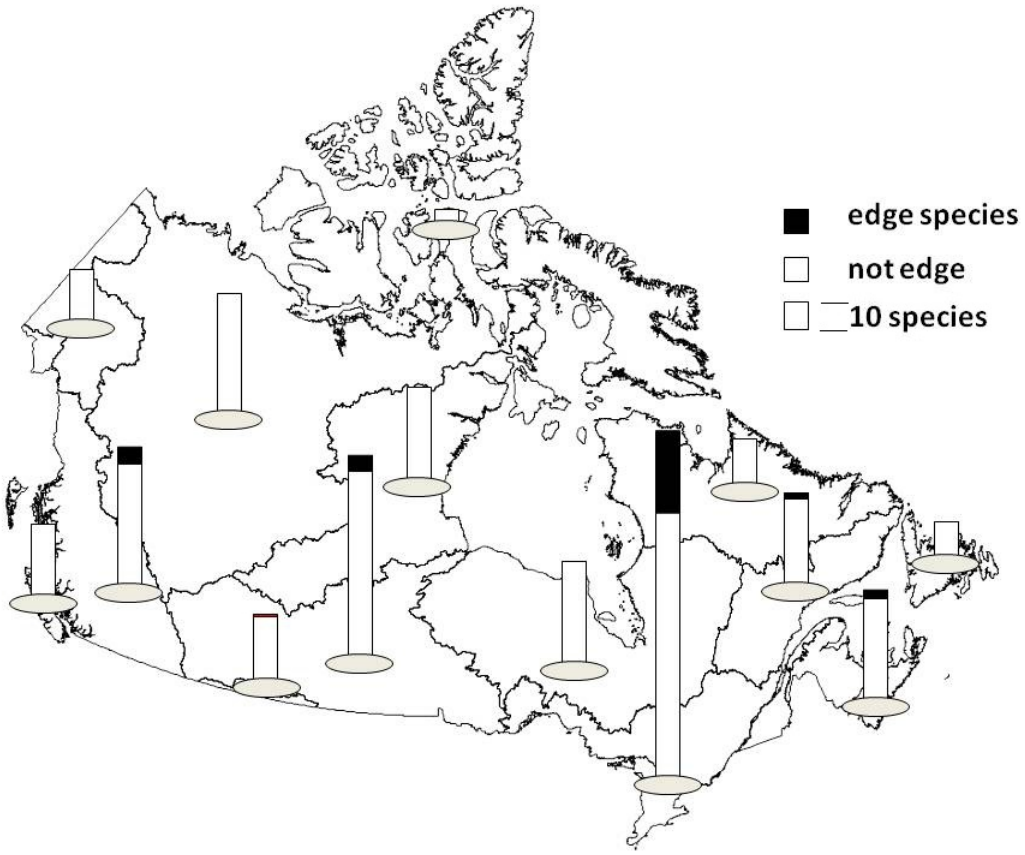

Figure 2.1 Number of freshwater fish species in Canada present in each of the COSEWIC national freshwater biogeoraphic zones, showing the number of species that are at the edge of their range in Canada. Modified from Dextrase and Mandrak (2006).

CHAPTER 3

PECTORAL FIN RAY AGING: AN EVALUATION OFA NON-LETHAL METHOD FOR AGING GARS AND ITS APPLICATION TO A POPULATION OF THE THREATENED SPOTTED GAR

Introduction

The Spotted Gar (*Lepisosteus oculatus*) is a fish species designated as Threatened under the Canadian *Species at Risk Act* (SARA). The species is distributed throughout the Mississippi River drainage with its northern limit extending into Canada (Figure 3.1). In Canada, *L. oculatus* inhabits three coastal wetlands of Lake Erie (Point Pelee, Rondeau Bay and Long Point Bay) with historic records from Lake St. Clair (COSEWIC 2005). The Threatened designation in Canada is due to their limited distribution and possible loss of critical habitat (COSEWIC 2005).

When preparing management strategies for species at risk, information is needed on life history traits, habitat associations, habitat availability and recovery targets (Rosenfeld and Hatfield 2006). This information is lacking for the Spotted Gar in Canada. Much of what is known about the Spotted Gar is based mainly on data gathered in the southern portion of its range (Love 2002, 2004). In this study, we attempted to fill in gaps in the life history of this species in Canada by conducting an age and growth study of the Spotted Gar in Rondeau Bay. Rondeau Bay is home to the largest of the known populations of Spotted Gar in Canada (COSEWIC 2005).

Various calcareous structures have been used to determine the age of fish specimens including otoliths, scales, opercula, and fin rays (Ihde and Chittenden 2002). Traditionally, branchiostegal rays have been used to age gar (Love 2004), though otoliths and sectioned scales have also been used (DiBenedetto 2009). The use of branchiostegal rays and otoliths requires sacrifice of the specimen to remove the structures and removal of a section of interlocking ganoid scales would leave the individual prone to infection.

For these reasons, we chose a non-lethal method, the use of pectoral fin ray cross sections, to age Spotted Gar specimens. The use of fin ray sections to age specimens is an effective method in several species including Common Carp (*Cyprinus carpio*; Phelps et al. 2007), Lake Whitefish (*Coregonus clupeaformis*; Mills and Beamish 1980), Muskellunge (*Esox masquinongy*; Brenden et al. 2006), Walleye Pollock (*Theragra chalcogramma*), Pacific Cod (*Gadus macrocephalus*), and Albacore (*Thunnus alalunga*; Beamish 1981). In addition, the removal of fin rays for aging has been shown to have no negative effects on the growth and survival of bull trout (*Salvelinus confluentus*; Zymonas and McMahon 2006).

The objectives of this study were to: determine if pectoral fin ray sections are suitable for aging gars; to compare the age and growth of the spotted gar population of Rondeau Bay with the age and growth of a spotted gar population in Lake Pontchartrain, Louisiana (Love 2004); and to compare the condition of individuals in the Rondeau Bay population with the standard for the species as reported in Bister et al. (2000). Because of a shorter growing season and colder temperatures, we predict that the spotted gar of Rondeau Bay will have a slower rate of growth than the spotted gar population studied by Love (2004) and be in poorer condition when compared to the standard for the population reported in Bister et al. (2000). Colder temperatures lead to reduced growth rates for many aquatic species (Angilletta et al. 2004).

Methods

Structure Comparison

We compared aging structures to test the validity of using pectoral fin rays to age Spotted Gar on 10 specimens collected from southwestern Michigan in October 2008. Five individuals were captured from Loon Lake in Branch County, Michigan (41.8689˚ N, -84.9427˚ W) and five were collected from Lake Pleasant in Hillsdale County, Michigan (41.8800˚ N, -84.5663˚ W). Michigan samples were collected using boat electrofishing. These individuals were sacrificed and the otoliths, branchiostegal rays, and first pectoral fin rays (clipped as close to the base as possible) were removed for aging. Individuals from these populations were chosen due to the similarity of climate between Michigan and Southern Ontario. The number of individuals used for validation is necessarily low as the species is also at risk (Special Concern) in Michigan. Following the method of Den Haas and Mandrak (2004), pectoral fin rays were embedded in epoxy resin and sectioned with a Buehler-Isomet low-speed saw to a thickness of 0.75 mm. These cross-sections were mounted on microscope slides and examined using a compound microscope at 400X magnification. Growth annuli were counted to estimate the age of the specimens (Figure 3.2). Branchiostegal rays were boiled until all flesh was easily removed and then air dried before aging using a dissecting microscope. Otoliths were ground into a thin transverse slice using GatorGrit 120-c waterproof paper (Mastercraft) and polished with 3M Lapping film, 261X, 30 micron. These thin sections were mounted to microscope slides using Crystalbond 509 (Electron Microscopy Sciences). Otoliths were viewed using magnification of 400X and growth annuli were counted. All structures were viewed and aged independently by two separate readers and

an index of precision was determined for each individual structure as in Den Haas and Mandrak (2004). The age as estimated by the first reader of the branchiostegal ray was used as the assigned age for all specimens. For all other age estimations the index of precision was calculated as equation [1]

$$\text{Index of precision} = \frac{|\,[\text{annuli counted on structure} - \text{assigned age}]\,|}{\text{assigned age}}$$

Based on the findings of Den Haas and Mandrak (2004), where the bottom 33% of structures had an index of precision score of 0.29 or higher, we will accept the method of aging using pectoral fin ray sections as valid if the average index of precision for the structure is less than 0.29. In addition to the index of precision, a chi-squared test was conducted to compare the observed (pectoral ray) age with the expected (branchiostegal ray) age for each of the two readers of pectoral fin rays.

Aging the Rondeau Bay Population

Rondeau Bay is a shallow (<3 m) coastal wetland along the north shore of the central basin of Lake Erie. The bay is characterized by clear water and abundant macrophyte growth. Spotted Gar was collected from 15 sites around Rondeau Bay using fine-mesh fyke nets (1.2 m hoops with 6.35 mm mesh), a non-lethal method of collection, 78 specimens were collected during May and June, 2007. Specimens were weighed to the nearest gram and their total length (mm) was measured. The first pectoral fin ray on the right side of each fish was then clipped as close to the base as possible for aging.

This technique of aging is non-lethal and all specimens were successfully released after handling. All animals were cared for in accordance with the Canadian Council on Animal Care guide to the care and use of experimental animals and this research was approved by the animal care committees of the University of Windsor and the Canadian Department of Fisheries and Oceans.

The fin rays were then prepared and read in the same manner as those from Michigan. Three sections from each specimen were aged independently of each other by the first reader. A second experienced reader also counted the growth marks, presumed to be annuli, to increase the precision of age estimation of the samples. Where disagreement between samples occurred, the most common age reported was used. The variance among reads of sections from the same individual was calculated.

To describe the growth of the Rondeau Bay population, regression analysis was conducted on the total length vs. age data set. The log-transformed total lengths were substituted into the standard weight equation developed by Bister et al. (2000) to determine the condition of individuals from the Rondeau population as compared to the standard for Spotted Gar.

Length-frequency plots were created for each age class. Upon inspection, each age class was divided into two size classes, based on the length-frequency distributions. As the fish could not be sacrificed to determine sex, and females are larger than males at the same age (Love 2004), the smaller size class individuals were presumed to be the males and the larger size class individuals was presumed to be the females of each age. Log transformations of the lengths, and regression analysis were then conducted for each

size class separately. Growth equations were then compared to the growth equations produced by Love (2004) by first extrapolating the raw data from a digital copy of Love's figure 3, using the computer software ImageJ (NIH image analysis software, http://rsbweb.nih.gov/ij/) followed by an analysis of covariance.

Results

Structure Comparison

There was a relatively good agreement among the various aging structures and readers (Table 3.1). The combined average index of precision for pectoral ray samples was 0.11, and for the otoliths was 0.14 (Table 3.1). The average index of precision for branchiostegal rays by the second reader was 0.03. Thus, the accepted standard of branchiostegal rays (lethal technique) is the most precise technique for aging Spotted Gar, followed by the use of sectioned pectoral rays (non-lethal technique) and sectioned otoliths (lethal technique) is the least precise. A Chi-squared test found no difference between the observed age (pectoral fin ray age) and the expected age based on the branchiostegal ray (Chi-square = 0.325, P = 0.99).

Aging the Rondeau Bay Population

Using non-lethal techniques, we captured, aged, and released 78 Spotted Gar in Rondeau Bay. Specimens collected ranged in age (3 to 10 years), total length (515 to 761 mm) and weight (0.52 to 1.94 kg). The length-frequency distribution appeared bimodal with fewer large than small specimens (Figure 3.3). The modal and most common age that was observed was six years. Although there was a significant relationship between

age and length ($Y = 18.52X + 493.62$; $P < 0.0001$), the amount of variation explained was low ($R^2 = 0.22$)(Figure 3.4).

When the Spotted Gar data from Rondeau Bay were separated into two size classes (presumed males and females), the slopes of the lines representing small size class ($Y = 0.022X + 6.21$; $R^2 = 0.43$; $P < 0.0001$) and large size class ($Y = 0.041X + 6.25$; $R^2 = 0.64$; $P < 0.0001$) were significantly different from one another ($P = 0.004$; Figure 3.4). A comparison of the age-length data from Rondeau Bay and Louisiana (Love 2004) showed that growth rates did not differ significantly for Rondeau large size class and Louisiana female ($P = 0.15$) or Rondeau small size class and Louisiana male ($P = 0.97$) Spotted Gar.

Bister et al. (2000) produced a standard weight equation for Spotted Gar, based on data collected from 47 populations of spotted gar across eight of the United States. When the log transformed total length for each specimen was substituted into this standard weight equation, we found that 73 of our 78 specimens were below the standard weight. Of the individuals that were over the standard weight for their length, two were 4 years old and the other individuals were 5, 8 and 10 years.

Discussion

The use of sectioned pectoral fin rays as a non-lethal method of aging is useful, particularly when dealing with species at risk or whenever sacrifice of the specimens is undesirable. We found that the method was precise when compared to the accepted standard method of aging using branchiostegal rays. The preparation of pectoral fin ray

samples is more time consuming than preparing branchiostegal rays, which only require boiling to remove flesh. Drying of the resin and sectioning of the samples is a fairly lengthy process; however, once the preparation is complete the age estimation is easily accomplished. Crowding of the growth annuli towards the outer edge of the ray, particularly in older specimens, may lead to underestimating the age in some cases. This was evidenced by one reader underestimating the age of our oldest specimen by two years compared to the branchiostegal ray. The second reader, however, was able to correctly determine the age of the oldest specimen using the pectoral fin ray; thus we urge caution when aging older specimens.

Growth rates differed by size classes that presumably represented sexes. The large amount of variability in the length-age data from Spotted Gar specimens from Rondeau Bay can be attributed to our inability to directly sex the fish using external characteristics in the field. Because definitive determination of sex in Lepisosteidae requires sacrifice of the fish and examination of the internal sex organs (Ferrara and Irwin 2001), we were unable to determine the sex of our individuals. Love (2004) showed that male and female Spotted Gar had differing length at age and rates of growth, with females growing larger and at a faster rate than males of the same age. Thus, a combined sample of males and females led to little correspondence of length and age. Additionally, it has been shown that there is substantial variation in growth within age cohorts of fish (Post and Parkinson 2001), contributing further to the variation in our length at age relationships.

The grouping of Rondeau Bay specimens based on the best estimation of sex (females larger and males smaller at age) exhibited similar results to the growth curves of

Spotted Gar from Lake Pontchartrain, Louisiana (Love 2004). Females from Rondeau Bay grew at a significantly higher rate than males from the same population consistent with the findings of Love (2004) in Louisiana. Interestingly, the rate of growth did not vary among populations for either male or female specimens between the Ontario and Louisiana populations, despite differences in latitude (42˚17'N for Rondeau Bay and 30˚11'N for Lake Pontchartrain). This is probably due to the high rate of individual variability in both the Rondeau Bay and Louisiana populations. Alternatively, the Rondeau Bay population may have adapted to the shortened growing season by increasing growth rate during the summer months to compensate for an extended winter, as was found by Conover and Present (1990) in the Atlantic Silverside (*Menidia menidia*).

The weight of most of the Rondeau Bay specimens was lower than predicted based on length using the standard length equation proposed by Bister et al. (2000). The reduced robustness of fish at a given length from the Rondeau Bay population may be attributed to the northern location. Many species do not actively feed in the colder months, and thus gar living at the northern latitudes may not have as much time to feed and increase their condition, as compared to a fish of the same size in southern latitudes. This would also lead to a longer inactive season, and thus these individuals would lose relatively more fat content over the winter than those in southern latitudes.

A possible consequence of the reduced weight at length of individuals compared to the southern populations is lower overall fecundity. Ferrara (2001) showed that fecundity of the spotted gar was positively correlated with total length and weight, suggesting that individuals in the northern population may produce fewer eggs than

individuals of similar length in the southern population. Lower female condition has also been shown to result in smaller egg size in the Atlantic Haddock (*Melanogrammus aeglefinus*) (Trippel and Neil 2004) and smaller larvae which may be less likely to survive. Lower male condition in the Atlantic Haddock resulted in lower fertilization success (Trippel and Neil 2004). Low body condition may also have survival implications. Low condition has been linked to an increase in disease susceptibility and severity of infection as reviewed by Beldomenico and Begon (2009). Thus, the Rondeau Bay population may have lower levels of reproduction and resistance to disease than southern populations.

Love (2004) found that Spotted Gar from Lake Pontchartrain, Louisiana reached sexual maturity at 1 year of age; however, none of the specimens captured from Rondeau Bay was younger than 3 years of age. Because our sampling method specifically targeted individuals moving into the shallows for spawning, the Spotted Gar in Rondeau Bay may delay maturation compared to those of more southern latitudes. Although our sampling method was passive, and smaller individuals may be less likely to be captured if they do not travel as far or frequently as larger individuals, the mesh size used was small enough to capture all but the smallest fishes. Thus, it is likely that smaller gar were not participating in the spawning behaviour that we targeted. Delayed maturity in Rondeau Bay populations can be attributed to the shorter growing season and colder average temperatures in northern than southern latitudes as was demonstrated with the Lake Trout (*Salvelinus namaycush*) by McDermis et al. (2010).

The maximum age of Spotted Gar in Rondeau Bay was found to be 10 years, which was the same as the maximum life expectancy in southern populations reported by

Ferrara (2001). This is in contrast to the expectation that individuals in colder climates would have slower growth rates, but a longer lifespan (Angilletta et al. 2004, Charnov and Gillooly 2004). Redmond (1964) however, aged an individual female Spotted Gar from Missouri at 18 years, suggesting that some exceptional individuals may exceed the maximum life expectancy of 10 years.

The high number of individuals in the 5 to 7 year age classes is indicative of strong year classes from the years of 2000 to 2002; however, it is not known at this time what has caused this variation in year classes. More research is needed to determine the long-term viability of the Rondeau Bay population of Spotted Gar, the largest of the Canadian populations. Future research to assure the survival of this top predator in Canada should include a determination of the habitat utilized by the species in Rondeau Bay as well as their diet preference in relation to the southern populations. Protection of the critical habitat as well as the important prey species will be an integral part of a management strategy for this species. Genetic divergence of the northern population from the southern population will also be of interest. Being isolated at the edge of their range, the northern population may have developed unique adaptations to survive. These adaptations will be important to preserve to ensure a future for the species in Canada.

Overall, we found that the Spotted Gar of Rondeau Bay have similar growth rates to those of Louisiana. However, delayed maturity and lower condition, combined with a lifespan that is not extended compared to gar of southern latitudes may lead to lower lifetime reproductive output, possibly contributing to the rarity of the species in Canada, and reinforcing its currently Threatened status.

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Table 3.1 Comparison of estimated age for Spotted Gar (*Lepisosteus oculatus*) specimens captured in southwestern Michigan using various structures. Ages in years determined by two separate readers from sectioned pectoral ray, branchiostegal ray and otolith are given for each specimen. The index of precision is shown in brackets for each structure. The age based on branchiostegal reader 1 is taken as the standard, thus index of precision is zero (perfect agreement) for all branchiostegal reader 1 ages.

Fish Number	Pectoral Ray Reader 1	Pectoral Ray Reader 2	Branchiostegal Reader 1	Branchiostegal Reader 2	Otolith Reader 1	Otolith Reader 2
118	4 (0.2)	4 (0.2)	5	5 (0)	7 (0.4)	8 (0.6)
120	7 (0)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
121	4 (0.2)	5 (0)	5	5 (0)	5 (0)	5 (0)
122	3 (0)	3 (0)	3	2 (0.33)	3 (0)	4 (0.33)
123	2 (0)	2 (0)	2	2 (0)	3 (0.5)	3 (0.5)
124	6 (0.14)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
125	2 (1)	1 (0)	1	1 (0)	1 (0)	1 (0)
127	4 (0)	4 (0)	4	4 (0)	4 (0)	4 (0)
128	12 (0.14)	14 (0)	14	14 (0)	14 (0)	14 (0)
130	7 (0.13)	7 (0.13)	8	8 (0)	9 (0.13)	9 (0.13)

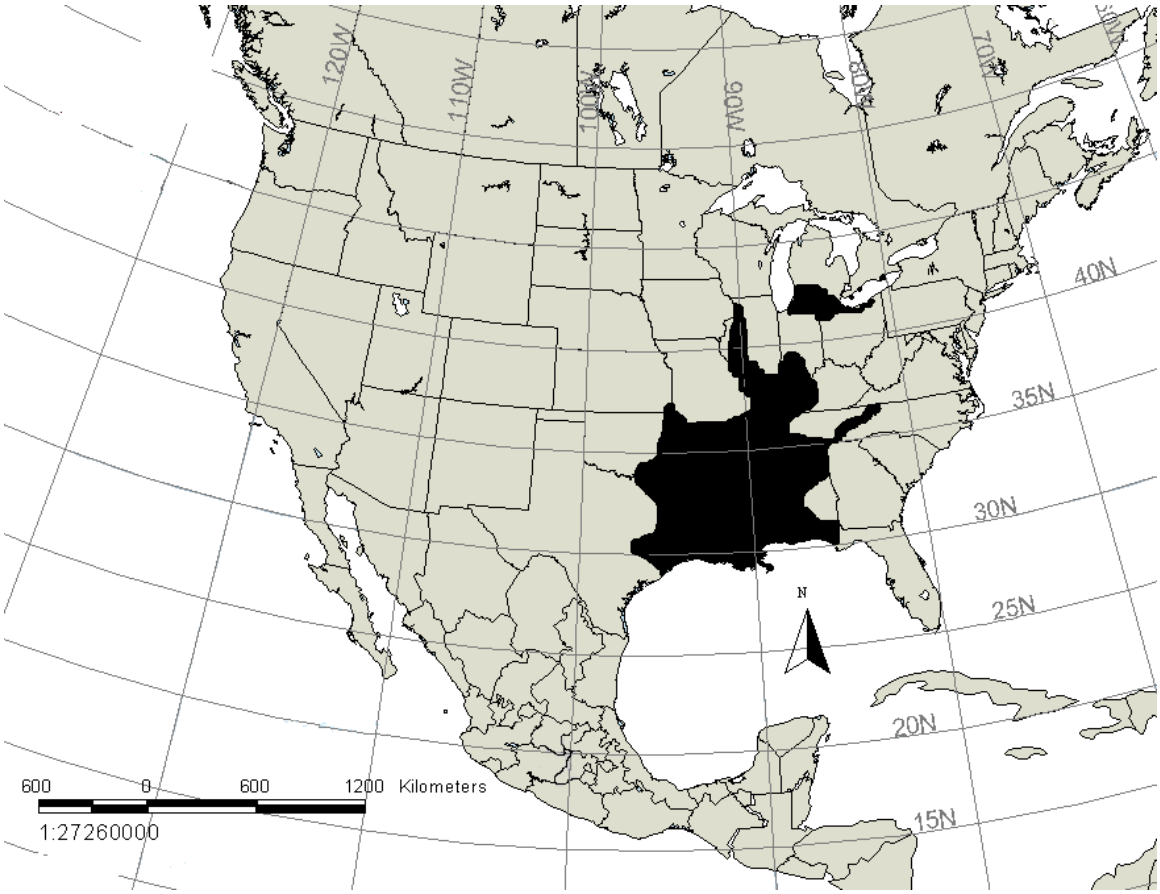

Figure 3.1 Range of the spotted gar, modified from Page and Burr (1991), to show all areas of known Canadian occurrences.

Figure 3.2 Cross section of pectoral fin ray of 7-year old spotted gar, viewed under magnification (400X) showing growth annuli.

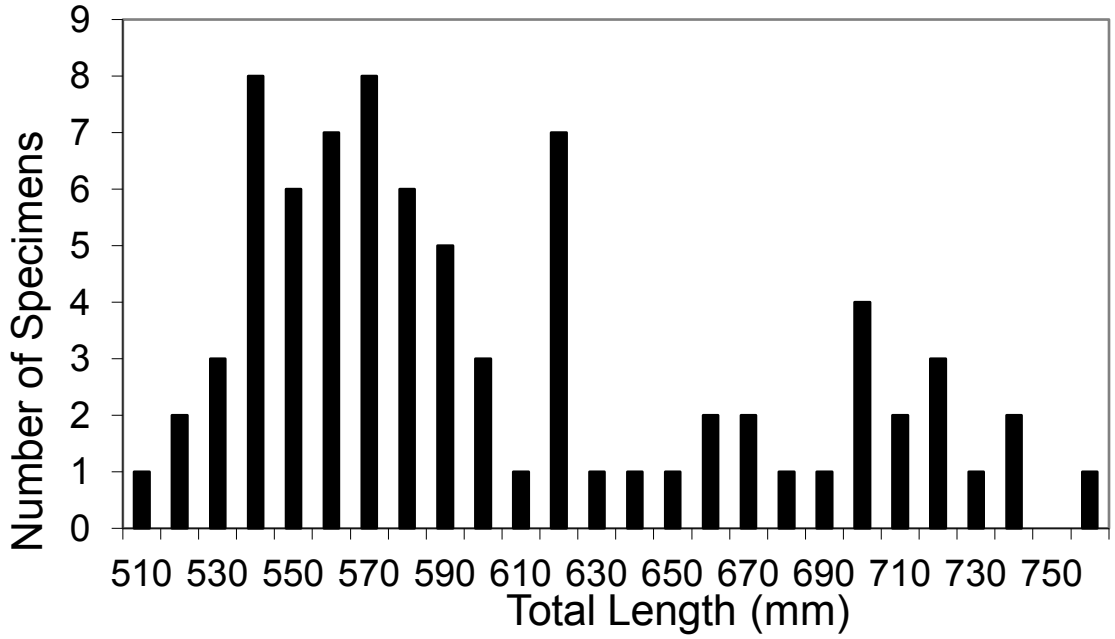

Figure 3.3 Length-frequency (total length) histogram for spotted gar captured in Rondeau Bay, Ontario, during 2007 sampling.

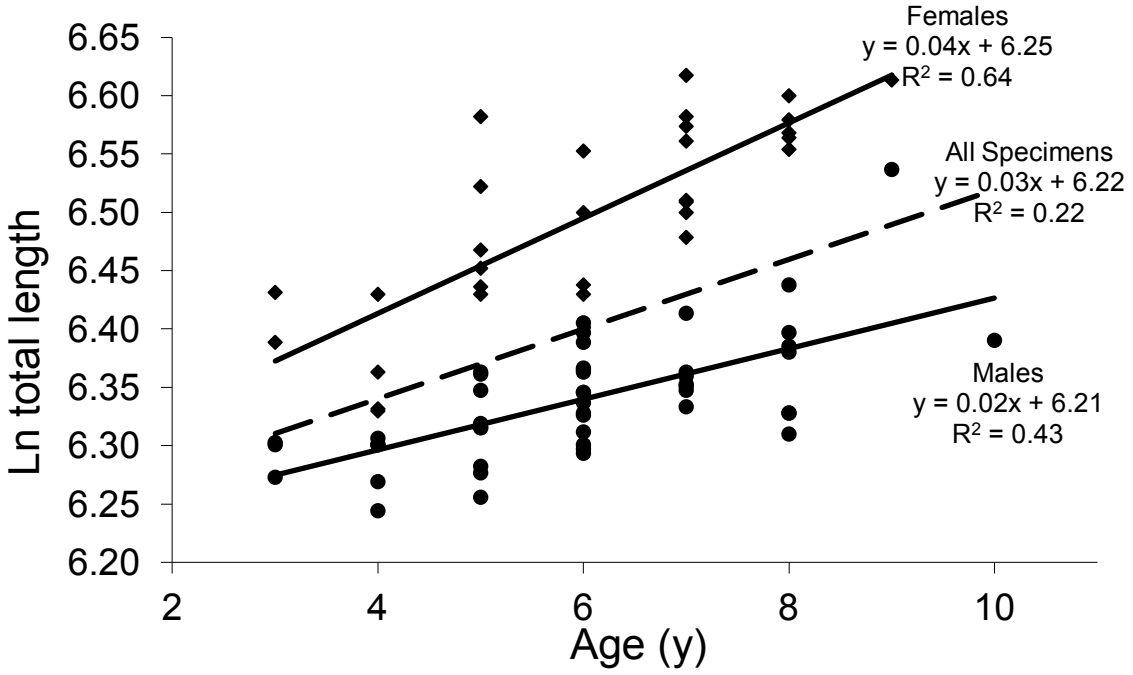

Figure 3.4 Natural log total length (mm) vs. age (years) of spotted gar captured in Rondeau Bay, Ontario, separated by presumed sex (see text for details). Male specimens are indicated by round dots and female specimens are indicated by diamonds. Dashed line indicates regression line for males and females combined.

CHAPTER 4

SPRING AND SUMMER DISTRIBUTION AND HABUTAT USE BY ADULT THREATENED SPOTTED GAR IN RONDEAU BAY, ONTARIO, USING RADIOTELEMETRY

Introduction

Preservation of the habitat that is used by a species at risk is paramount to the long-term survival of the species (Rosenfeld and Hatfield 2006). The Canadian *Species at Risk Act* defines this critical habitat for aquatic species as "spawning grounds and nursery, rearing, food supply, migration and any other areas on which aquatic species depend directly or indirectly in order to carry out their life processes" (*Species at Risk Act* 2002, s. 2(1)). Rosenfeld and Hatfield (2006) outlined four key information needs to identify critical habitat, including basic organism life history (and habitat associations), habitat availability, recovery targets, and habitat-abundance relationships.

Habitat associations may not be known for rare or at risk species and, thus, an effective means of determining which habitat is used by the species is needed. By definition, species at risk are rare, thus defining their critical habitat may be difficult (Nauman and Crawford 2009). One method of determining the habitat used by a specific life stage of a species is to monitor movements of individuals using radio telemetry. In this manner, the feeding, spawning, nursery and other important habitats can be determined for a species. This method has been used on a wide variety of taxa of species at risk, including bats (Bontadina et al. 2002), frogs (Lemckert and Brassil 2000), and fishes (Auer 1999). Radio-tagging and tracking in this manner has no negative effect on the behavior and swimming performance of fishes (Cooke 2003; Thorstad et al. 2000). Once habitat use by the species is determined, comparisons with availability of habitat types are made using an electivity index (Jacobs 1974) to show whether certain habitat intervals are preferred or avoided (Luttrell et al. 2002; Moyle and Baltz 1985).

The Spotted Gar *Lepisosteus oculatus* is a species designated as Threatened under the Canadian *Species at Risk Act*. This species is at the northern edge of its range in Canada, inhabiting three coastal wetlands of Lake Erie: Point Pelee; Long Point Bay; and, Rondeau Bay, the largest of the Canadian populations (COSEWIC 2005). Spotted Gar range as far south as the Gulf of Mexico, from eastern Texas in the west to the Florida panhandle in the east and is generally common south of the Great Lakes region (COSEWIC 2005). The Threatened designation in Canada is due to its limited distribution and the threats posed by pollution, turbidity, and habitat loss (COSEWIC 2005) and means that the Spotted Gar is likely to become endangered if steps are not taken to reverse the factors leading to its extirpation in Canada (COSEWIC 2010).

Although the movement and habitat use by the Spotted Gar was reported by Snedden et al. (1999) for a southern population in the Atchafalaya River Basin of Louisiana, there has yet to be any characterization of the habitat use by the species in Canada. The objectives of our study were to perform a radio-tracking survey of the Spotted Gar in Rondeau Bay, to describe the spring and summer distribution and critical habitat of this species in Canada, and to compare that habitat use with the Atchafalaya River Basin population studied by Snedden et al. (1999).

Methods

Study Site

Our study was conducted in Rondeau Bay, a shallow (maximum depth of 3 m) coastal wetland on the north shore of the central basin of Lake Erie (Figure 4.1).

Rondeau Bay is characterized by abundant submerged macrophyte growth, and its area (approximately 37 km^2) is nearly enclosed. The bay is bounded on the east by Rondeau Provincial Park and by the town of Erieau in the south, with the remainder of the area bordered by agricultural land with some residential development (Figure 4.1). There is a navigational channel in the southern portion of the bay at Erieau that provides connectivity to the central basin of Lake Erie.

Specimen Collection and Tagging

Individual Spotted Gar specimens were collected from May 17 to May 23, 2007. Thirty-seven specimens were captured using 1.2 m fine mesh fyke nets (6.35 mm bar mesh) set for approximately 24 hours and retrieved in the morning. Nets were set in shallow areas adjacent to shore, targeting spawning related movements (Figure 4.1b). After specimens were weighed (kg) and measured for total length (mm), fish were anesthetized in a 0.015% clove oil solution (3 mL clove oil emulsified with 5 mL ethanol, in 20 L water). Radio tags, with unique frequencies (Table 4.1), were attached externally to the dorsal musculature immediately behind the posterior insertion of the dorsal fin, following the procedure of Snedden et al. (1999). Tagged specimens ranged in length from 515 mm to 745 mm and weighed from 0.53 to 1.94 kg. The radio tags, manufactured by Holohil Systems Limited (Model PD-2), measured 23 mm X 12 mm X 6 mm, with an antenna 24 cm long; battery life was approximately four months. Tag weight (3.8 g) was <1% of the body weight of the smallest specimen. Small tag size and location of attachment at the base of the dorsal fin ensured that the swimming ability of specimens would not be impeded. Handling, surgeries, and recovery were conducted immediately at the site of capture.

Specimens were held in a recovery bin after surgery until they were able to maintain equilibrium. They were then released back into the bay at the capture site. All animal handling and surgeries were approved by the animal care committees of the University of Windsor and the Canada Centre for Inland Waters.

Tracking of Specimens and Distribution Mapping

The movement and subsequent location of specimens were tracked from a boat, using a Lotek tracking receiver set to cycle through the tag frequencies. Once a specimen's signal was located, the position was homed in and a hand-held GPS unit was used to determine the coordinates. Water depth (m), surface temperature (˚C), pH and conductivity (μs/cm) were measured using a Hydrolab Surveyor 4a with Datasonde 5. Additionally, aquatic macrophyte samples were taken when present and brought back to the lab for identification to the genus level. Once fish were located, their tag frequency was removed from the cycle list in the receiver so that specimens were located a maximum of once per tracking bout.

Tracking of specimens was conducted from the end of May through September, 2007, on at least three days per week and up to five days per week. Multiple tracking bouts were conducted over a 24 hour period on July 11 and July 25, 2007. Tracking effort was concentrated within Rondeau Bay; however, several attempts were made to locate fish outside the bay without success. Once tracking was completed, ARCMap GIS software was used to map all location coordinates for each individual (Figure 4.2a). All the locations where Spotted Gar were tracked in Rondeau Bay were noted (Figure 4.2b). We employed a modification of the technique used by McGrath and Austin (2009) to

determine if the number of times a specimen was located was sufficient to describe its distribution. A series of minimum convex polygon that enclosed all these points was created (cf. Winter 1977). Minimum convex polygons were built after each tracking point was sequentially added to the map (instead of daily tallies as in McGrath and Austin 2009). The area of the polygons was calculated using ARCMap. Once all points had been mapped and the area of each polygon measured, we plotted the area of cumulative minimum convex polygon against number of times a specimen was located. The leveling out of the curve for an individual specimen indicates that there are sufficient data points to describe its distribution.

Several individuals exhibited a distinct clustering of points (four or more points in proximity) where they were located several times in the summer. To determine whether specimens were associated with nearshore or offshore habitats, the distance from shore to the closest of these clustered points was measured for each individual. Also, the farthest linear distance between two tracking locations and the maximum distance from point of capture were measured for each specimen as a surrogate for home range. Regression analysis was used to determine the relationship between: 1) fish size (total fish length, weight) and distance from shore to clustered points; 2) fish size (total fish length, weight) and maximum distance from capture; and, 3) fish size (total fish length, weight) and maximum distance between points.

Habitat Variables

Tracking locations were divided into two groups based on season: spring (May and June), which includes the spawning period for this species, and summer (July to September). ARCMap was used to interpolate habitat values for the entire area of Rondeau Bay by inverse distance weighting based on the values collected at tracking locations. Habitat layers were created for each of the measure variables separately by season. These habitat layers were then compared to the observed habitat variables at all Spotted Gar locations to calculate electivity indices (Jacobs 1974). The electivity index (D) for each interval of a variable's distribution is calculated as follows:

$$D = [r-p] / [(r+p) -2rp],$$

Where: r is the proportion of individuals using the interval and p is the proportion of the overall habitat that has this value (Luttrell et al. 2002). These electivity indices are interpreted according to Moyle and Baltz (1985) where: a value from -1.00 to -0.50 indicates strong avoidance, -0.49 to -0.26 indicates moderate avoidance, -0.25 to 0.25 indicates neutral selection, 0.26 to 0.49 indicates moderate selection and 0.50 to 1.00 indicates strong selection.

Population Size and Area of Suitable Habitat

In May 2009 a mark-recapture study was conducted in Lake Pond a marsh at Point Pelee National Park. The Point Pelee marsh is a coastal wetland of Lake Erie with similar habitat to Rondeau Bay. The contiguous surface area of the marsh is approximately 220 ha. and has no connection to the main basin of Lake Erie. Spotted Gar were captured using 1.2 m fine mesh fyke nets (6.35 mm mesh) set overnight.

Captured specimens (n=93) were marked using PIT tags and released immediately after handling. A total of 99 Spotted Gar was captured and released, 6 of these were recaptured during the sampling. Based on this sampling, the total population of Spotted Gar in the Point Pelee marsh was estimated to be 483 individuals, with a density of 2.2 individuals per ha.

To estimate the population size of Spotted Gar in Rondeau Bay, the population density estimate of 2.2 individuals per ha for the Point Pelee marsh was used and assuming similar habitat and population density at the locations, this density was multiplied by the area of Rondeau Bay to estimate total population.

Results

Tracking and Distribution Mapping

Of the 37 radio-tagged individuals, 35 were located at least once (Table 4.1). One tag was presumed lost when the individual was tracked on consecutive days to the same location in very shallow water and no fish was evident. All subsequent locations for this tag were removed from the analysis. The fate of the second tag which was not located is unknown. Each individual was located an average ± SD of 6.19 ± 4.96 occasions, for a total of 224 discrete locations.

When the cumulative area of minimum convex polygon was plotted against number of times located, the curve appeared to level off for 10 individuals, (Figure 4.4)

indicating that the tracking effort was sufficient to describe the overall distribution for these specimens.

There was no significant relationship between fish length and offshore distance of clustered points (P = 0.17). The mean ± SD offshore distance of these clusters was 1.77 ± 1.58 km. There was a significant negative relationship between the natural log-transformed weight of specimen and offshore distance of the clusters ($\log_e$ (offshore distance) = -0.68 $\log_e$(weight) + 5.02 (R^2 = 0.36, P = 0.02).

When all specimens were considered, the mean ± SD farthest distance from capture and mean ± SD farthest distance between two points were 2.95 ± 1.76 km and 3.47 ± 2.25 km, respectively. Regression analysis revealed no significant relationship for the natural log-transformed data of length vs. farthest distance from capture (P = 0.17) and length vs. farthest distance between points (P = 0.19). There was, however, a marginally significant relationship between natural log-transformed weight and farthest distance from capture and between natural log-transformed weight and farthest distance between locations. These relationships were $\log_e$ (distance from capture) = 1.02 $\log_e$(weight) – 5.94 (R^2 = 0.13, P = 0.033) and $\log_e$ (distance between points) = 1.08 $\log_e$(weight) – 6.18 (R^2 = 0.13, P = 0.036).

Habitat Variables

Interpolated raster layers were created for each habitat variable (example: Figure 4.3). The electivity indices showed strong positive selection by the Spotted Gar for several habitat intervals in spring (Table 4.2) and summer (Table 4.3). In spring Spotted Gar exhibited a preference for both the shallowest(< 0.5 m) and the deepest (> 2.5 m),

waters, areas with no macrophyte growth, waters with conductivity levels > 325 μS, or < 225 μS, and pH values < 8.5. The habitat interval of moderate depths (1.00 m – 1.49 m) was strongly avoided. In summer, habitats strongly selected by the Spotted Gar were the deepest depths (>2.5 m) and the shallowest depths (<0.5 m), areas with two or more macrophyte genera present, and waters with pH between 8.0 and 8.5.

Of the 224 locations to which Spotted Gar were tracked, 201 sites (90%) had some form of aquatic vegetation present. Seven sites had emergent vegetation only, nine sites had both emergent and submerged vegetation and 185 sites had submerged vegetation only. A large proportion of the sites contained complex, or highly branched, vegetation. It was common to have sites represented by several genera of plants (Table 4.4).

A spawning event was witnessed on June 12. This spawning activity took place in a mixed bed of macrophytes that included *Myriophyllum* spp. and *Ceratophyllum* spp. located 391 m from shore. The spawning event consisted of a single large female surrounded by 3 three smaller males thrashing around in the shallow vegetation.

Population Size and Area of Suitable Habitat

Based on the population density estimate (2.2/ha) from the Point Pelee marsh and total area of Rondeau Bay (3215 ha), the population of Spotted Gar in Rondeau Bay is approximately 8121 individuals. The area of suitable habitat, based on our raster interpolation of vegetation complexity (Figure 4.3), conservatively determined by the proportion of Rondeau Bay with two or more macrophyte genera is 1543 ha. A less conservative estimate, the total proportion of the bay with either two or more macrophyte

genera or no macrophytes present, is 1884 ha. These areas were chosen as surrogates for suitable habitat area because Spotted Gar feeding success has been shown to depend on the macrophyte complexity of the cover present (Ostrand et al. 2004). Additionally, most Spotted Gar were found in areas with two or more macrophyte genera present (Table 4.4). Areas with no macrophytes present were moderately selected by Spotted Gar in the spring (Table 4.2). Other habitat variables, such as pH, temperature and conductivity, were not used to identify habitat area because they varied with changing weather conditions.

Discussion

The Spotted Gar specimens tracked in this study were most often found associated with aquatic vegetation. This association with aquatic macrophytes as cover shows an adaptation to local conditions in Rondeau Bay when compared to the Spotted Gar population of Lower Atchafalaya River, Louisiana, where fish were mainly associated with flooded timber (Snedden et al. (1999). In Rondeau Bay, Spotted Gar were often found in mixed beds of complex macrophytes. Like the timber in the Snedden et al. (1999) study, complex macrophyte beds created a three-dimensional environment in which the Spotted Gar could hide and forage. This habitat type (specifically vegetation density) has been shown to be important for the feeding success of Spotted Gar (Ostrand et al. 2004). The potential loss of habitat is one of the limiting factors for the recovery of Spotted Gar populations in Canada (COSEWIC 2005). Specifically, the removal of aquatic vegetation by both physical and chemical means represents a high impact activity

that disturbs Spotted Gar in Rondeau Bay (Bouvier and Mandrak 2010). Removal of aquatic vegetation should be curtailed given the findings that Spotted Gar in Rondeau Bay are dependent on aquatic macrophytes throughout the spring and summer periods.

There was also strong selection for areas without vegetation in the spring. Interestingly, our findings showed that only 11% of Rondeau Bay lacked vegetation in the spring. These unvegetated areas may be used for post-spawn feeding since spring spawning minnows (eg. Spottail Shiner (*Notropis hudsonius*)) present in sandy bottomed areas (Scott and Crossman, 1998) provide ample prey for Spotted Gar.

Early in the season, Spotted Gar were often found near shore. Movement into the shallows was likely due to the spawning behavior of the species. The Spotted Gar is known to spawn in spring in shallow water among aquatic vegetation (Redmond 1964). In the summer, Spotted Gar tended to move offshore and several individuals were repeatedly tracked to the same location. Similarly, Snedden et al. (1999) found that Spotted Gar established defined home ranges in the summer.

In the Atchafalaya River basin, Snedden et al. (1999) found that Spotted Gar tended to migrate into flooded areas in the spring, followed by the establishment of home ranges for the duration of the high water stage. The average distance from shore to the site of repeated location for Spotted Gar specimens in Rondeau Bay was much farther (mean ± SD = 1.77 ± 1.58 km) than that reported by Snedden et al. (1999), where 48% of all Spotted Gar movements were within 10 m from shore. This difference in behavior likely results from habitat differences between the two areas. Rondeau Bay is shallow, with an extended littoral zone and macrophyte cover throughout, while the Atchafalaya

River basin is narrower and has depths ranging from 3 - 5 m (low water stage in the Snedden et al. (1999) study area). The Atchafalaya River basin, unlike Rondea Bay, generally lacks aquatic vegetation (Snedden et al. 1999). Evidently, Spotted Gar are relating to specific depths and cover, rather than shoreline features in Rondeau Bay.

Our habitat layers were created based on a relatively small number of points compared to the size of Rondeau Bay. The limitations in our method are apparent in cases where there were no observed values in a particular range. In such cases, the interpolated habitat layer also lacks values in the range. Observations on which interpolations were based were well spread throughout the bay. Given the lack of available habitat maps and associated data for our study we were limited to interpolating habitat values for the entire study area.

The moderate preference for spring surface temperatures (20 - 23˚C) is indicative of the preferred spawning temperature of Spotted Gar in spring. Snedden et al. (1999) reported that spawning related movements began when temperatures reached 15˚C. Boudreaux (2005) reported spawning activity in a laboratory at a mean temperature of 20.6˚C.

The strong selection for high surface temperature interval (> 26˚C) in the summer for the specimens in Rondeau Bay likely reflects preferred feeding temperatures. This temperature was much higher than the preferred water temperature of 16˚C reported by Coker et al. (2001) for Spotted Gar in Canada. The physostomous gas bladder, common to all gar species, allows the Spotted Gar to obtain atmospheric oxygen and, thus, provides an advantage over many other predatory species in warm waters and

corresponding low oxygen concentrations that often result. Smatresk and Cameron (1982) showed that Spotted Gar increase their rate of air breathing when temperatures are increased and the use of the physostomous gas bladder is significantly higher at 30˚C than at 20˚C. Our study also showed preference for low temperatures (17 – 19.9˚C) later in the sampling period. This finding was influenced by individuals inhabiting offshore areas in the early fall.

Conservation of the Spotted Gar, a native top predator, in Canada will hinge on the protection of its critical habitat for all life stages. Our study indicates Spotted Gar use emergent and submerged aquatic macrophyte beds in both the nearshore and offshore areas of Rondeau Bay for feeding, cover and spawning. Long-term survival of the species in Canada will require at least 1400 adult Spotted Gar (Young and Koops 2010) and at least 360 ha of suitable habitat (DFO 2010). We show that the population of Spotted Gar in Rondeau Bay is large enough (8121 individuals) and has sufficient suitable habitat (1543 to 1884 ha) to be viable in the long term. Although this population estimate is based on Point Pelee marsh data, Point Pelee and Rondeau Bay are similar, albeit at different size scales. Based on the similarity of habitats, the population density should be similar in the two locations.

Our sampling failed to collect any specimens less than three years old, which is the presumed age of maturity for Spotted Gar (Glass et al. 2011). Thus, additional studies are required to identify the critical habitat for young of the year, juvenile, and subadult life stages. Nevertheless, our current findings will be used by the Spotted Gar recovery team to define critical habitat and recovery targets for the Spotted Gar recovery

strategy, leading to protection of aquatic macrophytes and other critical areas of Rondeau Bay. These actions will assist in the conservation of the species.

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Table 4.1 Capture date, time at liberty and number of times located for Spotted Gar specimens in Rondeau Bay

Radio Tag Frequency	Date Tagged	Days at Liberty	Days Located	Total Locations
151.242	23/05/2007	130	1	1
151.270	23/05/2007	130	13	15
151.299	23/05/2007	130	9	9
151.320	23/05/2007	130	1	1
151.340	23/05/2007	130	7	7
151.360	23/05/2007	130	2	2
151.380	24/05/2007	129	10	11
151.400	24/05/2007	129	8	9
151.420	24/05/2007	129	2	2
151.440	24/05/2007	129	7	7
151.460	17/05/2007	136	2	29
151.481	17/05/2007	136	9	9
151.500	17/05/2007	136	10	10
151.521	17/05/2007	136	2	2
151.541	17/05/2007	136	7	7
151.560	17/05/2007	136	0	0
151.579	17/05/2007	136	4	4

151.600	17/05/2007	136	6	6
151.620	17/05/2007	136	4	4
151.637	17/05/2007	136	0	0
151.661	17/05/2007	136	2	2
151.680	17/05/2007	136	2	2
151.700	17/05/2007	136	7	7
151.720	17/05/2007	136	9	9
151.740	17/05/2007	136	7	7
151.762	17/05/2007	136	3	3
151.780	17/05/2007	136	2	2
151.800	18/05/2007	135	6	6
151.820	31/05/2007	122	20	24
151.840	23/05/2007	130	5	5
151.860	23/05/2007	130	12	14
151.880	23/05/2007	130	15	16
151.900	23/05/2007	130	4	4
151.921	23/05/2007	130	3	3
151.942	23/05/2007	130	2	2
151.961	23/05/2007	130	3	3
151.980	23/05/2007	130	7	7

Table 4.2 Electivity indices and level of selection for habitat variable intervals in May and June.

Habitat Variable	Habitat Interval	Electivity Index[a]	Selection Level
Macrophyte Growth	No Macrophytes	0.78	Strong selection
Macrophyte Growth	Single Macrophyte	-0.36	Moderate avoidance
Macrophyte Growth	Mixed Macrophytes	-0.44	Moderate avoidance
Depth (m)	< 0.50	0.90	Strong selection
Depth (m)	0.50 – 0.99	0.29	Moderate selection
Depth (m)	1.00 – 1.49	-0.67	Strong avoidance
Depth (m)	1.50 – 1.99	-0.72	Strong avoidance
Depth (m)	2.00 – 2.49	-0.40	Moderate avoidance
Depth (m)	≥ 2.50	0.84	Strong selection
Temperature (˚C)	17.00 – 19.99	NA	NA
Temperature (˚C)	20.00 – 22.99	0.25	Neutral selection
Temperature (˚C)	23.00 – 25.99	-0.3	Moderate avoidance
Temperature (˚C)	≥ 26.00	0.24	Neutral selection
Conductivity (µS)	< 225.0	NA	NA
Conductivity (µS)	225.0 – 249.9	0.86	Strong selection
Conductivity (µS)	250.0 – 274.9	-0.04	Neutral selection
Conductivity (µS)	275.0 – 299.9	-0.57	Strong avoidance

Conductivity (µS)	300.0 – 324.9	-0.13	Neutral selection
Conductivity (µS)	325.0 – 349.9	0.67	Strong selection
Conductivity (µS)	≥ 350.0	0.97	Strong selection
pH	< 8.0	0.99	Strong selection
pH	8.0 – 8.49	0.57	Strong selection
pH	8.50 – 8.99	-0.50	Moderate avoidance
pH	9.0 – 9.49	-0.33	Moderate avoidance
pH	≥ 9.50	0.74	Strong selection

[a]Values from -1.00 to -0.50 indicate strong avoidance, -0.49 to -0.26 moderate avoidance, -0.25 to 0.25 neutral selection, 0.26 to 0.49 moderate selection and 0.50 to 1.00 strong selection (Moyle and Baltz 1985). NA indicates that no values were recorded in that range in field observations and thus did not appear in the interpolated layer.

Table 4.3 Electivity indices and level of selection for habitat variable intervals in July through September.

Habitat Variable	Habitat Interval	Electivity Index[a]	Selection Level
Macrophyte Growth	No Macrophytes	-0.32	Moderate avoidance
Macrophyte Growth	Single Macrophyte	-0.46	Moderate avoidance
Macrophyte Growth	Mixed Macrophytes	0.50	Strong selection
Depth (m)	< 0.50	0.64	Strong selection
Depth (m)	0.50 – 0.99	0.04	Neutral selection
Depth (m)	1.00 – 1.49	-0.61	Strong avoidance
Depth (m)	1.50 – 1.99	-0.08	Neutral selection
Depth (m)	2.00 – 2.49	0.42	Moderate selection
Depth (m)	≥ 2.50	0.87	Strong selection
Temperature (˚C)	17.00 – 19.99	0.63	Strong selection
Temperature (˚C)	20.00 – 22.99	0.05	Neutral selection
Temperature (˚C)	23.00 – 25.99	-0.40	Moderate avoidance
Temperature (˚C)	≥ 26.00	0.51	Strong selection
Conductivity (µS)	< 225.0	0.65	Strong selection
Conductivity (µS)	225.0 – 249.9	-0.56	Strong avoidance

Conductivity (µS)	250.0 – 274.9	0.29	Moderate selection
Conductivity (µS)	275.0 – 299.9	0.91	Strong selection
Conductivity (µS)	300.0 – 324.9	NA	NA
Conductivity (µS)	325.0 – 349.9	NA	NA
Conductivity (µS)	≥ 350.0	NA	NA
pH	< 8.0	NA	NA
pH	8.0 – 8.49	0.94	Strong selection
pH	8.50 – 8.99	0.34	Moderate selection
pH	9.0 – 9.49	-0.25	Moderate avoidance
pH	≥ 9.50	0.09	Neutral selection

[a]Values from -1.00 to -0.50 indicate strong avoidance, -0.49 to -0.26 moderate avoidance, -0.25 to 0.25 neutral selection, 0.26 to 0.49 moderate selection and 0.50 to 1.00 strong selection (Moyle and Baltz 1985). NA indicates that no values were recorded in that range in field observations and thus did not appear in the interpolated layer.

Table 4.4 Composition of submerged macrophytes present at Spotted Gar tracking locations. *indicates a complex, or highly branched, macrophyte type.

Genus	Number of Sites Present	Sites as Lone Species	Sites Dominant Species in Mixed Bed	Sites Secondary Species in Mixed Bed
Chara *	68	21	39	8
*Potamageton**	86	5	34	47
*Myriophyllum**	61	6	25	30
*Ceratophyllum**	20	1	5	14
*Elodea**	4	0	0	4
Valisneria	59	1	2	56
Lemna	1	0	0	1
None Present	22	NA	NA	NA

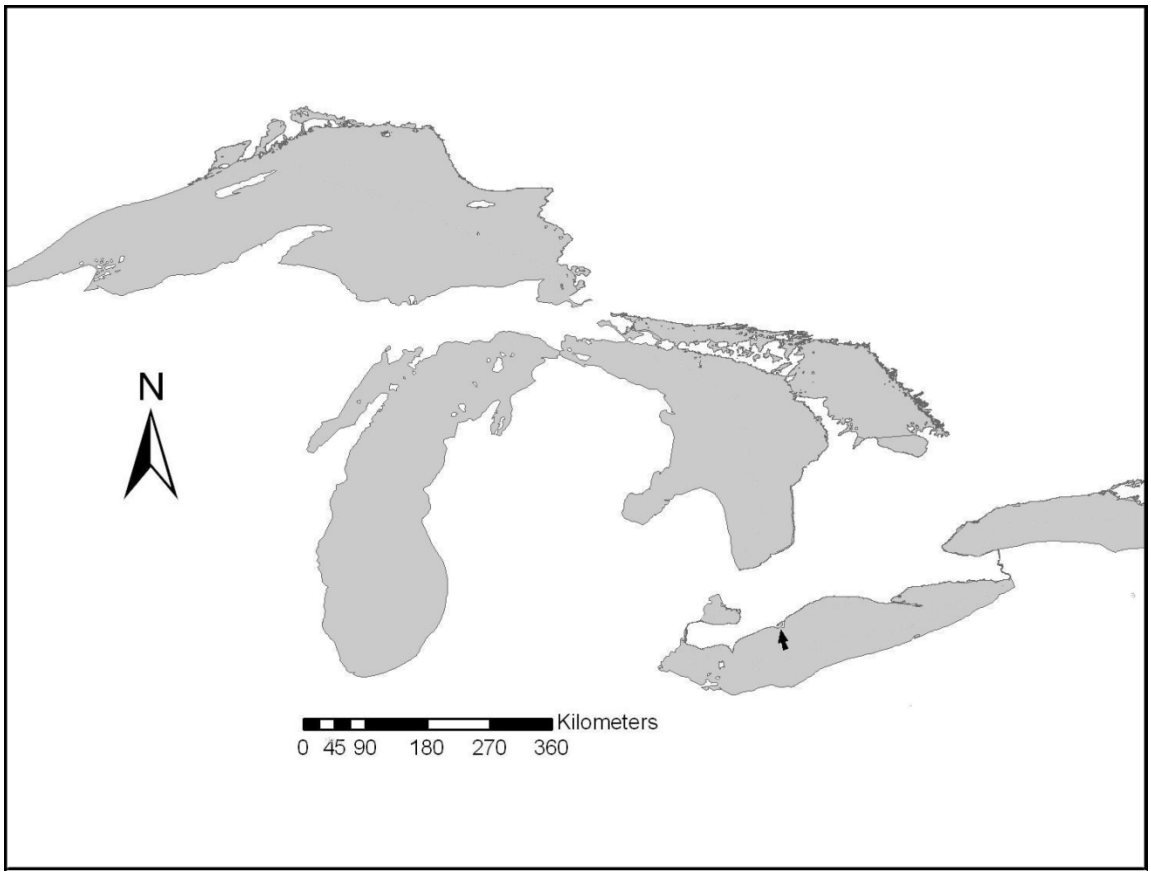

Figure 4.1A Location of Rondeau Bay, indicated by black arrow.

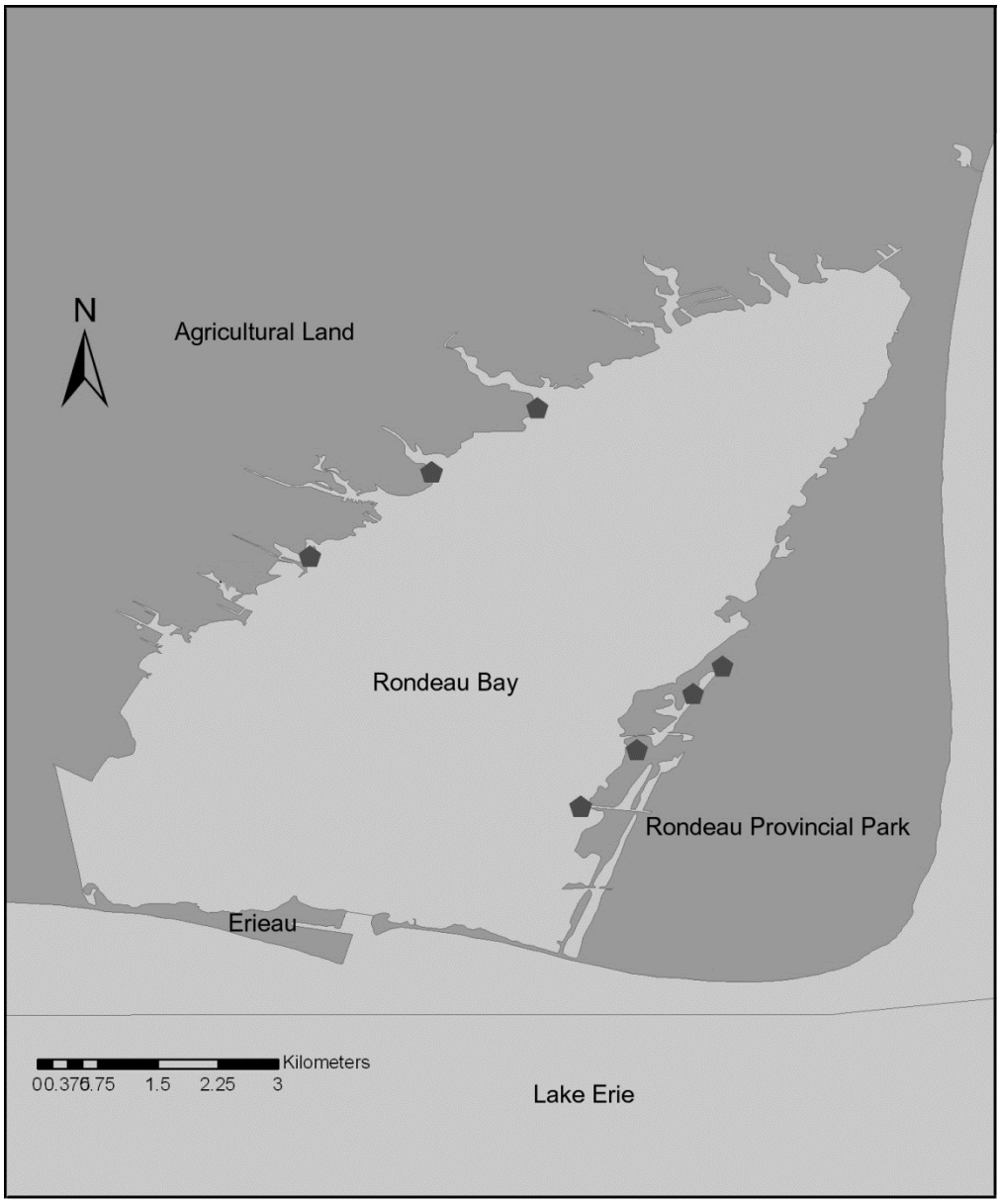

Figure 4.1B Map of Rondeau Bay showing locations where Spotted Gar were captured, tagged and released.

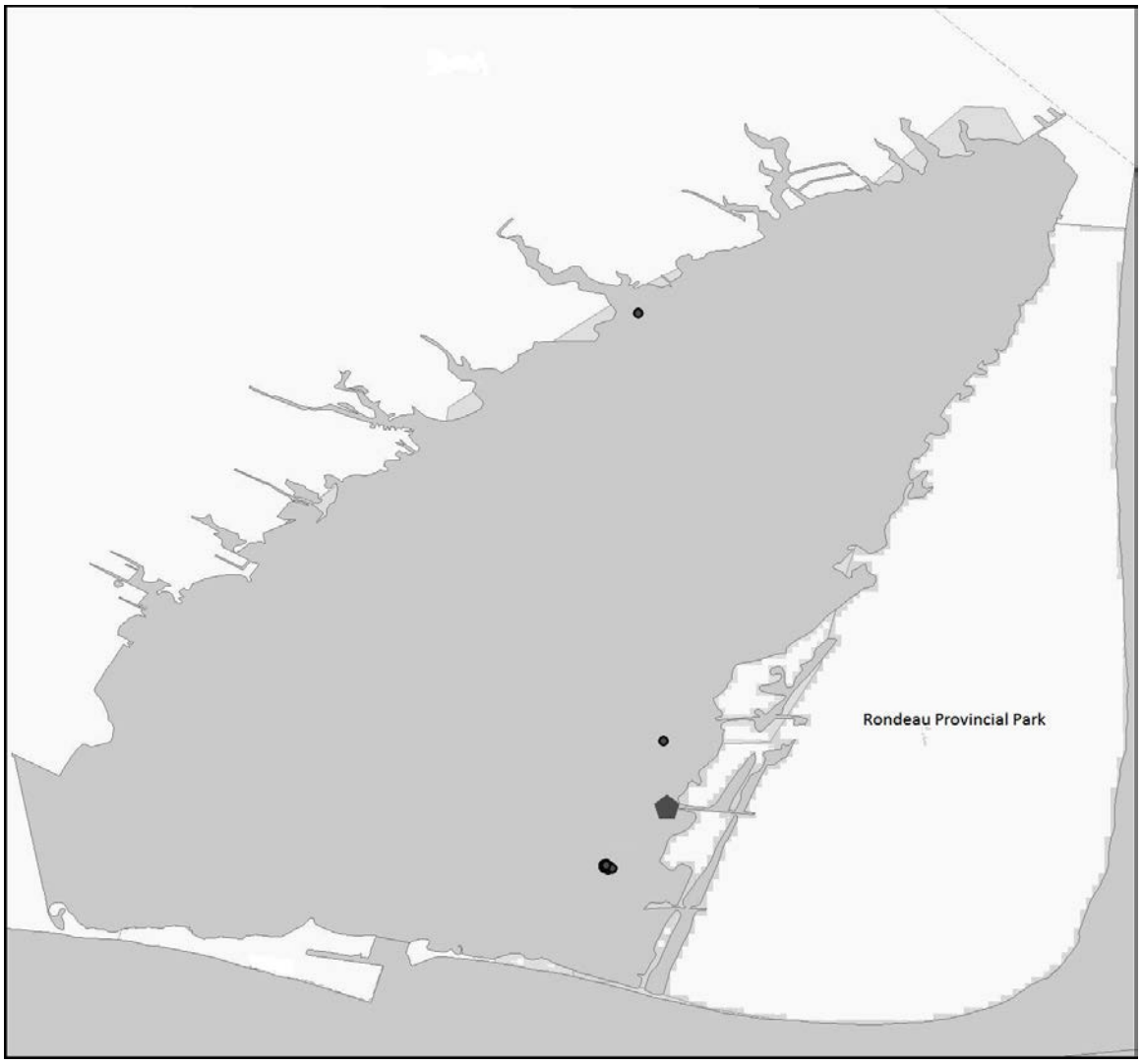

Figure 4.2A Locations of a single specimen, tag number 151.270, determined by radio-tracking, in Rondeau Bay during spring and summer 2007.

Figure 4.2B All tracking locations of radio-tagged Spotted Gar specimens in Rondeau Bay, spring and summer 2007.

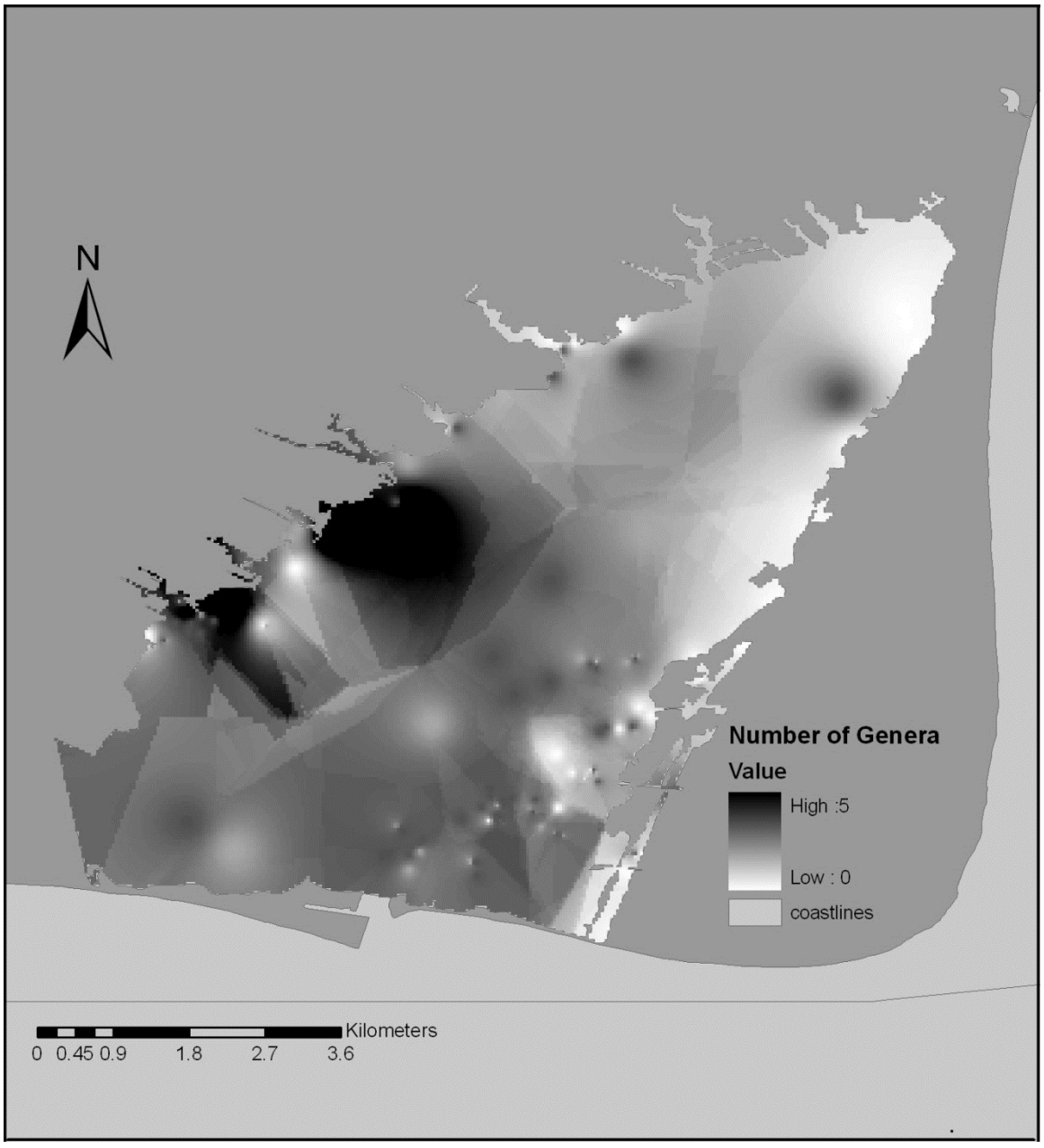

Figure 4.3 Interpolated raster of the number of aquatic macrophyte genera present in Rondeau Bay. Darker areas indicate more macrophyte types present, white areas indicate total lack of macrophyte growth.

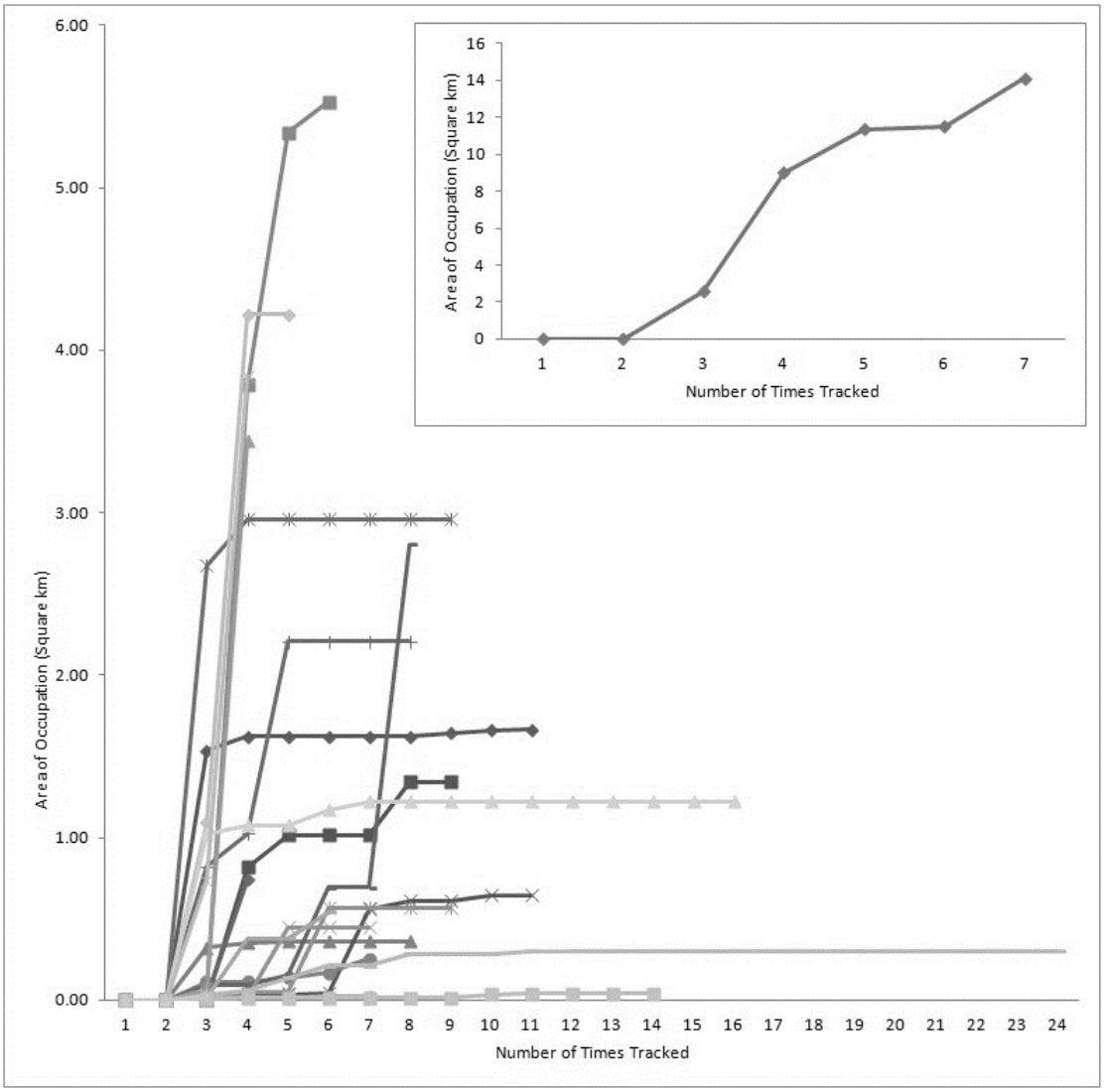

Figure 4.4 **.** Plot of cumulative maximum convex polygon area versus number of times located. Inset shows individual with tag number 151.541(see Table 4.1).

CHAPTER 5

CONSERVATION IMPLICATIONS OF GENETIC STRUCTURE AMONG CORE AND NORTHERN EDGE POPULATIONS OF SPOTTED GAR (*Lepisosteus oculatus*), AN ANCIENT FISH SPECIES, BASED ON MICROSATELLITE ANALYSIS

Introduction

Conservation of the range-wide genetic diversity of a species is often cited as an important objective for the conservation strategy for species (e.g. Reed and Frankham 2003). This need to conserve genetic diversity has led to calls for the protection of wildlife, not only at the species level, but at the level of genetically distinct populations (Jelks et al. 2008). The Canadian Species at Risk Act, in fact, protects not only species and sub-species, but recognized distinct populations as well (*Species at Risk Act* s.2(2)).

Populations at the edge of a species' range may carry a disproportionately high amount of the genetic variation within the species (e.g. Munwes et al. 2010). Edge populations tend to be smaller and more isolated than core populations, thus genetic drift often plays a larger role in driving the differentiation of edge populations (Garcia-Ramos and Kirkpatrick 1997). Additionally, populations at the edge of the species' range often live at the extremes of the environmental conditions in which the species can survive, leading to increased or differential selection pressures for these edge populations compared to those in the central range (Case and Taper 2000).

Advancements in the field of molecular genetics, particularly the genotyping of individuals at polymorphic microsatellite loci, permit high resolution in detecting genetic diversity (Estoup et al. 1998). This enables researchers to quantify the range-wide genetic diversity of a species and identify those populations that are distinct, and thus, of higher conservation importance. Microsatellite analyses have been used to characterize the population genetic diversity in several fish species at risk including the Razorback Sucker, *Xyrauchen texanus* (Dowling et al. 2012), Atlantic Salmon, *Salmo salar* (Horreo et al. 2011) and Atlantic Sturgeon, *Acipenser oxyrinchus oxyrinchus* (King et al. 2001).

The Spotted Gar (*Lepisosetues oculatus*) is a fish species of the family Lepisosteidae (Gars). The Spotted Gar is native to the Mississippi and Great Lakes drainages of North America and ranges from the Gulf of Mexico northward to the Great Lakes region (Page and Burr 2011). The species is at the northern edge of its range in Canada and is listed as Threatened in Canada under the Canadian *Species at Risk Act* and the *Endangered Species Act* of Ontario. The Threatened designation in Canada stems from the species' limited distribution and potential for habitat loss in the region (COSEWIC 2005). In Canada, the Spotted Gar is found in three coastal wetlands of Lake Erie: Long Point Bay; Point Pelee; and Rondeau Bay, the Canadian waterbody with the most individuals present (COSEWIC 2005). The Spotted Gar has been assigned a conservation status of Special Concern or higher in all of the American states in the Great Lakes basin (Natureserve 2012), but is generally common south of the Great Lakes region (Natureserve 2012). To date, there has been no comprehensive study of the range-wide genetic diversity for this species.

The objectives of this study are: to conduct a survey of the genetic diversity of the Spotted Gar throughout its range on the basis of microsatellite loci to determine if the populations at the northern edge of the species' range are distinct, and thus, deserving of their conservation status; and, to determine the temporal stability of the population genetic structure at the Rondeau Bay sample site, an urgent action proposed in the Spotted Gar Recovery Strategy (Staton et al. 2012).

Methods

To obtain tissue samples for this study, we contacted educational and government institutions throughout the range of the Spotted Gar and requested tissue samples. Additionally, specimens were collected by the authors from each of the three known Canadian populations as well as populations in Michigan, Louisiana, and Missouri. Specimens were collected by various methods including boat electrofishing (Michigan, Missouri, Ontario), 1.2 m fine mesh fyke nets (Ontario), and gill nets (Louisiana). In total, fin tissue samples of 681 specimens were collected from eight different sampling locations (Table 5.1, Figure 5.1) and preserved in 95% ethanol. Once all samples had been obtained, DNA was extracted following the protocol of Elphinstone et al. (2003).

Eight microsatellite loci developed for Alligator Gar (*Atractosteus spatula*) were PCR amplified for all specimens (Moyer et al. 2009). PCR reactions were conducted in 12.5 µL reactions containing 0.5 M dye labelled forward primer, 0.5 M reverse primer, 1X PCR buffer, 200 µM of each dNTP, 0.5 U Taq polymerase and various volumes of 25 mM $MgCl_2$ (Table 5.2). The thermal cycler reaction conditions were as follows: initial denaturation at 94 ºC for 120 s, followed by 35 cycles of 94 ºC for 15 s, various annealing temperatures (Table 5.2) for 15 s, 72 ºC for 30 s, and a final extension period of 60 s at 72 ºC.

Dye-labelled PCR products were visualized using a LiCor 4300 DNA analyzer (Li-Cor Biosciences Inc.) with manufacturers' size standards (50 – 350 bp). Individual genotypes were determined by scoring alleles using GENE IMAGIR v.4.05 (Scanalytics Inc.).

Genotype data were tested for allele scoring error, outlier alleles, discrepancies between observed and expected allele step size, and large allele gaps using Microsatellite Analyzer (MSA v4.05) software (Dieringer and Schlötterer 2003). All pairs of loci were tested for linkage disequilibrium using ARLEQUIN v.3.01(Excoffier et al. 2005). Departures from Hardy-Weinberg Equilibrium (HWE) were tested in ARLEQUIN using the Markov-chain Monte Carlo method with 100 000 dememorisation steps and 1 000 000 Markov chain steps. Sequential Bonferroni correction was applied in all instances of simultaneous tests (HWE departure, Pairwise F_{ST}) to correct for multiple simultaneous tests (Rice 1989).

Population divergence

Pairwise F_{ST} values (Weir and Cockerham 1984) were calculated for each sampling location to determine genetic differentiation using ARLEQUIN. The analysis program STRUCTURE v.2.3 (Pritchard et al. 2000), which uses a model-based clustering method to infer genetic structure, was used to determine the number of population genetic clusters and infer whether distinct populations were evident. STRUCTURE was run with a 500 000 burn-in period and 500 000 Monte Carlo Markov Chain generations, with 3 iterations and admixture allowed in the simulation. The total allowable number of populations for the simulation ranged from K = 1 to K = 9 (the number of sampling locations + 1). The second order rate of change (ΔK) of the LnP(D) function was used to select the most appropriate number of clusters (Evanno et al. 2005) and was calculated using Structure Harvester (Dent and vonHoldt 2012). STRUCTURE output was compiled using CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) and visualized using DISTRUCT v. 1.1 (Rosenberg 2004). Once the populations had been partitioned into

their respective groups, the process was repeated within groups of populations to determine the presence of any substructure in the population groups. Within-site genetic structure was further characterized by calculating observed (H_O) and expected (H_E) heterozygosities and allelic richness (A) for each sample site using ARLEQUIN and the presence of recent bottleneck effect was tested for using BOTTLENECK v.1.2.02, employing a Wilcoxon signed-rank test and a stepwise mutation model.

Temporal stability at Rondeau Bay sample site

To determine the natal year for specimens collected in Rondeau Bay, Ontario, pectoral fin rays were collected from 273 individuals. These pectoral fin rays were used to age specimens in the manner of Glass et al. (2011). All of these individuals were genotyped and included in the population level analyses described above. Once the age of each individual was identified, we assigned individuals to groups based on their natal year and population assignment from the STRUCTURE analysis. To determine if the genetic structure at the Rondeau Bay site is stable over time, we calculated pairwise F_{ST} between the populations and between all pairs of natal years within each assigned population using ARLEQUIN.

Results

Each of the loci was polymorphic and the number of alleles ranged from 5 to 19 (Table 5.2). When the sequential Bonferroni correction was applied to HWE departure tests, 15 of the 64 possible combinations showed significant deviation from Hardy-Weinberg equilibrium and three additional loci were monomorphic within the Michigan population, a finding that can be attributed to the small sample size of 10 individuals

collected from Michigan. Each of the Louisiana, Missouri and Mississippi (Townsend Lake) sites had a single locus that deviated from HWE, although the loci in question differed among each locale. The Canadian sample sites at Point Pelee and Rondeau Bay both showed multiple loci that deviated significantly from Hardy-Weinberg equilibrium. Five of 8 loci deviated in the Point Pelee site and seven of the 8 loci deviated in the the Rondeau Bay site.

Population Divergence

Pairiwse F_{ST} values among sampling sites ranged from -0.010 to 0.297 (Table 5.3). Each of the pairwise F_{ST} comparisons showed significant divergence after Bonferroni correction, except for the comparison between the Missouri and Mississippi (Townsend Lake) populations.

When STRUCTURE was used to investigate the number of population clusters across all samples, the largest ΔK of the LnP(D) function was shown for $K = 4$ populations ($\Delta K = 140.93$). The sample sites that grouped together in the clusters showed a geographic distinction with the first cluster comprised of all of the southern populations grouping together (Louisiana, Mississippi, Missouri), while all of the northern sample sites comprised the other three groups (Figure 5.2). Because the southern sample sites were grouped in a distinct separate cluster, we then further investigated the substructure by analysing the southern and northern sample sites grouped separately. The largest ΔK for the southern cluster was calculated for a $K = 2$ populations ($\Delta K = 165.93$) and had two populations with no admixture between them spread among all sample sites (Figure 5.2). When the northern population cluster was

investigated the highest ΔK was shown for K = 2 populations (ΔK = 577.07). This cluster analysis showed that Michigan and Point Pelee each had populations distinct from the other, whereas, Rondeau Bay and Long Point had individuals that shared genotypes with both the Michigan and Point Pelee populations.

The allelic richness (A) ranged from 1 to 15 alleles at a single locus per site (Table 5.4) with observed (H_O) and expected (H_E) heterozygosities ranging from 0.00 to 1.00 and 0.103 to 0.939, respectively. The results of the Wilcoxon signed-rank tests showed no evidence of a bottleneck, in the form of excess heterozygosity, for any of the sample sites. There was, however, a significant deficiency of heterozygosity found in the Rondeau Bay site ($p = 0.0098$).

Temporal Stability of Rondeau Bay site

When specimens from the Rondeau Bay site were aged, their ages ranged from 2 to 10 years, and their corresponding natal year ranged from 1998 to 2007. Once the aged individuals were grouped by assigned population, the F_{ST} between the assigned populations within Rondeau Bay showed significant divergence ($F_{ST} = 0.086$, $p < 0.001$). When natal years within each assigned population were compared, all pairwise F_{ST} values (Bonferroni corrected) showed no significant differentiation, indicating that the population genetic structure of the Rondeau Bay population was stable over the time period from 1998 to 2007.

Discussion

The results of our analysis reveal significant genetic structure present across the range of the Spotted Gar. We found divergence between the populations in the core

southern range of the Spotted Gar and the populations at the northern edge of the species' range. The divergence of the edge populations from the southern core population may be the result of isolation and subsequent lack of gene flow (Garcia-Ramos and Kirkpatrick 1997), coupled with increased selection pressure (Case and Taper 2000). Edge populations typically have populations that are much smaller than populations at the core of the species' range and, thus, experience a corresponding increase in genetic drift (Vucetich and Waite 2003). As edge populations are prone to increased selection pressure, isolation, and genetic drift, compared to core populations they are more evolutionarily dynamic (Lesica and Allendorf 1995), promoting the divergence of edge populations from core populations.

Our analyses also revealed divergence among the northern sample sites. Point Pelee and Michigan were each grouped separately and Rondeau Bay and Long Point Bay had individuals of genotypes from both population clusters. The Rondeau Bay and Point Pelee sites have very little, if any, contemporary gene flow between each other but there is shared ancestry between these two sites that are more than 50 km apart. Rondeau Bay and Long Point Bay are open to Lake Erie, whereas, the marsh at Point Pelee is formed by a barrier beach and is largely isolated from the western basin of Lake Erie due to low water levels, although periodic breaches do occur (Surette 2006). Since 1973, seven breaches have are known to have occurred and another eight unrecorded breaches are predicted to have occurred (Surette 2006). Given the infrequent nature of breach events, the unlikely scenario of a breach happening when there are Spotted Gar migrants in the vicinity given the distance and lack of suitable habitat between Point Pelee and the closest site occupied by Spotted Gar (Rondeau Bay), the likelihood of natural migration

between these populations is very small. Additionally, radio-tracking Spotted Gar in Rondeau Bay throughout the spring and summer showed no individuals had left the bay for the open water of the central basin of Lake Erie (Glass et al. 2012).

The Rondeau Bay site is particularly important because it carries all of the genetic diversity found in the north (Figure 5.1) and exhibits temporal stability. The temporal stability of the genetic structure is expected as this species is long-lived (Ferrara 2001, Glass et al. 2011) and will spawn in multiple years once sexual maturity is reached (Redmond 1964). Generations overlap maintaining the genetic structure over time. The temporal stability of the genetic structure and large population size for the Rondeau Bay sample site indicate that the Spotted Gar should be viable in the long-term at this site, provided that sufficient habitat is protected.

Maintenance of the overall diversity of genetic structure within the species requires protection of the northern populations. As populations at the northern edge of a species' range have adapted to the colder climate in which they are found, compared to those at the central and southern portions of a species range, and given that edge populations tend to contain more dispersive morphs (Phillips et al. 2007), northern edge populations will be vital to the northward migration of species as climate warms and new habitat becomes available for colonization (e.g. Chu et al. 2005).

Although the microsatellite loci we utilized showed no evidence of linkage disequilibrium, making them suitable for this analysis, we found significant divergence from HWE at several sample sites. The deviation from HWE of the Michigan sample site is likely attributed to sampling error, due to the small number of individuals (10)

sampled. The other two sites that showed significant divergence from HWE (Point Pelee and Rondeau Bay) are coastal wetlands of Lake Erie, at the northern edge of the species' range. Sampling error is likely not the cause of the deviations in this case as these sites had the highest sampling effort in our study; 93 individuals were collected from the Point Pelee site and 415 individuals were sampled from the Rondeau Bay site. In the case of the Point Pelee sample site, divergence from HWE can be attributed to its isolation. The sample site is usually isolated from other populations by low water levels and unsuitable habitat and, as a result, the population has not reached mutation – drift or migration – drift equilibrium. The deviation from HWE in the Rondeau Bay sample site can be attributed to the Wahlund effect as the result of our STRUCTURE analysis indicates the presence of multiple populations at this site. Combining multiple populations that have different allele frequencies will cause significant divergence from HWE when they are analyzed as a single population.

Conservation Implications

Our study clearly shows that the northern edge populations of the Spotted Gar are genetically distinct from the population in the southern portion of the species' range. This finding supports the continued conservation of this species in northern areas, such as Michigan and Canada. Protection of peripheral populations, as advocated by Fraser (2000), is a key component of maintaining genetic diversity within this species. Preservation of the genetically distinct populations of aquatic species, as suggested by Jelks et al. (2008) and mandated by the Canadian *Species at Risk Act* (s.2(2)) should be considered for edge populations, regardless of whether the species is abundant in other jurisdictions in the core of its range. Additionally, the divergence among Canadian

populations points to a lack of gene flow and subsequent genetic drift, as predicted for edge populations by (Garcia-Ramos and Kirkpatrick 1997). Lack of gene flow implies that in the case of the Spotted Gar, there is little potential for a rescue effect, where edge populations are supported by an influx of migrants from core populations. The lack of rescue effect is also a consideration for listing of a species for conservation priority in Canada (COSEWIC 2010), further strengthening the argument to maintain protection of the Canadian populations of this species.

The Spotted Gar population at the Rondeau Bay sample site is particularly important conservationally, given its large number of individuals and that it carries all of the genetic diversity found in the north. Several threats with high potential to impact this population have been identified including habitat modifications, vegetation removal, nutrient loading and turbidity loading from terrestrial sources (Bouvier and Mandrak 2010, Staton et al. 2012). To date, the critical habitat of the Spotted Gar has been identified in Rondeau Bay (Staton et al. 2012) and the protection of this habitat will be necessary for the persistence of this genetically diverse population, which is distinct from the southern core populations. The protection of critical habitat by eliminating the practice of vegetation removal in critical areas will be necessary to accomplish the goal of protecting this population (Staton et al. 2012). The conservation of this population will benefit the species as a whole by maintaining a significant portion of the overall genetic diversity within the species.

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Table 5.1 Location and number of Spotted Gar sampled from each sample site.

Site	Site Name	Waterbody	Latitude	Longitude	No. of Samples
LA	Louisiana	Lac des Allemands	29.9151° N	90.5728° W	40
MS1	Mississippi (1)	Pascagoula River	30.7749° N	88.6888° W	11
MS2	Mississippi (2)	Townsend Lake/ Jackson Lk North	33.1439° N	90.4110° W	56
MO	Missouri	Mingo Creek	36.97966° N	90.2047° W	49
MI	Michigan	Loon Lake / Lake Pleasant	41.8689° N	84.9427° W	10
PP	Point Pelee	Lake Erie	41.9655° N	82.5094° W	93
RB	Rondeau Bay	Lake Erie	42.2873° N	81.8978° W	415
LPB	Long Point Bay	Lake Erie	42.6145° N	80.4503° W	7

Table 5.2 PCR forward primers and reaction conditions for amplification of microsatellite loci. Primers were originally developed by Moyer et al. (2009).

Primer	$MgCl_2$ (µL)	Ta(°C)	Number of alleles	Size Range (bp)
Asp010	1.0	58.3	5	287 – 293
Asp012	1.0	60.2	5	206 – 218
Asp029	1.0	58.3	5	116 – 124
Asp046	1.1	60.2	9	183 – 199
Asp057	1.0	60.2	14	148 – 187
Asp066	1.0	62.0	19	225 – 279
Asp095	1.0	63.5	14	182 – 224
Asp096	0.75	58.3	7	104 – 114

Table 5.3 Pairwise F_{ST} comparisons among all pairs of Spotted Gar sample sites. Values in bold indicate a significant difference between the site pair.

Site	LA	Long Pt.	MI	MS (1)	MS (2)	MO	Pt. Pelee	Rondeau
LA	0							
Long Pt.	**0.1391**	0						
MI	**0.2041**	**0.2973**	0					
MS (1)	**0.0683**	**0.0802**	**0.1363**	0				
MS (2)	**0.0386**	**0.1056**	**0.2612**	**0.0440**	0			
MO	**0.0330**	**0.0873**	**0.1849**	**0.0464**	0.0102	0		
Pt. Pelee	**0.2203**	**0.1754**	**0.2101**	**0.1894**	**0.1238**	**0.1502**	0	
Rondeau	**0.1723**	**0.1553**	**0.1192**	**0.1143**	**0.1493**	**0.1093**	**0.0764**	0

Table 5.4 Allelic richness (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) for eight microsatellite loci at each sample site

	Asp010			Asp012		
Sample Site	A	H_O	H_E	A	H_O	H_E
Louisiana	4	0.65789	0.62667	3	0.16216	0.19993
Mississippi 1	3	0.90000	0.67368	3	0.45455	0.39394
Mississippi 2	3	0.56250	0.66022	2	0.10870	0.14214
Missouri	3	0.55000	0.60981	3	0.10638	0.10318
Michigan	2	0.50000	0.39474	1	0	NA
Pt. Pelee	4	0.68966	0.68190	2	0.35484	0.29346
Rondeau	4	0.52000	0.56733	5	0.12773	0.14188
Long Pt	3	0.66667	0.54545	3	0.40000	0.60000

	Asp029			Asp046		
Sample Site	A	H_O	H_E	A	H_O	H_E
Louisiana	3	0.34375	0.37775	4	0.63889	0.71322
Mississippi 1	3	0.27273	0.25541	4	0.50000	0.66842
Mississippi 2	2	0.41379	0.37266	5	0.68182	0.72544
Missouri	3	0.69767	0.49549	5	0.65000	0.76044
Michigan	1	0	NA	3	0.20000	0.54211
Pt. Pelee	3	0.31646	0.48174	4	0.39326	0.64680
Rondeau	5	0.20339	0.43449	8	0.49524	0.73120
Long Pt	2	0.40000	0.35556	4	0.42857	0.69231

	Asp057			Asp066		
Sample Site	A	H_O	H_E	A	H_O	H_E
Louisiana	12	0.84615	0.84049	14	0.84615	0.88578
Mississippi 1	5	0.66667	0.83007	12	0.90909	0.93939
Mississippi 2	10	0.82609	0.81749	13	0.84906	0.89218
Missouri	9	0.72917	0.84298	14	0.87500	0.89934
Michigan	1	0	NA	5	0.55556	0.67974
Pt. Pelee	6	0.55435	0.65491	5	0.65934	0.69455
Rondeau	10	0.22899	0.37894	15	0.68269	0.79549
Long Pt	4	0.14286	0.67033	6	0.83333	0.86364

	Asp095			Asp096		
Sample Site	A	H_O	H_E	A	H_O	H_E
Louisiana	12	0.77500	0.82500	3	0.15152	0.33986
Mississippi 1	7	1.00000	0.85714	4	0.14286	0.71429
Mississippi 2	10	0.71429	0.85227	3	0.27273	0.45772
Missouri	10	0.72727	0.88671	3	0.25000	0.42179
Michigan	5	0.77778	0.71895	2	0.40000	0.35556
Pt. Pelee	4	0.54237	0.61495	4	0.40000	0.59213
Rondeau	9	0.46333	0.73391	5	0.15169	0.72727
Long Pt	2	0.40000	0.53333	2	0.33333	0.33333

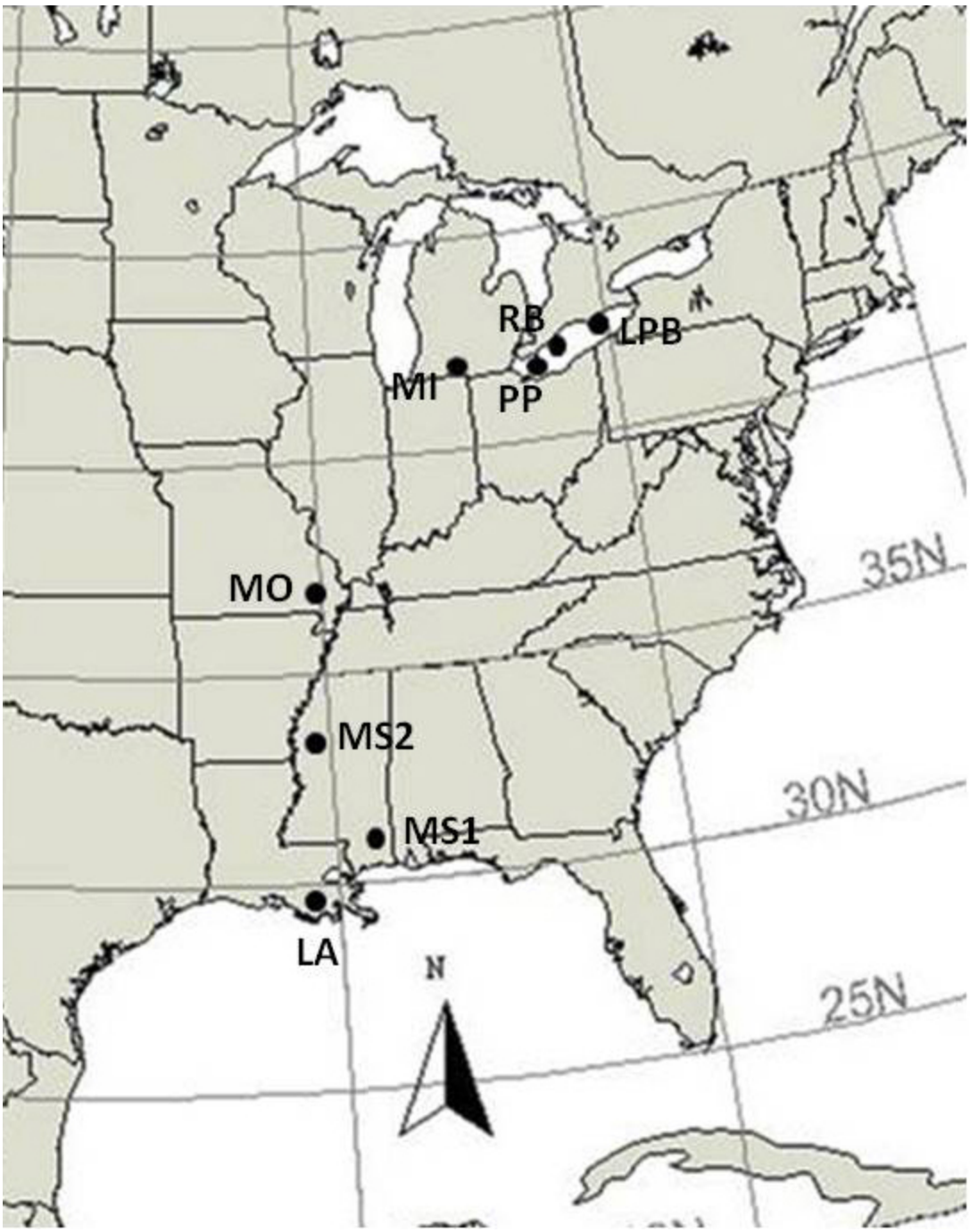

Figure 5.1 Spotted Gar sample locations, indicated by black dots on the map (see Table 5.1 for key to labels)

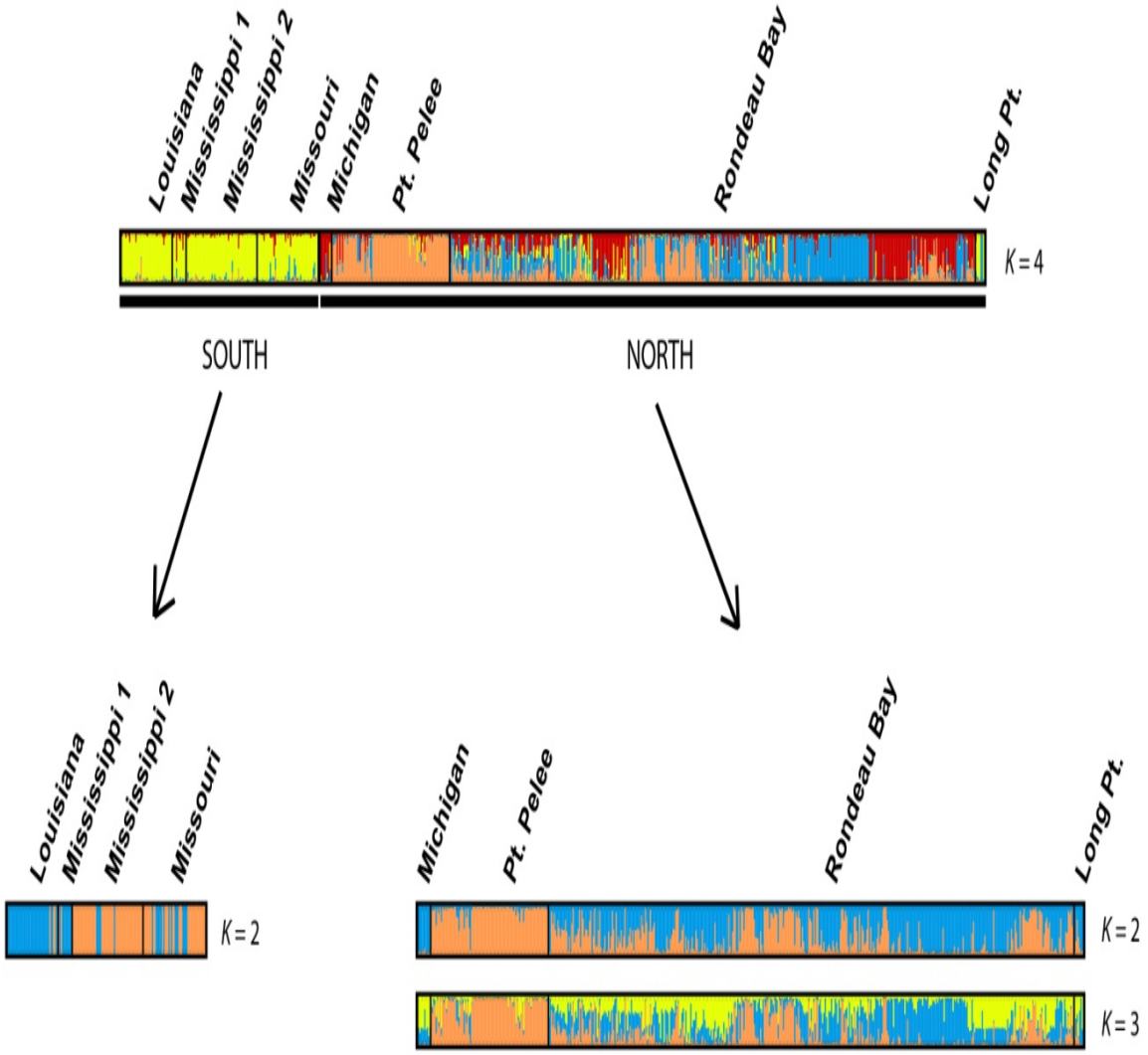

Figure 5.2 Population genetic structure of Spotted Gar determined by Bayesian clustering assignment using STRUCTURE showing all eight sampled populations throughout North America for K = 4. Southern group population substructure for K = 2 and northern group substructure for K = 2 and K = 3 are also shown.

CHAPTER 6

DNA SEQUENCES REVEAL PRESENCE OF THREATENED SPOTTED GAR (Lepisosteus oculatus) IN A CANADIAN COMMERCIAL FISH MARKET

Introduction

The sequencing of mitochondrial genes has been widely used to identify individual specimens to the species level (Hubert et al. 2008, Ward et al. 2005). Differences in mitochondrial DNA sequence have also proven useful for phylogeographic studies within a species or species group (Avise et al. 1987). Using these molecular techniques, it may be possible to identify the population of probable origin for specimens, provided there is enough diversity within the sequence among populations and that potential source populations are adequately sampled (Muirhead et al. 2008). This approach is particularly valuable where some, but not all, populations of a species are of conservation concern and the species is exploited commercially, or where the identification and origin of a commercial specimen is not readily apparent. For example, Venegas-Anaya et al. (2008) used mitochondrial DNA sequences for a phylogeographic study of the caiman (*Caiman crocodilus)*, a species that is exploited both in the pet trade and for its skin. The phylogeographic analyses of that species will help identify illegal trade and harvest of the caiman (Venegas-Anaya et al. 2008). Mitochondrial DNA sequences have also been used to identify the species of origin for whale products (Palumbi and Cipriano 1998) and seafood products (Wong and Hanner 2008) that have been sold commercially.

The family Lepisosteidae (gars) is a group of ancient fishes that arose around 110 million years ago, and contains seven extant members classified in two genera, *Atractosteus* and *Lepisosteus* (Grande 2010). All of the living species of gar are found in the western hemisphere, ranging from Central America through the Great Lakes basin, while fossil specimens have also been found in Europe, India and Africa (Nelson 2006).

Originally, all gars were grouped within a single genus – *Lepisosteus;* Wiley (1967) published the first phylogeny for the Lepisosteidae that included two separate genera. More recently, Grande (2010) published a phylogeny of all of the extant and fossil gars based on morphology. The first molecular-based phylogenies of the Lepisosteidae were created by Wright et al. (2012) using Cytochrome oxidase subunit I (CO1) and a suite of nuclear genes. Although the phylogenies based on nuclear DNA markers were not well resolved there was general agreement among molecular and morphological phylogenies (Grande 2010, Wright et al. 2012). The chief discrepancy among the phylogenies was the placement of the Shortnose Gar (*Lepisosteus platostomus*) (Wright et al. 2012) with respect to the Longnose Gar (*Lepisosteus osseus*) and the Spotted Gar (*Lepisosteus oculatus*) / Florida Gar (*Lepisosteus platyrhincus*) clade.

Two species of gar are found in Canada; the Longnose Gar is common and widespread throughout the Great Lakes region and the Spotted Gar is only found in southern Ontario (Scott and Crossman 1998). The Spotted Gar is classified as a Threatened species in Canada under the Canadian *Species at Risk Act*, and the Ontario *Endangered Species Act*, due to the species' limited distribution in Canada and the potential for habitat loss (COSEWIC 2005). Additionally, the potential exists for the Spotted Gar to be included in commercial fishing bycatch (Bouvier and Mandrak 2010), thereby increasing the prospects for Spotted Gar to find their way into commercial trade in both the aquarium and live food fish markets alongside the closely related and nearly morphologically identical species, the Florida Gar. Both federal (Section 32(2)) and provincial (Section 9(1)) acts prohibit the possession, collection, buying, selling, or trading of threatened species with only the provincial act exempting individuals obtained

from outside Ontario (Section 9(2)). Therefore, it is important to be able to confirm the species-level identification and geographic origin of any individuals found in trade. The purpose of this study is to create a phylogeny of the Lepisosteidae that can be used to effectively identify the origin of unknown commercial gar specimens and to help to resolve the placement of Shortnose Gar within the genus *Lepisosteus*.

Methods

Government agencies and academic institutions throughout the range of the Spotted Gar and other gar species were contacted to provide tissue samples. In this manner samples of a single population of Florida Gar and Shortnose Gar were received from Miccosukee Fish and Wildlife Department (Miccosukee tribe of Indians of Florida) and the Wisconsin Department of Natural Resources respectively. Spotted Gar samples were provided from two populations in Mississippi by Tulane University and Mississippi State University, as well as from single population in Tennessee by the University of Tennessee. Spotted Gar populations were sampled by the authors in Louisiana, Missouri, Michigan and Ontario. Ontario samples were collected from three populations in Lake Erie wetlands (Point Pelee, Long Point Bay and Rondeau Bay) along with a single sample from Hamilton Harbour in Lake Ontario. Longnose Gar samples were collected by the authors from a single population in Rondeau Bay. Four individual gars, three juveniles and one adult, were purchased from a pet shop in Kitchener, Ontario. All of the pet shop gars were labelled and sold as Spotted Gar. One additional commercial specimen was obtained from a live food fish market in Toronto, Ontario. All tissue

samples were preserved in 95% ethanol and DNA was extracted following the protocol of Elphinstone et al. (2003).

PCR amplification was used to amplify segments of the mitochondrial genes cytochrome oxidase subunit I (COI), the cytochrome b (Cytb) and the nuclear myosin heavy chain 6 (Myh6) gene. All PCR reactions were conducted in 25 µL volumes, with 0.5 M primer concentrations, 2.5 mM $MgCl_2$, 1X PCR buffer, 1 U of TaqPolymerase (ABI) and 100 µM of each dNTP. For COI reactions, primers COX1-2F (5´-TCGACTAATCATAAAGATATCGGCAC -3´) and COX1-2R (5´-ACTTCAGGGTGACCGAAGAATCAGAA- 3´) were used with the following reaction conditions: initial denaturation at 95ºC for 2 min, followed by 35 cycles of 94ºC for 30 s, 54ºC for 30 s and 72ºC for 1 min, with a final extension period of 72ºC for 10 min. For Cytb amplification the forward primer CYT-B-5 (5´-GGCAAATAGGAARTATCATTC-3´) and reverse primer 1RS (5´-TGACTTGAARAACCACCGTTG-3´) were used. Reaction conditions were an initial denaturation at 95ºC for 2 min, followed by 35 cycles of 94ºC for 30 s, 46ºC for 30 s and 72ºC for 1 min, with a final extension period of 72ºC for 10 min. For Myh6 amplification forward primer Myh6F459 (5´-CATMTTYTCCATCTCAGATAATGX-3´) and reverse primer Myh6R1325 (5´-ATTCTCACCACCATCCAGTTGAA-3´) were used with the following reaction conditions: initial denaturation at 94ºC for 2 min, followed by 40 cycles of 94ºC for 15 s, 54.3ºC for 30 s and 72ºC for 30 s, with a final extension period of 72ºC for 5 min. PCR products of the two mitochondrial genes were purified using Agencourt AmpPure (Beckman Coulter, Inc) as per manufacturer's instructions. Approximately 800 bp PCR products of the Myh6 gene were purified by gel extraction using the QIAquick gel

extraction kit (Qiagen) as per manufacturer's instructions. Purified PCR products were sequenced at the Genome Quebec Innovation Centre at McGill University.

COI and Cytb sequences for the three species in the genus *Atractosteus*: the Alligator Gar (*Atractosteus spatula*), the Cuban Gar (*Atractosteus tritoechus*) and the Tropical Gar (*Atractosteus tropicus*) were obtained from GenBank, along with sequences for the Bowfin (*Amia calva*) that was included as an outgroup to the gar family. Sequences were aligned and inspected using Sequencher 4.9 (Gene Codes Corp, Ann Arbor MI), and trimmed to 610 bp length for COI, 613 bp for Cytb and 744 bp for Myh6. As mtDNA is free from recombination and inherited as a single unit, COI and Cytb sequences for each individual were combined into a single longer sequence prior to phylogenetic analysis. Nucleotide model selection was conducted using likelihood scores and both AIC and Bayesian selection criteria in jModel Test (Posada 2008). Phylogenetic trees were generated using both neighbour-joining (Tamura-Nei) and maximum likelihood (HKY) methods with 1000 bootstraps in MEGA4 (Tamura et al. 2007) and PhyMLv3.0 (Guindon and Gascuel 2003).

Results and Discussion

The combined mitochondrial sequences produced a well resolved tree with uniformly high bootstrap values (Figure 6.1). The topological relationships within the genus *Lepisosteus* support the relationships shown by Wright et al. (2012), with Shortnose Gar (*L. platostomus*) grouping in a clade with Longnose Gar (*L. osseus*), in contrast to the phylogeny produced by Grande (2010) based on skeletal anatomy that had

Longnose Gar closely related to the other two *Lepisosteus* with Shortnose Gar more distantly related. This finding, along with that of Wright et al. (2012) suggests that the currently accepted topology of this genus based on skeletal morphology should be revisited to further examine the position of the Shortnose Gar relative to the other members of the *Lepisosteus* genus.

The phylogeny produced by the nuclear Myh6 gene sequences was unable to resolve the relationships within the *Lepisosteus* genus (Figure 6.2). This result was anticipated given the findings of Wright et al. (2012), where it was found that several nuclear genes including Myh6 produced varied and frequently unresolved topologies for the family Lepisosteidae. Once all seven nuclear genes were sequenced and collectively analyzed by Wright et al. (2012), the tree was resolved and the topology showed the expected relationships (apart from the placement of Shortnose Gar as mentioned previously) compared to the phylogeny based on skeletal morphology (Grande 2010). When the COI sequences were added, bootstrap values were even higher (Wright et al. 2012) than with the nuclear genes alone. This finding, along with the high bootstrap values of our combined mitochondrial tree, points to the mitochondrial sequences providing a more suitable tool for describing the relationships among this family of fishes.

Four mitochondrial haplotypes were recovered for the Spotted Gar: two exclusively in Missouri and Louisiana, a third from Missouri and Tennessee, and a widespread fourth found in Louisiana, Mississippi and throughout all of the northern (MI, ON) sampling locations. All northern caught specimens shared this widespread haplotype. The diversity of haplotypes in the southern portion of the species' range

compared to the north suggests a range contraction as a result of Pleistocene glaciation, followed by colonization of the northern range via a single successful haplotype. This pattern of reduced haplotype diversity in previously glaciated areas compared to non-glaciated regions has been documented in both fishes (Ray et al. 2006) and invertebrates (Weider and Hobaek 1997).

The results of the present study demonstrate a similar pattern of post-glacial colonization in other *Lepisosteus* species as well. Two haplotypes were recovered from Florida Gar and a single haplotype found for Longnose Gar. The Florida Gar is limited to Florida and Georgia and would not have been affected by glaciations while the Longnose Gar is the most northerly distributed of the gar species (Page and Burr 2011) and likely experienced a similar range contraction and post-glacial expansion regime as suggested for the Spotted Gar. As we included only a single population of Longnose Gar, further sampling throughout the geographic range of the Longnose Gar is needed to determine whether this is an artifact of sampling or if the Longnose Gar shows a similar pattern of post-glacial colonization as the Spotted Gar. The Shortnose Gar, sampled in Wisconsin, showed higher levels of haplotype diversity with three different haplotypes found in the single population.

The four 'pet shop' samples were found to share the most common Florida Gar haplotype, indicating that although these specimens were labelled and sold as "Spotted Gar", they were in fact Florida Gar, welcome news for the Threatened Canadian population. The live food fish market sample, however, was a Spotted Gar. This specimen shared the Spotted Gar haplotype common to all northern populations indicating that this specimen could have been collected illegally in Canadian waters.

While the population of origin cannot be unequivocally determined due to this haplotype being found in Lousiana, Michigan and Mississippi, the likely source of this individual is Long Point Bay in Lake Erie which supports a commercial trap net coarse fishery. Spotted Gar has been reported as bycatch in this live food fish industry (Gislason et al. 2010). This finding, along with the identification of Spotted Gar found in the live fish market in Toronto affirms the concern of Bouvier and Mandrak (2010) that commercial fishing is a potential risk to the Spotted Gar in Canada. Clearly, there is a need for increased education among commercial fishers with respect to species at risk, and in particular Spotted Gar, especially in areas where they may be encountered

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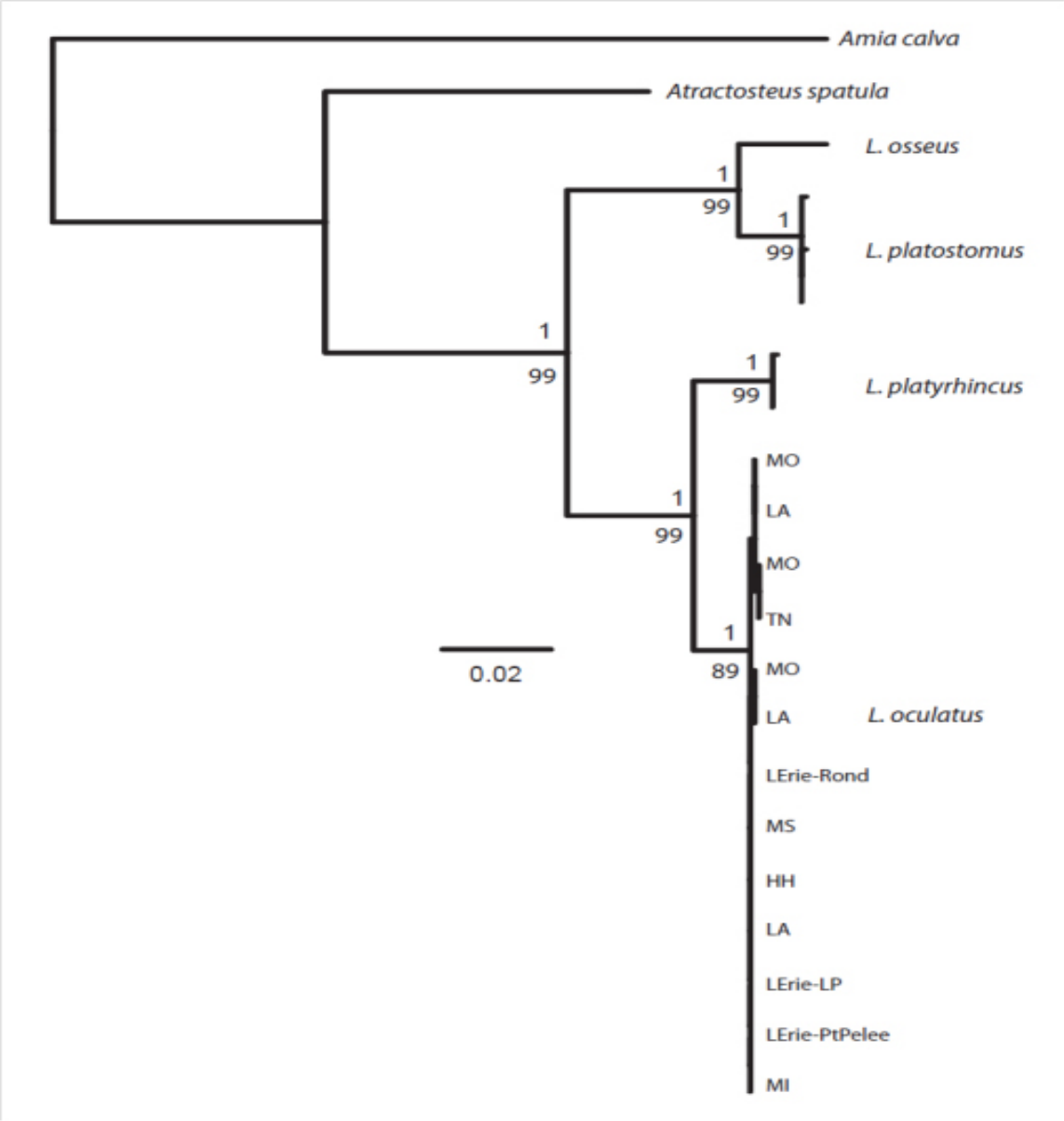

Figure 6.1 Phylogenetic tree for the family Lepisosteidae including bootsrap values for maximum likelihood (top) and neighbour-joining (bottom) tree construction methods, derived from combined COI and Cytb sequences. Population abbreviations for *L. oculatus* specimens: LErie-Rond, LErie-LP and LErie-PtPelee correspond to Ontario samples collected in Rondeau Bay, Long Point Bay and Point Pelee respectively. HH corresponds to individual collected in Hamilton Harbour of Lake Ontario. All other abbreviations correspond to the state in which specimens were collected.

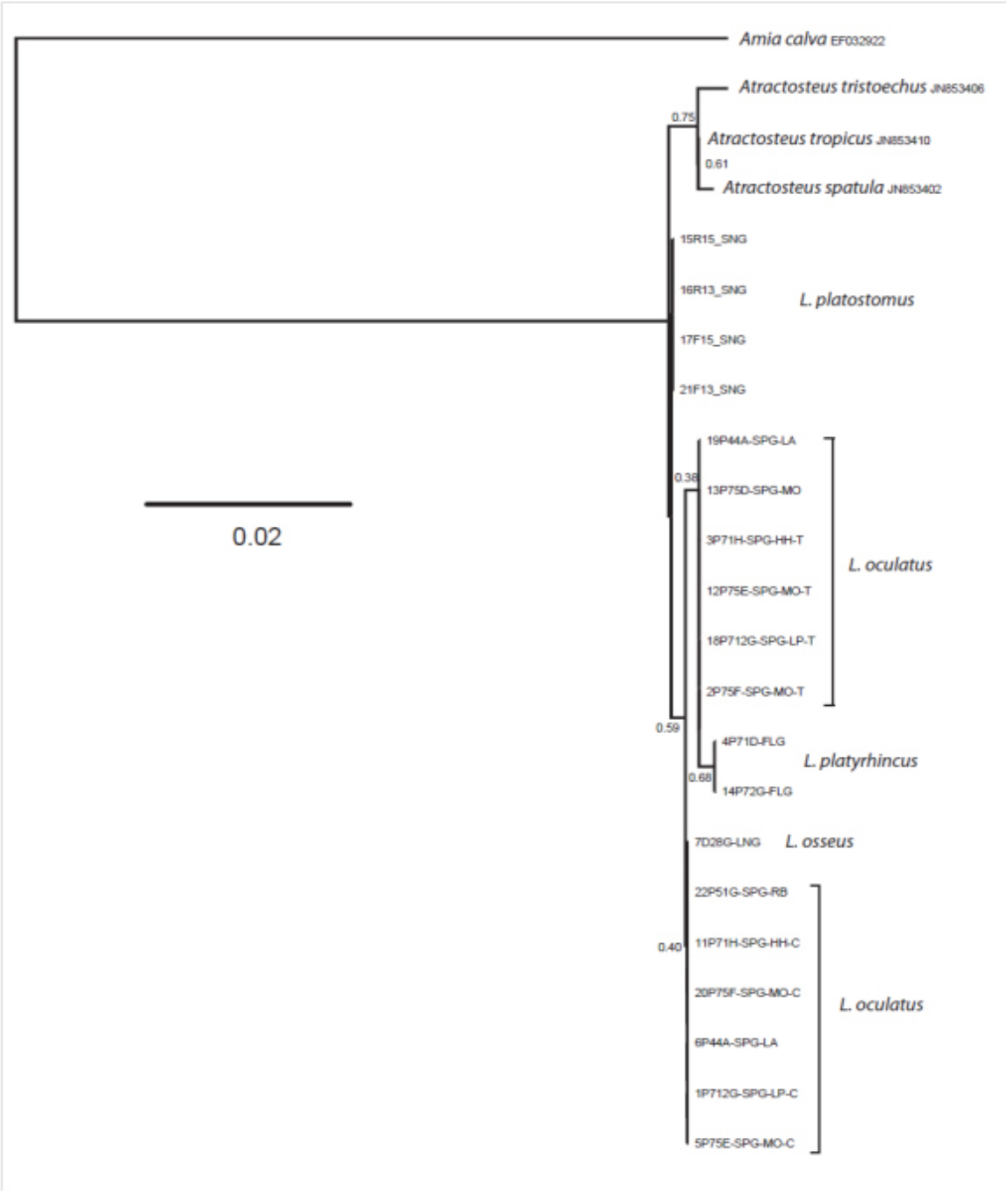

Figure 6.2 Neighbour-joining phylogenetic tree for the family Lepisosteidae derived from Myh6 sequences

CHAPTER 7

GENERAL DISCUSSION

Discussion: Summary and Implications

The conservation of populations of species that are at the edge of their range is controversial topic in the field of conservation biology. Assigning conservation priority to edge populations can be a less efficient use of conservation funds than if core populations are prioritized (Rodrigues and Gaston 2002). However, edge populations should be protected where genetic divergence is present so that the range-wide genetic diversity of the species in maintained (Reed and Frankham 2003). It has been argued that a jurisdiction has a moral obligation to protect all diversity within its boundaries, despite the conservation status of the species elsewhere (Arponen 2012). My research seeks to inform this debate by examining the freshwater fish species at risk in Canada, particularly those at the edge of their range.

Canada provides a good opportunity to examine the conservation of edge populations. Many species in North America range northward into southern Canada and are considered to be species at risk in this country. I examined the ecological and life history traits of Canadian freshwater fish species and compared the traits of species listed as at risk and those not at risk, including whether they are at the edge of their range in Canada. This analysis showed that a species at the edge of its range in Canada was more likely to be listed as a species at risk in Canada and afforded legal protection. I also found that species with endemic distributions were more likely to be listed as species at risk. The protection of endemic species is often cited as a conservation priority (Myers et al. 2000, Wilson et al. 2006).

Several arguments have been forwarded as to why the protection of species at the edge of their range is not sound conservation practice. Opponents to the protection of

edge populations argue that edge populations are naturally small due to normal meta-population dynamics leading to periodic extinctions and recolonization (Hanski 1982, Hanski and Gyllenberg 1993). Opponents also argue that edge populations are often areas of low habitat quality and act as population "sinks", and these edge populations must receive a constant flow of migrants to persist over time (Gaggiotti and Smouse 1996). Because of these reasons, protecting populations based on political boundaries, rather than ecological characteristics of the population, often results in less efficient allocation of conservation funds (Hunter and Hutchinson 1994). In contrast to the expectation of small population size for edge populations, I demonstrated in chapter 4 that the Spotted Gar population in Rondeau Bay is estimated to be over 8000 individuals. I also demonstrated the northern edge populations of Spotted Gar are genetically distinct from the southern core populations, which would not be observed if a continuous flow of migrants was present. Additionally, in chapter five, I also determined the temporal stability of the genetic structure at the Rondeau Bay site. The genetic structure within the two assigned genetic groups in Rondeau Bay was found to be stable between the years of 1998 – 2007. The investigation of the temporal stability was outlined as an urgent priority area of research in the Spotted Gar recovery strategy (Staton et al. 2012). The finding that the genetic structure at this site, along with the large number of individuals in Rondeau Bay, indicates that the Spotted Gar population should be viable at this site in the long term.

A central argument for the protection of edge populations is the protection of the range wide diversity within a species (Reed and Frankham 2003). Because edge populations are often small and isolated, they experience increased genetic drift

compared to populations at the core of the species' range (Bunnell et al. 2004). Additionally, edge populations often experience harsh environmental conditions leading to increased selection pressure compared to core populations (Case and Taper 2000). Living under these conditions, edge populations can evolve traits divergent from core populations, such as increased phenotypic plasticity (Chevin and Lande 2011). Increased genetic diversity compared to core populations may also be present (e.g. Munwes et al. 2010). Significant genetic differentiation was found across the range of the Spotted Gar. The sample sites across the southern core of the species' range clustered together as a single population, separate from the northern sample sites. Within the northern sites, the Point Pelee and the Michigan sites were divergent from each other; whereas, the Rondeau Bay site had individuals that demonstrated shared ancestry with both the Michigan and Point Pelee populations. Rondeau Bay is a particularly important site because it carries all of the diversity found in the north. Additionally, there is evidence of a shared ancestry between the Spotted Gar at the Point Pelee and Rondeau Bay sites with a lack of contemporary gene flow between them. The lack of gene flow precludes a rescue effect for the Point Pelee population. The finding that the northern edge populations of Spotted Gar are distinct from populations in the southern core of the species' range reaffirms the need to protect these northern edge populations to maintain the overall genetic diversity of the species.

Edge populations may also be locally abundant and play an important ecological role. Zalewska-Galosz et al. (2012), for example, showed that the aquatic plant *Potamogeton polygonifolius* was very abundant at the edge of its range. I demonstrated that the Spotted Gar population in Rondeau Bay is quite large, containing an estimated

number of individuals in excess of 8000, supporting the prediction of locally abundant edge populations.

Edge populations have also been shown to have more dispersive morphs compared to core populations (Phillips et al. 2007). Parmesan and Yohe (2003) described a poleward shift in species' ranges in response to climate change. Edge populations, containing more dispersive morphs and geographically positioned at the northern extent of species' distributions will be important for the colonization of habitats that become available due to climate change. The Spotted Gar is one of several species that has been predicted to increase its distribution in Canada in response to a warming climate (Mandrak 1989).

The continued protection of species at the edge of their range can be justified where populations are distinct (Jelks et al. 2008) as I have shown for the northern populations of Spotted Gar based on microsatellite analyses. Other mitigating factors specific to edge populations should also be considered. Channell and Lomolino (2000) showed that when a species declines in abundance it will tend to persist at the edge of its range, while disappearing from the core of the range. This pattern of change in distribution as a species declines in abundance is particularly apparent where human activity is a major cause in the species' decline (Channell and Lomolino 2000). This makes the protection of edge populations particularly important. In some cases, edge populations are upstream of core populations such that reverse gene flow exists from the edge to core populations. Under these conditions the edge population will have a greater impact on the genetic diversity of the species than the core populations (Pringle et al. 2011), and should be given conservation priority.

Throughout my thesis, I have demonstrated numerous local adaptations in the northern edge populations of Spotted Gar in Canada. Adaptations to life history were demonstrated in the later age at maturity and lower lifetime reproductive output in Spotted Gar from the northern edge populations. I found that there was no difference in the length at age between the Canadian population of Spotted Gar in Rondeau Bay and a population from Lake Pontchartrain, Louisiana (Love 2004), despite a similar life expectancy. The Spotted Gar in Rondeau Bay may have adapted to the shortened growing season at the northern edge of their range by increasing its summer growth rate; this adaptation was reported in the Atlantic Silverside, *Menidia menidia* (Conover and Present 1990).

Adaptations to habitat selection were also seen. By radio-tracking Spotted Gar specimens in Rondeau Bay, I found that adult Spotted Gar in the northern edge population inhabited areas farther offshore (1.77 km from shore) than in core populations. Specifically, most movement of individuals in a southern core population was within 10 m of shore (Snedden et al. 1999). I also found that adult Rondeau Bay Spotted Gar utilized different habitat than Spotted Gar in the southern population. Spotted Gar in the Rondeau Bay population preferred aquatic vegetation for cover, whereas Spotted Gar in the Atchafalaya River Basin, Louisiana, were most often associated with flooded timber (Snedden et al. 1999). The finding that the Spotted Gar in Rondeau Bay prefer macrophyte cover is also important because it will be used by the Spotted Gar recovery team to define the critical habitat of the species in Rondeau Bay (Staton et al. 2012). The protection of aquatic macrophytes in Rondeau Bay, an area that has been subjected to

macrophyte removal in the past, will be important for the survival of the Rondeau Bay population of Spotted Gar.

Genetic divergence between the northern edge populations and populations in the southern core of the species' range also was demonstrated when the northern edge populations were found to be distinct from the southern core populations. These local adaptations make it imperative to protect the northern edge populations of Spotted Gar, particularly the Rondeau Bay population which carries all of the genetic diversity present in the north. Evidence of local adaptation in edge populations shows the importance of protecting edge populations to preserve the overall diversity of species.

Several key assumptions were required to come to the conclusion that local adaptation exists in the Canadian populations of Spotted Gar compared to core populations of the species. The first assumption is that the southern populations from Louisiana with which I compared the Canadian populations are, in fact, core populations. These populations in Louisiana, though at the southern edge of the species' range may be considered to be core populations based on their high abundance, where the Spotted Gar is one of the most abundant species in the system (Snedden et al. 1999). The southern boundary of the Spotted Gar's range is delineated by the presence of the Gulf of Mexico, rather than ecologically, or environmentally, marginal conditions for the species.

Additionally, the Spotted Gar is a relatively understudied species such that studies on the ecology and life history with which to compare the Canadian populations are scarce. In fact, the studies based on Louisiana populations of Spotted Gar used for

comparison in chapters three and four are the only other published accounts of the age and growth (Love 2004), and home range (Snedden et al. 1999), respectively.

The second assumption is that the observed differences in age and growth, home range and habitat use are in fact adaptations that are heritable, rather than adaptive responses within the normal range of plasticity for these traits. To determine if the differences seen in the age and growth of Spotted Gar from the core and edge populations is the result of inherited differences or merely the result of the differing environments in northern populations persist, a common garden experiment would be beneficial. In the common garden experiment specimens from both populations are reared under the same environmental conditions. Any differences in growth could then be attributed to inherited differences, rather than environmental conditions. Because the Spotted Gar is a Threatened species in Canada, this experiment would likely have to be conducted in another jurisdiction.

Despite the assumptions made in these instances, and the downfalls of studying a Threatened species, the results of this study remain compelling. The northern edge populations of Spotted Gar are genetically distinct and geographically separated from core populations, with little or no gene flow between them. The northern edge populations are also found in unique habitats compared to core populations. These characteristics are all traits that COSEWIC uses to define designatable conservation units (COSEWIC 2010), thus the Canadian populations of Spotted Gar meet the criteria required for listing as a species at risk in Canada.

Future Research

My research has raised important questions about future research specific to the Spotted Gar. My study of the habitat use of Spotted Gar was concentrated on the spring and summer distribution of adult specimens. The habitat use during the fall and over the winter is not known for this species in Canada. Additionally, the habitat use by other life stages will be an important area of study to ensure that the critical habitat for all life stages can be protected. Additionally, the critical habitat for the Spotted Gar should be defined in both Long Point Bay and Point Pelee to ensure that enough habitat is preserved for continued survival of this species at both locations.

The pattern of post-glacial colonization by a single successful haplotype of Spotted Gar raises interesting questions given the presence of multiple potential colonizing haplotypes in the south. This pattern of post-glacial colonization has also been shown in both fishes (Ray et al. 2006) and invertebrates (Weider and Hobaek 1997). I found a single haplotype in the Longnose Gar (*Lepisosteus osseus*) population that was sampled, whereas multiple haplotypes were found in the population of Shortnose Gar, despite these species being closely related and both inhabiting northern areas. Further sampling of Longnose Gar and Shortnose Gar throughout their ranges would show whether the Longnose Gar shares the same pattern of colonization as the Spotted Gar. Further study to explain the differences in colonization pattern between the Spotted Gar and Shortnose Gar (and potentially Longnose Gar as well) would be of interest to describe the phylogeographic relationships among this group of ancient fishes and shed light on post-glacial colonization in North American fishes.

In addition, further surveys of the aquarium trade and commercial fish markets for the presence of species at risk will be important to determine the extent to which these species make their way into the commercial fish trade. Spotted Gar has previously been reported as a bycatch species in the commercial trap-net fishery in Long Point Bay (Gislason et al. 2010). If more Spotted Gar specimens or other species at risk are found in the commercial trade, the impact of this mortality should be estimated to determine the risk it poses.

Overall, I have demonstrated several local adaptations in northern edge populations of Spotted Gar. These edge populations are of high conservation importance to maintain the overall diversity and evolutionary potential and, ultimately, survival of the species (Fraser 2000).

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APPENDICES

Appendix A Variables for ecological and distributional traits of freshwater fish species in Canada

Variable	Type	Codes (categorical) / Units (continuous)
Conservation listing	categorical	0 = not listed 1 = listed as a species at risk
Edge distribution	categorical	0 = no edge distribution in Canada 1 = edge distribution in Canada
Single watershed	categorical	0 = found in more than one watershed 1 = found in a single watershed in Canada
Length at maturity	continuous	cm
Age at maturity	integer	years
Maximum length	continuous	cm
Maximum age	integer	years
Riverine	categorical	0 = does not typically inhabit riverine environment 1 = typically inhabits riverine environment
Anadromous	categorical	0 = not an anadromous species 1 = anadromous species
Stream spawner	categorical	0 = does not spawn in streams / rivers 1 = spawns in streams / rivers
Benthic juvenile	categorical	0 = no benthic juvenile stage 1 = benthic juvenile stage
Benthic adult	categorical	0 = no benthic adult stage 1 = benthic adult stage
Juvenile feeding guild	categorical	0 = filter feeding 1 = invertivore 2 = piscivore 3 = herbivore / algaevore
Adult feeding guild	categorical	0 = non-feeding adult 1 = invertivore 2 = piscivore 3 = parasitic 4 = herbivore / algaevore
Nest building	categorical	0 = adults do not construct nest 1 = adult construct nest for spawning
Human exploitation	categorical	0 = not exploited by humans 1 = exploited by humans

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PUBLICATIONS

Glass, W.R., Walter, R.P., Heath. D.D., Mandrak, N.E. and L.D.Corkum. DNA sequences reveal presence of Threatened Spotted Gar (*Lepisosteus oculatus*) in Canadian commercial fish market. Conservation Genetics. *Submitted – submission number COGE-D-12-00273*

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