Analysis of the American Mink (*Neovison vison*) Harvest Decline and Genetic Introgression

by

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GENERAL ABSTRACT

Analysis of the American Mink (*Neovison vison*) Harvest Decline and Genetic Introgression

Krista Shofstall

The American mink (*Neovison vison*) is endemic to North America where they have been domesticated over the course of 150 years by the fur industry. These domestic mink have been escaping from farms around the world and in North America while the harvests of wild mink across Canada are in decline. In this thesis, I used a combination of environmental data, spatial data, and genetics to better understand the declines. A multiple linear regression and a tree regression indicated that muskrat harvest growth rate, road density, and annual precipitation had the most effect on the mink harvest. To study the genetic introgression of domestic and wild mink, a 300 basepair fragment of the mitochondrial control region was used to determine regional differences between the wild and domestic populations of Nova Scotia and Ontario. Color differences and the direction of introgression were also studied. Significant differences between Nova Scotia wild, Ontario wild, and domestic mink were found. A pairwise Φ st test was used to determine directional introgression and resulted in an introgression of the hybrids towards the wild population. Together these results provide a better understanding of the decline in mink harvest although further research is needed to assess the direct impact of domestic escapees on the environment and on the wild population in North America. Prevention of domestic escapees is needed to stop hybridization which is important for the preservation of the species and to prevent further risk of outbreeding depression.

Keywords: American mink, domestic, harvest data, introgression, *Neovison vison*, mitochondrial DNA, Ontario, population decline

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GENERAL INTRODUCTION

The American mink (*Neovison vison* previously known as *Mustela vison*) is a member of the family Mustelidae and is endemic to North America (Wilson and Mittermeier 2009). It is sexually dimorphic; males are 10% larger and 50% heavier than females (Larivière 1999, Macdonald and Harrington 2003, Wilson and Mittermeier 2009). Male mink are 33-43cm from head-body and weigh 850-1805g, while female mink are 30-40cm and weigh 450-840g (Wilson and Mittermeier 2009). Mink have elongated bodies and are dark brown in coloration with a white patch on chin, throat, or chest (Larivière 1999, Wilson and Mittermeier 2009) but other colors may be observed (Larivière 1999).

The mink is a semi-aquatic carnivore that occurs throughout Canada and the United States except for the deserts in southwestern part of the U.S. (IUCN). It is found near small creeks, streams, rivers, lakes, wetlands, swamps, marches, and along coastal beaches (Larivière 1999, Wilson and Mittermeier 2009). Mink live in riparian vegetation and tend not to move far from water sources (Melero et al. 2008). Rarely mink can be found in areas away from water if prey is very abundant (Larivière 1999). Mink favor aquatic habitats near wood cover or dense vegetation with little to no human disturbance (Previtali et al. 1998, Melero et al. 2008). In coastal environments, mink select vegetated tidal slopes that are protected from waves and in the prairies, mink select large wetlands with large areas of open water (Larivière 1999).

American mink mate early in the year between February and April (Larivière 1999, Macdonald and Harrington 2003, Wilson and Mittermeier 2009). They have a short period of delayed implantation, up to 35 days (Macdonald and Harrington 2003). The gestation period is 39-70 days (Wilson and Mittermeier 2009) with a litter of 1-10 kits around April-May (Larivière 1999, Wilson and Mittermeier 2009). These litters are often of mixed paternity

(Macdonald and Harrington 2003). The kits are weaned after 5-6 weeks and begin to hunt at 7-8 weeks (Wilson and Mittermeier 2009). Juvenile mink disperse in the first fall (Larivi è re 1999). Females become sexually mature at 12 months and males at 18 months (Larivi è re 1999).

The American mink home range is often linear and ranges from 1 to 15.9 km, which they hold until death (Melero et al. 2008). Males typically have larger home ranges then females. Home ranges rarely overlap and never overlap over core habitat areas (Melero et al. 2008). Core habitats are areas which consist of den sites, dense vegetation, and are usually in close proximity to preferred feeding areas (Melero et al. 2008). Mink prey on birds, insects, fish, crustaceans, molluscs, amphibians, small mammals, and have been known to eat deer and even other mustelids (Shier and Boyce 2009). Mink are typically generalist predators that specialize on muskrats in areas of low prey diversity and/or in winter (Erb et al. 2001). Mink diets change according to their habitats (i.e. coast, marsh, streams), season, sex, and longitude (Erb et al. 2001, Macdonald and Harrington 2003, Shier and Boyce 2009). Mink diet also depends on the size of the individual mink because larger mink tend to take larger prey (Macdonald and Harrington 2003, Shier and Boyce 2009). There are large differences in the diet of mink between habitats. For example, along the coasts mink eat primarily crab, molluscs, and fish (Shier and Boyce 2009), whereas along lakes and ponds they eat primarily birds and amphibians (Wilson and Mittermeier 2009). In Northern Canada, mink rely on muskrat as a primary prey item (Proulx et al. 1987, Shier and Boyce 2009) and in areas where otters occur they tend to shift their diets away from fish completely (Previtali et al. 1998).

The American mink is an ecologically and economically important fur-bearer species. In North America, mink have been trapped for centuries and today they are trapped across their entire native range (Bluett et al. 2006). Canadian fur trappers established mink farms in 1866

(Hansen 1996, Kruska 1996) and now there are around 2 million domestic mink on farms across Canada (Nituch 2011). Mink farms spread to Europe in the 1920s (Macdonald and Harrington 2003), South America in the 1940s (Previtali et al. 1998), and more recently to Asia (Hau and Xu 2016). The worldwide mink fur industry produces 30 million pelts annually (Mason 2001).

In North America, mink farms are usually on good mink habitat (Joergensen 1985). The domesticated mink is kept in a long row of separate wire mesh cages with a nest box (Mason 2001). The domestic mink is provided with water and paste-like food that is placed on the top of the wire mesh cages (Mason 2001). The food contains a variety of recycled grains, eggs, fish and meats that are blended together into a food paste. Domestic mink are bred with 4-5 females to a single male between December-March (Canadian Mink Breeders Association 2016). The females are always placed into the pen of the male mink (Canadian Mink Breeders Association 2016). Gestation of domestic mink last between 40-70 days and lights are used to extend the daylight period to shorten the gestation period (Canadian Mink Breeders Association 2016)

Domestic mink are actively selected for larger size, fur quality, color uniformity (Belliveau et al. 1999), high productivity, behavior, as well as other morphological characteristics (Joergensen 1985). This intense selection for desirable traits under the mink farms uniform environmental and nutritional conditions has led to unintended morphological changes (Belliveau et al. 1999). These traits include reduced sexual dimorphism in skull size and shape (Lynch and Hayden 1995), differences in baculum size and shape (Schulte-Hostedde and Bowman unpub. data), and reduced brain size (Kruska 1996).

Mink color is selected by individual farms and market demand (Joergensen 1985, Belliveau et al. 1999). Many colors are line bred because of their recessive nature (Joergensen

1985, Kidd et al. 2009). The black fur color is a result of intense selection from darker brown fur (Joergensen 1985, Belliveau et al. 1999). To explore new colors and mink traits, the mink are exposed to line breeding and positive assortative mating (Belliveau et al. 1999). This intense selection for color phases has favored various mutations and a decline in reproductive performance (Belliveau et al. 1999). The number of kits a domestic mink has varies with color (Canadian Mink Breeders Association 2016). The color of domestic mink affects behavior and likely affects other fitness traits.

The artificial selection of captive populations can result in evolutionary divergence between wild and domestic individuals (Norén et al. 2005). Domesticated animals readily escape from farm environments (Naylor et al. 2005, Kidd et al. 2009, Norén et al. 2009) and large-scale introduction of domesticated animals has affected natural populations around the world (Wiseman et al. 2000, Macdonald and Harrington 2003, Naylor et al. 2005, Bonesi and Palazon 2007, Norén et al. 2009, Randi 2008, Nituch et al. 2011, Beauclerc et al. 2013). Feral mink have caused dramatic ecological effects in Europe and South America. The ecosystem changes caused by feral mink are well documented across Europe and South America (Previtali et al. 1998, Macdonald and Harrington 2003). In most areas where escaped domestic American mink occur they are associated with conservation problems (Macdonald and Harrington 2003, Bonesi and Palazon 2007).

Feral mink have greatly affected native prey species and are hindering the conservation of some endangered species (Macdonald and Harrington 2003). Feral mink in Europe have been the sole cause for the water vole extinction in Scotland, and mink are negatively affecting the nesting success of many ground nesting/burrow nesting birds (Macdonald and Harrington 2003, Bonesi and Palazon 2007). They are also a major concern for the conservation of endemic birds

and rodents in Argentina (Macdonald and Harrington 2003) and have caused severe declines in amphibian populations (Carlsson et al. 2010). Feral mink are also competing with native mustelid species such as polecats (*Mustela putorius*), and European otters (*Lutra lutra*), and may have contributed to the dramatic decline of the endangered European mink (*Mustela lutreola*; Previtali et al.1998, Lodé et al. 2001).

The ecological effects of escaped domestic mink in North America have been understudied or overlooked entirely (Nituch et al. 2011). Several recent studies have shown that domestic mink hybridize with wild mink (Kidd et al. 2009, Beauclerc et al. 2013, Bowman et al. 2017) and domestic mink can transfer Aleutian mink disease virus (AMDV) to the wild population (Nituch et al. 2011). There are no data to suggest that feral mink or hybrid mink have diets that are different than wild mink (Shier and Boyce 2009). The domestic mink has undergone behavioral changes to become tamer (Joergensen 1985) and there is no evidence that escaped mink behave differently when they are in the wild. However, when studying wild mink, escaped mink and mink ranches are often not taken into account and this may result in conflicting or misleading inferences about mink ecology.

Domestic escapees must also be taken into account when assessing harvest data of wild mink because the mink harvest is supplemented by escaped domestic mink (Bowman et al. 2007). It is unclear whether wild mink were ever abundant on the Canadian landscape or whether the seemingly abundant wild mink have been domestic escapees over the last 100 years (Bowman et al. 2007). Bowman et al. (2007) also suggest that the wild mink population had a historic decline but were possibly unable to fully recover because of the presence of domestic mink. A steady influx of escaped domestic mink into the wild decreases fitness of the wild population and could lead to a decline in the wild population (Randi 2008, Zalewski et al. 2010).

One of the objectives of this thesis is to more fully understand the mink harvest decline in Ontario. In order to understand this decline, I examined the effects of mink farms and other environmental variables to determine their effects on the mink harvest in each Wildlife Landscape Zone (WLZ).

The other objectives of this study were to determine what mitochondrial haplotypes occur in Ontario and Nova Scotia and whether the different groups (wild or farm) could be differentiated using these haplotypes. If these different groups could be differentiated the direction of introgression could be tested. The direction of introgression is relevant to conservation because of the possibility of outbreeding depression (Wiseman et al. 2000).

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CHAPTER 1: Decline of American mink (*Neovison vison*) harvest in Ontario Canada.

ABSTRACT

The American mink (*Neovison vison*) is harvested across Canada. This harvest has been in decline but the cause is unknown. There are several possible reasons for the mink harvest decline including a decline in the muskrat populations, climatic changes, habitat disturbance, and mink farm density. I hypothesize that the mink harvest decline in Ontario is caused by a combination of factors related to food source decline, trends in mink farms, and environmental changes. The harvest data were converted to a growth rate and the selected variables were z-transformed and analyzed with both multiple linear regression and tree regression. The multiple linear regression associated muskrat harvest growth rate and road density with the mink harvest growth rate, suggesting a positive association between the number of muskrat and mink, and a negative association between road density and mink harvest. The tree regression showed that the areas where mink harvest is the greatest are areas that have low road density and high annual precipitation. Mink farm density was not significant but should not be disregarded as it still may be an important factor. The largest declines in mink harvest were in northern Ontario closely followed by southern Ontario. Central Ontario had the least decline in the harvest and even showed some harvest growth. Habitat management such as water level regulations and road mitigation strategies are needed to stop the decline of muskrat and mink.

Keywords: American mink, common muskrat, harvest data, *Neovison vison*, mink farms, multiple linear regression, Ontario, population decline, roads, tree regression.

INTRODUCTION

The American mink (*Neovison vision*) is harvested for their fur in almost every state and province across their native range (Bluett et al. 2006). Wildlife managers use these harvest data as an index of population status (McDonald and Harris 1999, Bluett et al. 2006, Bowman et al. 2007). However, harvest data are considered to be an indirect estimate of population size and therefore may be inaccurate and lacking in precision (Brzeziński et al. 2010). Harvest data may be affected by trapping effort and differences in effort can be caused by changes in fur price, hunting management such as bag limits, or even weather (McDonald and Harris 1999). The quantitative methods to determine the relative or absolute abundance of species across large spatial scales are lacking (Bluett et al. 2006) but for fur-bearer species, harvest data can be used to determine the general population trends (Bluett et al. 2006, Bowman et al. 2007, Erb et al. 2001, Holmengen et al. 2009, Shier and Boyce 2009, Viljugrein et al. 2001). Harvest data have been shown to be a reliable index of population cycles (Viljugrein et al. 2001), and long-term population trends (Carlsson et al. 2010). Though a consideration of trapping effort when using large-scale harvest data is important as trapping effort changes overtime (McDonald and Harris 1999).

The current mink harvest decline in Canada is evident in these data (Bowman et al. 2007) even with the supplementation of escaped domestic mink from fur farms (Bowman et al. 2007, Zalewski et al. 2010). Most of the mink declines across North America have been attributed to contamination by organochlorine chemicals or mercury in the waterways to which mink are extremely sensitive (Osowski et al 1995). These contaminants affect mink reproductive success and, if in high enough concentrations, can lead to poisoning (Osowski et al. 1995). In Ontario, this currently does not seem to be the case (Bowman et al. 2007, Gorman 2007). However, it is

possible that water contamination could have caused widespread historic declines in the wild mink population prior to 1968 (Bowman et al. 2007). Several possible contemporary factors affecting the mink harvest decline in Ontario include the decline in the muskrat harvest, climatic changes, habitat disturbance, and mink farm density.

Common Muskrat

The common muskrat (*Ondatra zibethicus*) is an important prey item for mink (Viljugrein et al 2001, Shier and Boyce 2009). Muskrat harvest in eastern North America has declined by 75% over the last 30 years (Roberts and Crimmins 2010) and along Lake Ontario muskrats have a very low population density (Greenhorn et al. 2017). The importance of muskrat in the mink diet depends on the availability of other prey, water levels, and habitat type (Proulx et al. 1987, Erb et al. 2001, Shier and Boyce 2009). External factors, such as precipitation, affect mink and muskrat populations similarly (Holmengen et al. 2009) and water levels are of extreme importance to the survival of muskrats and this could be similar for mink (Bellrose and Low 1943).

Habitat

The most important habitat characteristic for mink is water. American mink do not go very far from water sources such as wetlands and waterways (Melero et al. 2008, Hodder et al. 2017). During winter many waterways and wetlands freeze and this tends to restrict mink to unfrozen riparian areas in the home range (Hodder et al. 2017).Water level and changes in the water level have both direct and indirect impacts on this species and can be seasonal (Bellrose and Brown 1941, Kroger 1973). Changes in water levels affect prey availability for mink (Proulx et al. 1987) and management of water levels can reduce the abundance of muskrats (Greenhorn

et al. 2017). Dams can create rapid fluctuations in the water level that can alternatively expose or submerge portions of riverbeds (Bellrose and Low 1943, Kroger 1973). Severe fluctuations in water level affect the survival of surrounding animal and vegetative species (Bellrose and Brown 1941, Bellrose and Low 1943).

The vegetation around a water source is important for mink (Racey and Euler 1983, Previtali et al. 1998, Melero et al. 2008) because mink require dense areas of cover near waterways for protection from weather, denning and protection from competitors such as martens and otters (Racey and Euler 1983, Previtali et al. 1998, Hodder et al. 2017). Riparian vegetation is removed when lands nearby are developed or roads are built (Racey and Euler 1983). Vegetation can also be removed by flooding regimes or other natural disasters such as windstorms (Racey and Euler 1983). Human activity has an effect on vegetation but the activity also affects mink ranges and use of core habitats (Melero et al. 2008).

Latitude and Longitude

Spatial location has an effect on the diet and habitat availability of mink (Erb et al. 2001). In Canada, prey richness is larger in the southeastern part of the country and coastal mink have a larger proportion of fish and crustaceans in their diet (Holmengen et al. 2009). In northern latitudes mink prey almost exclusively on muskrats (Proulx et al. 1987, Shier and Boyce 2009). Latitude in Ontario is also a predictor for probability of domestic mink occurrence (Beauclerc et al. 2013). South of latitude 43.13^{o}N, all mink sampled are most likely to be domestic individuals (Beauclerc et al. 2013).

Mink farms

In Ontario, many mink are known to escape from farms (Bowman at al. 2007, Kidd et al. 2009, Beauclerc et al. 2013) and it is assumed that their diet is the same as that of the wild mink, although there is currently no data to support this (Shier and Boyce 2009). Mink farms in North America are usually located in good mink habitat and there has been a positive correlation found between the density of mink farms and the number of mink trapped per year (Bowman et al. 2007). The escapees have been found to remain close to the mink farm (Beauclerc et al. 2013). The territory size and dispersal ability of feral mink is closely related to the size and density of mink farms (Hua and Xu 2016).

Feral mink have caused dramatic ecological effects in Europe and South America, which may be happening in North America. However, it may be less obvious because this is the American mink's native range (Beauclerc et al. 2013).The ecosystem changes caused by feral mink are well documented across Europe and South America (Previtali et al. 1998, Macdonald and Harrington 2003). Feral mink have greatly affected native prey species such as the water vole (*Arvicola* spp.), avian prey such as the coot, and several eider duck species (Macdonald and Harrington 2003). They have also led to major concern for the conservation of endemic birds and rodents in Argentina (Macdonald and Harrington 2003). Feral mink in Europe have been the sole cause for the water vole extinction and are negatively affecting the nesting success of many ground nesting birds (Macdonald and Harrington 2003). These feral mink are also competing with native mustelid species such as polecats (*Mustela putorius*), European otters (*Lutra lutra*), and are responsible for the dramatic decline of European mink (*Mustela lutreola*; Previtali et al.1998). A steady influx of escaped domestic mink into the wild decreases fitness of the wild population and could lead to a decline in the wild population (Zalewski et al. 2010)

The effects of mink farms and domestic mink on the wild population in North American are largely unknown (Bowman et al 2007). Domestic mink make up a fairly large proportion of the wild population captured (Kidd et al. 2009, Beauclerc et al. 2013, Bowman et al. 2017), and mink farms and escaped domestic mink help facilitate the spread of the Aleutian mink disease virus (AMDV; Nituch et al. 2011, Nituch et al. 2012) introducing a higher risk of potential pathogen transfer to the wild mink population (Bowman et al. 2017). The spreading of AMDV from domestic mink to wild mink may be a cause of the decline in the wild population (Nituch et al. 2011). This disease can also be transferred to other species which has potential negative effects for the wildlife communities surrounding the farms (Nituch et al. 2015).

Given this information, I hypothesized that the decline in mink harvest in Ontario is caused by a combination of factors related to muskrat population decline, and density of mink farms. I predict where muskrats harvests are in substantial decline, mink harvest will also be in decline. I also predict that I can detect an effect of mink farms on mink harvest trends in neighboring areas.

METHODS

Harvest Data and MNR Zones

Harvest data for American mink and muskrat were used to create an index of population trends under the assumption that variation in trapping effort was spatially uniform across Ontario. While temporal variation in effort was certainly present due to changing prices, I obscured this effect by pooling data across years. I assumed that remaining spatial variation was due to environmental factors rather than spatial variation in effort. Harvest data were provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF). The data consisted of the

number of individual animals captured by commercial trappers per year and summarized across provincial Wildlife Landscape Zones (WLZ). The trapping records were analyzed from 1981-2010, with the 1986, 1989, and 1991 years missing. The growth rate of mink harvest (mink) and the growth rate of muskrat harvest (muskrat) were calculated per year ($r= \ln(N_{t+1}/N_t)$) and the average change in growth rate was taken for each of the 35 WLZs to remove the temporal effects of trapping effort. WLZ boundaries were provided by the OMNRF and projected in North America Lambert Conformal Conic.

Environmental Variables

Temperature and precipitation data were used to determine changes that can affect mink habitat over time. The data were provided by the OMNRF and included the Annual Mean Temperature (AMT), Minimum Temperature Coldest Period (MinTCP), Mean Temperature Coldest Quarter (MTCQ), Precipitation Coldest Quarter (PCQ), Annual Precipitation (AP), and Growing Degree Days (GDD). Climate normals (1980 to 2010) were summarized for each of the 35 WLZs. A correlation matrix was calculated using Excel to determine correlation coefficients and exclude highly correlated environmental variables from the mink harvest growth rate model.

Road Density

Road density was used as a proxy for development and human disturbance. To calculate road density, a road layer from 2011 was obtained from the scholars-geoportal on the Laurentian University library website (http://geo1.scholarsportal.info/). The road layer was transformed into North America Lambert Conformal Conic then merged with the WLZ layer. Roads along the waterline had to be selected by aerial reconnaissance (lasso) in ArcMap 10.4.1 and added to the road lengths per zone. The road lengths were used to calculate road density per zone by dividing road length by zone area in km^2.

Latitude and Longitude

Latitude and Longitude were calculated on WLZ layer in North America Lambert Conformal Conic by taking the centroid of each WLZ using the features to point data management tool in ArcMap 10.4.1.

Mink Farm Density

Mink farm data were received from the OMNRF which included the number of farms per census division from 1986-2001 from the Canadian census. Farms per county were averaged over the 4 census years (1986, 1991, 1996, 2001) then divided by county area. The county area was calculated in ArcMap 10.4.1. using a North America Lambert Conformal Conic projection (Statistics Canada). The census division and WLZ was overlaid and merged to calculate the area of the census division that lay within each zone. Then a weighted average was calculated from the mink farm density per census division by multiplying the mink farm density per census division by the percent of the census division area in each zone. The percent of the census division area in the zone was summed for each zone to get the weighted average of mink farms per zone.

Statistical Analysis

A second correlation matrix was conducted in Excel and highly correlated variables removed from regression models. The variables were z-transformed and two types of regressions were run in R (R Core Team 2016), a multiple linear regression and a tree regression using package tree (Ripley 2016). A multiple linear regression is a commonly used method for spatial models of multiple variables (Patriche et al. 2012) and is used for hypothesis testing. A tree regression identifies optimum separation points within predictor variables (Patriche et al 2012).

Then separates into a number of groups what is characterized by the maximum internal homogeneity and maximum external differentiation (Patriche et al. 2012).

RESULTS

The harvest of American mink in Ontario declined (Figure 1.1, Figure 1.2) across all zones except for one which had a slightly positive growth rate of 0.005 (Figure 1.2). The environmental variables were highly correlated (0.73-0.99 r; Table 1.1). Two environmental variables were selected (annual precipitation (AP) and growing degree days (GDD)) for the regression models. These two variables were selected because there was a high correlation (r>0.96) with the other environmental variables which were excluded. Latitude was highly correlated with other variables and thus was excluded from this study (Table 1.2).

I used a multiple linear regression and tree regression to determine which of these variables (AP, GDD, muskrat, farm density, road density, and longitude) had an effect on the mink harvest growth rate (mink). The multiple linear regression was significant ($F=7.237$, d.f.=6 and 28, $p=<0.001$, $R^2=0.52$; Table 1.3). The regression slope for muskrat was 0.408 indicating that the greater number of muskrat was associated with a greater number of mink ($t=2.860$, $p=0.008$). Road density had a negative coefficient of -0.393 and this indicates that the greater density of roads was associated with fewer mink ($t=-1.730$, $p=0.095$). Annual precipitation ($t=1.400$, $p=0.173$), growing degree days ($t=0.097$, $p=0.923$), farm density ($t=0.511$, $p=0.613$), and longitude ($t=-0.036$, $p=0.971$) did not have a significant effect on the mink harvest.

The tree regression showed slightly different results (Figure 1.3). This could be because a tree regression represents non-linearities in the data better than the multiple linear regression (Patriche et al. 2012). Muskrat harvest growth rate (Figure 1.4), annual precipitation (Figure 1.5), and road density (Figure 1.6) were found to have the most effect on mink. The first split was in

the muskrat data where z<-1.174 (<-0.169 muskrat harvest growth rate) very low mink numbers occurred (z=-1.796, -0.241 mink harvest growth rate). For z>-1.174 (<-0.169 muskrat), mink was associated with AP and road density (Figure 1.3). The best areas for mink were areas with low road densities (z<-0.138, <0.438 km^2 road density) and high AP (z>-0.177, >791.497 mm AP). Low road density and high AP was most prominent in central Ontario (Figure 1.7).

DISCUSSION

American mink harvests in Ontario declined over the assessment period. I hypothesized that mink harvest decline in Ontario was caused by a combination of factors related to food source decline, density of mink farms, and environmental changes and this was partially supported by the results. The mink harvest decline was found to be affected by a combination of different factors including muskrat harvest growth rate, annual precipitation (AP), and road density.

Common Muskrat

Muskrat harvest growth rate had the largest affect in both models for the decline in the mink harvest growth rate. The initial prediction was supported by the results, where muskrat harvests are in substantial decline mink harvest were also in decline. This was observed in northern Ontario and in one zone in southern Ontario where the muskrat rate was in a substantial decline (<-0.169 muskrat harvest growth rate). Some independent evidence suggests that muskrat populations have declined in Ontario (Greenhorn et al. 2017). However, the relationship between mink and muskrat harvest may also be due to trapper effort and lack of reporting. This result does reflect the results from previous studies that in specific regions, such as northern Ontario, mink become a specialist predator and respond rapidly to the fluctuations in the muskrat populations (Erb et al. 2001). The muskrat is important for the winter survival of mink,

particularly at northern latitudes (Proulx et al. 1987, Shier and Boyce 2009). In southern Ontario the muskrat is less prevalent in the mink's diet, which has been associated with greater prey species richness in that area (Shier and Boyce 2009). Muskrat are important for the winter diet of mink (Shier and Boyce 2009).

Environmental Variation

The second split in the tree regression was annual precipitation whereby an annual precipitation below 705.78 mm affected mink in northern Ontario and was associated with the second greatest decline in mink. An annual precipitation greater than 705.78 mm but less than 791.5 mm affected mink in north central Ontario whereas an annual precipitation above 791.5 mm affected the mink in central Ontario; this area had the least decline in the mink rate suggesting that precipitation is very important for the survival of mink. Fluctuations in precipitation can cause disturbances in mink habitat (Viljugrein et al. 2001). Severe fluctuations such as drought reduce the amount of habitat available (Viljugrein et al. 2001) and lowers water levels dramatically (Bellrose and Brown 1941). Water is a very important habitat characteristic for mink which tends not to go very far from water sources (Melero et al. 2008, Hodder et al. 2017).

Roads

Roads were found to affect the mink harvest growth rate in both models. The highest road density in Ontario is in the south. The tree regression showed that a road density greater than 0.438 roads/km^2 affected the mink rate more negatively than if the road density was less than 0.438 road/km^2. Mink tend to use habitats that are the least disturbed by humans (Previtali et al. 1998). A relatively small increase in development has been shown to cause rapid declines of

mink activity (Racey and Euler 1983). Southern Ontario showed very high road densities and a single road could impact populations up to 2 km away (Findlay and Bourdages 2000).

Mink Farms

Mink farms in our models had little to no effect on the mink harvest growth rate. This could be because the harvest data included all wild caught mink including escaped domestic mink. The amount of domestic mink in the wild is a problem and actions should be taken to prevent domestic escapes. Thirty-six percent of all wild captures in southwestern Ontario were domestic mink and another 28% were domestic-wild hybrids (Beauclerc et al. 2013). An earlier study found that upwards of 78% of all wild caught mink in southern Ontario were of domestic origin (Kidd et al. 2009). These domestic individuals could have vastly negative impacts on the wild population and could have affected the decline of the wild mink population (Bowman et al. 2007). These escaped domestic mink could be detected in the harvest data (Bowman et al. 2007). As the density of farms increase the more domestic mink will supplement the harvest data but as domestics increase this may have a directly negative impact on the wild population through increased competition, disease, and hybridization (Bowman et al. 2007).

This problem in the harvest data is further compounded by the decrease in the density of mink farms over the last 30 years. Declines in farms can also affect the harvest data by lessening the number of escapees in the data that would be seen as a decline (Bowman et al. 2007). The wild mink could still be under the negative impact of previous escapees and the decline in the wild populations may be more severe than suggested by the harvest data.

Mink farms were not an important factor affecting the mink harvest growth rate in this study. However, mink farms should not be disregarded and they may still be having a direct or indirect impact on wild mink population that was hidden in this study by the supplementation of

domestic mink and the decline in the number of mink farms. Mink harvests are supplemented by domestics though the amount of escapes is difficult to calculate because of under reporting by the farms. Bowman et al. (2007) calculated that domestic mink increase the harvest by 4.7% per year. Even with this supplementation mink harvest growth rates are in decline throughout Ontario. This was also observed in a study looking at the impacts by feral mink on wild mink across Canada (Bowman et al. 2007).

The areas where the mink growth rate was highest were in areas that had low road density and high annual precipitation but it must be noted that these areas have no mink farms. Further research is needed to assess the direct impact of domestic escapees on the environment and on the wild population. Declines in the muskrat harvest growth rate, change in annual precipitation, and high road density have significant effects on the mink harvest growth rate. Habitat management strategies such as better water level management and mitigation of roads are needed to stop the decline of muskrat and mink populations.

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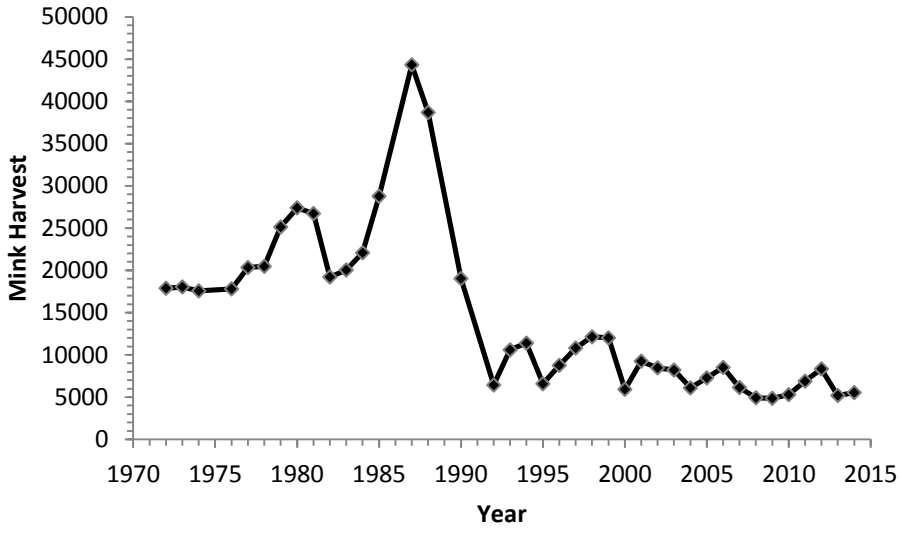

Figure 1.1. Total American mink (*Neovison vison*) harvest in Ontario from 1972-2014.

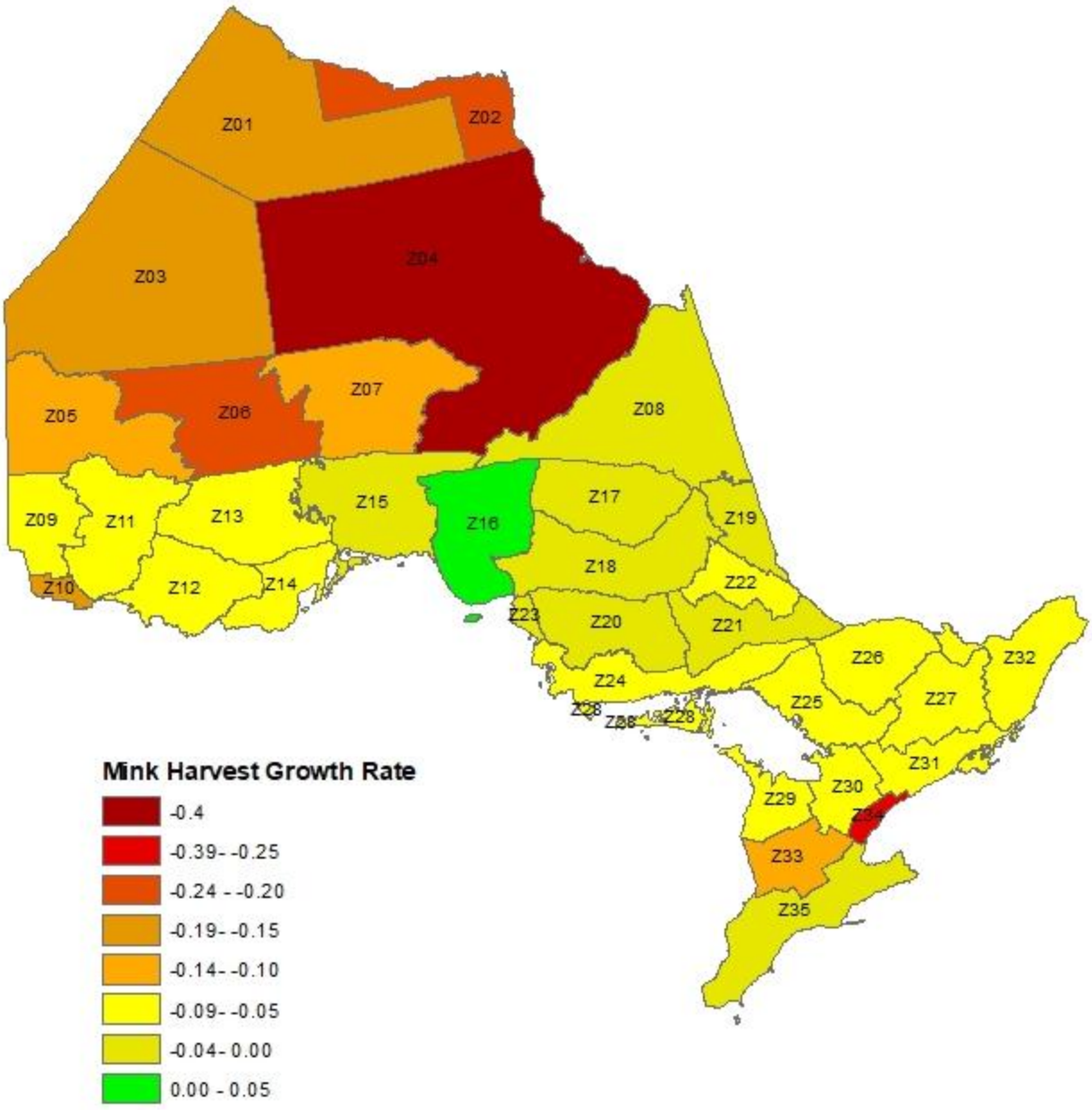

Figure 1.2. Ontario Ministry of Natural Resources and Forestry (OMNRF) Zone map of Ontario in North America Lambert Conformal Conic shows the American mink (*Neovison vison*) harvest growth rate per individual zone from 1981-2010.

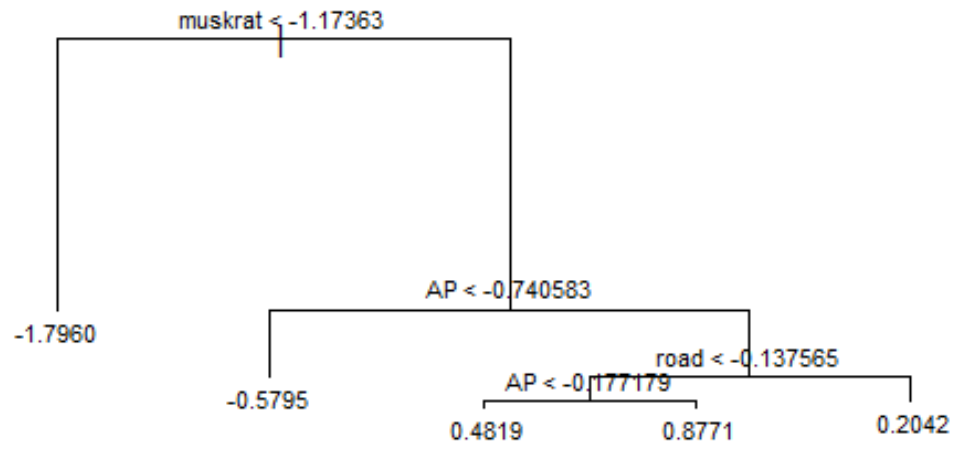

Figure 1.3. Regression tree of the probability of decline of American mink (*Neovison vison*) harvest growth rate in Ontario from 1981-2010. Muskrat is the muskrat harvest growth rate, AP is annual precipitation, and road is the road density value. All values shown were z-transformed. The values at each terminal node (branch tips) are the average mink harvest growth rate which coincides with the variable splits above the terminal node.

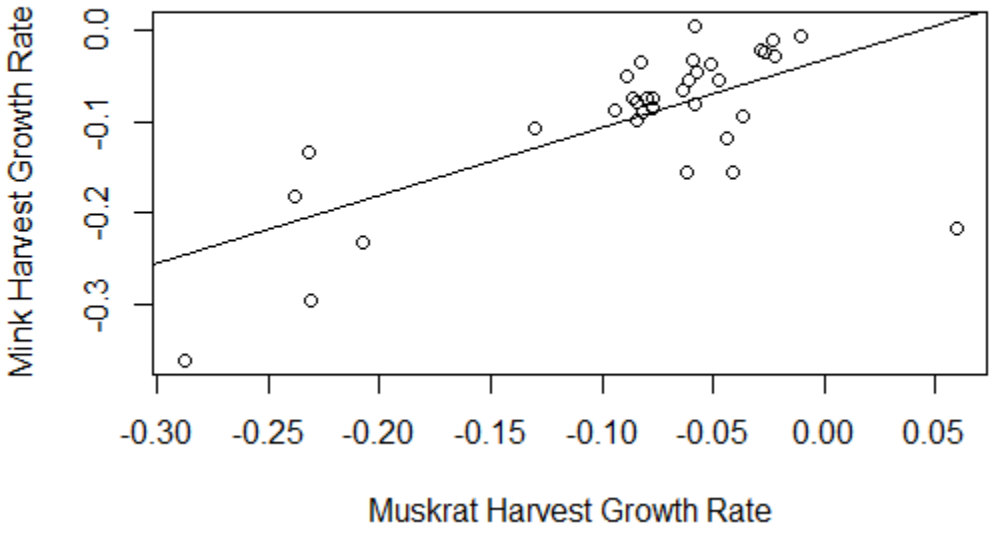

Figure 1.4. Scatter plot of harvest data from 1981-2010 between American mink (*Neovison vison*) harvest growth rate and common muskrat (*Ondatra zibethicus*) harvest growth rate in Ontario (R^2=0.424).

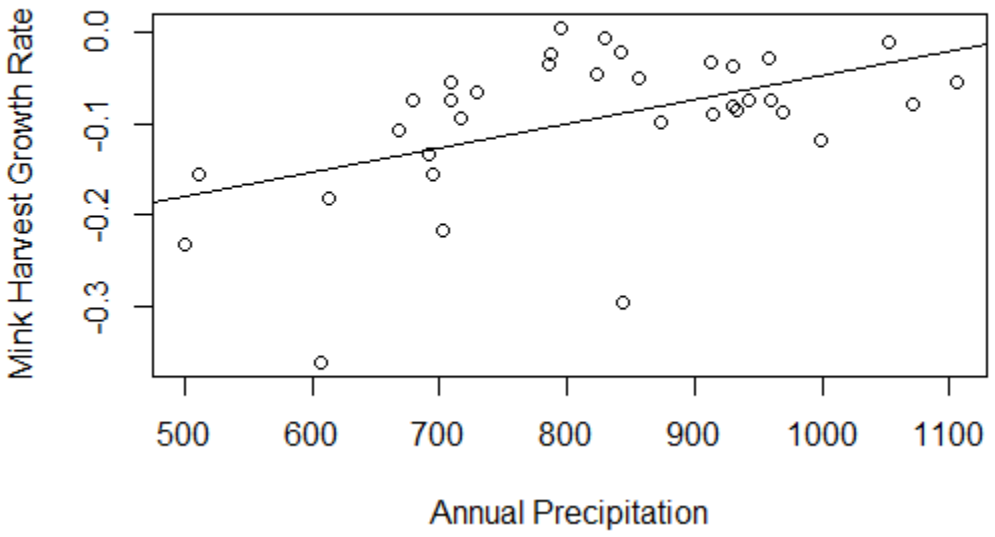

Figure 1.5. Scatter plot of harvest and environmental data from 1981-2010 between American mink (*Neovison vison*) harvest growth rate and annual precipitation in Ontario (R^2=0.224).

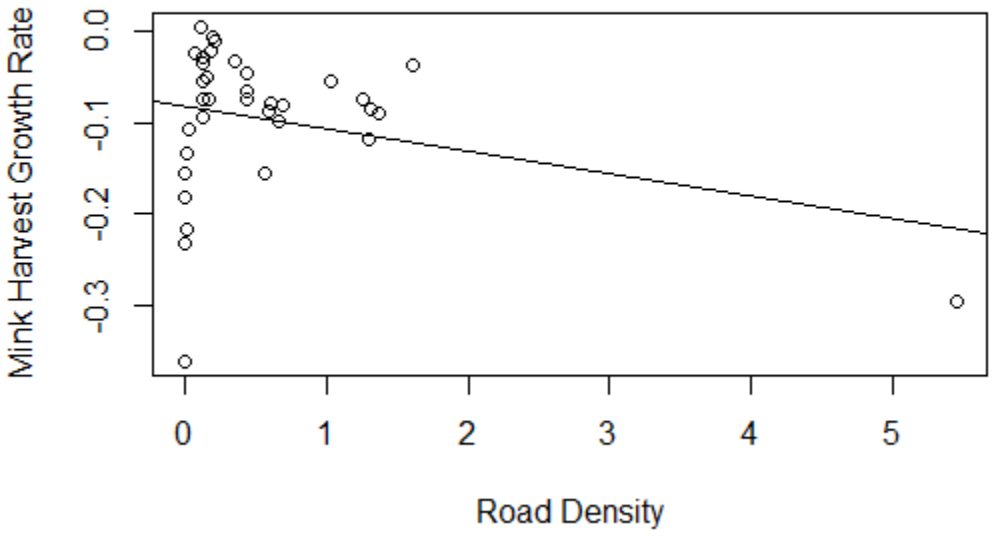

Figure 1.6. Scatter plot of harvest data from 1981-2010 and Ontario road density from 2011 between American mink (*Neovison vison*) harvest growth rate and log road density (R^2=0.059).

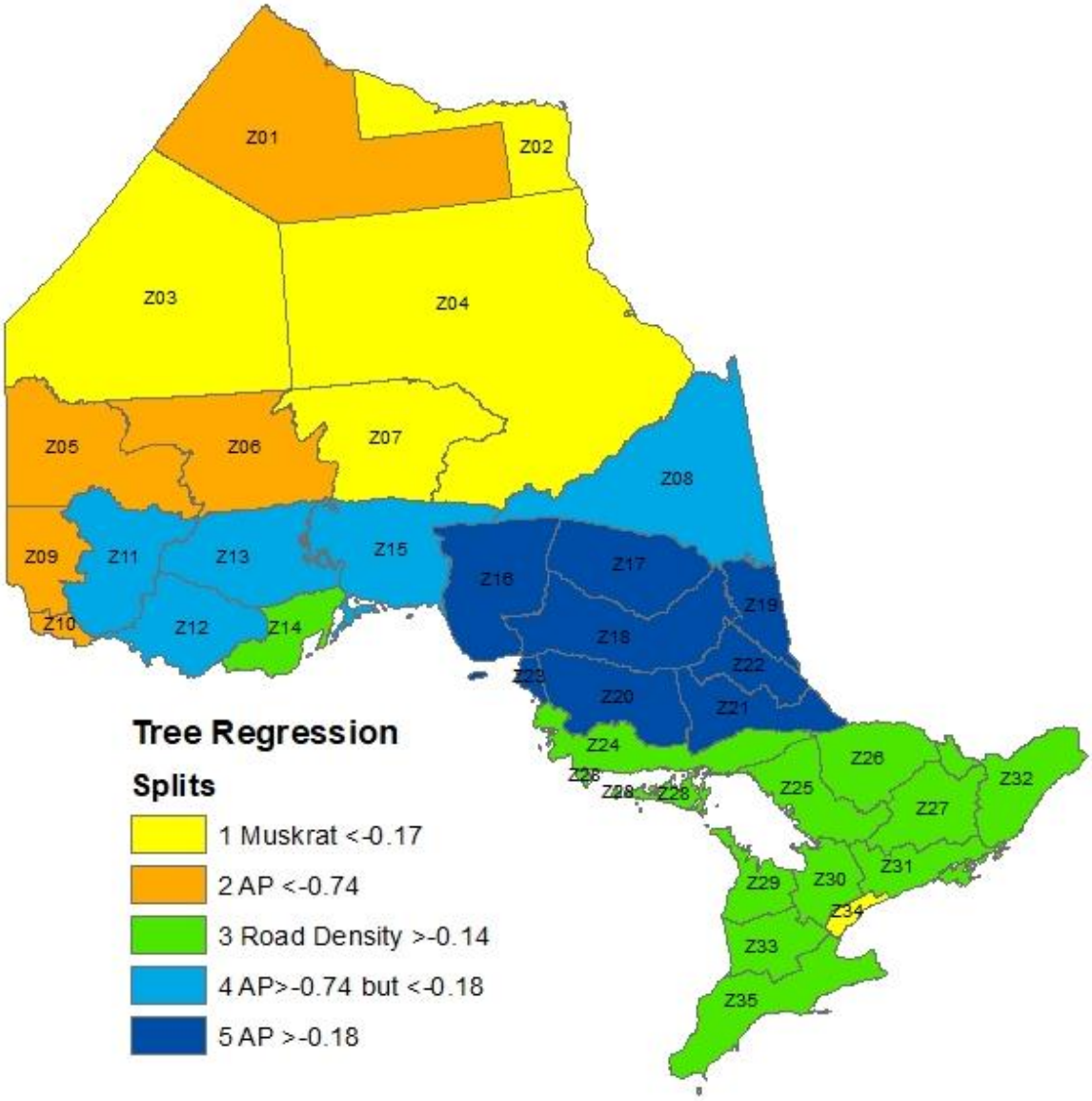

Figure 1.7. A visual representation of the regression tree of the areas that were separated at each variable separation split using the Wildlife Landscape Zones (WLZ) map of Ontario. The data were z-transformed. Muskrat is the muskrat harvest growth rate. AP is annual precipitation (mm) and road density (km^2).

Table 1.1. Correlation matrix of environmental variables for the Wildlife Landscape Zones (WLZ) in Ontario. The environmental variables are Annual Mean Temperature (AMT), Minimum Temperature Coldest Period (MinTCP), Mean Temperature Coldest Quarter (MTCQ), Precipitation Coldest Quarter (PCQ), Annual Precipitation (AP), Growing Degree Days (GDD) from 1981-2010.

	AMT	AP	GDD	MinTCP	MTCQ	PCQ
AMT	1					
AP	0.79	1				
GDD	0.98	0.75	1			
MinTCP	0.97	0.78	0.98	1		
MTCQ	0.99	0.83	0.97	0.99	1	
PCQ	0.75	0.96	0.74	0.79	0.82	1

Table 1.2. Correlation matrix of the variables from years 1981-2010. AP (annual precipitation), GDD (growing degree days), and muskrat (muskrat harvest growth rate).

	AP	GDD	Muskrat	Farm Density	Road Density	Longitude	Latitude
AP	1						
GDD	0.75	1					
Muskrat	0.38	0.14	1				
Farm Density	0.35	0.59	0.06	1			
Road Density	0.33	0.69	-0.22	0.44	1		
Longitude	0.69	0.56	0.06	0.30	0.43	1	
Latitude	-0.86	-0.94	-0.29	-0.50	-0.60	-0.62	1

Table 1.3.Multiple linear regression output using z-transformed data from 1981-2010.

Variable	Estimate	Standard Error	T-value	P-value
Intercept	3.19E-16	0.117	0.000	1
Muskrat Harvest Growth Rate	0.408	0.143	2.860	0.008
Annual Precipitation	0.417	0.298	1.400	0.173
Growing Degree Days	0.03	0.275	0.097	0.923
Farm Density	0.07	0.146	0.511	0.613
Road Density	-0.339	0.196	-1.730	0.095
Longitude	-0.007	0.193	-0.036	0.971

CHAPTER 2: Mitochondrial DNA analysis and the directionality of the domestic, wild American mink (*Neovison vison*) introgression in Ontario and Nova Scotia.

ABSTRACT

The American mink (*Neovison vison*) is an invasive species in many parts of the world because of deliberate releases and accidental escapes from mink farms. In North America, domestic mink that have escaped can interact with wild conspecifics. Domestic and wild mink are phenotypically and genetically distinct populations that are known to hybridize and introgress. The wild mink population has declined in recent years and hybridization with the domestic mink may be one of the causes. I used part of the maternally-inherited mitochondrial DNA control region to test for population differentiation and introgression. I hypothesized that bi-directional hybridization is occurring and that the hybrids would backcross into both of the parental populations (wild and domestic mink). I also hypothesized that the more natural domestic color phases will have higher fitness in the wild. In particular, I predicted that: (1) the domestic population will have low genetic diversity because of the intense artificial selection and line breeding domestic mink are exposed to; (2) mink will have haplotypes specific to the source population (wild or farm) from lack of gene flow between groups and populations; (3) domestic and wild mink will have unbiased directional gene flow with hybrids breeding into both parental populations; and (4) the natural mink color phases (brown, mahogany) will be related to the hybrids because other color phases have lost the natural camouflage of the species and are more inbred. To this end, I examined a 300 basepair fragment of the mtDNA control region. I sequenced 319 individuals of wild, domestic, and hybrid origin from both Ontario and Nova Scotia and found 63 haplotypes within our study area comprised of Ontario and Nova Scotia. There was an overlap of domestic and wild haplotypes but when separated into population by census division this overlap was not as profound. The pairwise Φ_{st} values showed differences between populations and the AMOVA showed significant differences between groups (Nova

Scotia wild, Ontario wild, and domestic mink; p=0.04). Directional hybridization was found using pairwise Φ_{st} values showing that hybrids were related to the wild population (Φ_{st}=0.006, p=0.13). Mitigation of this introgression is important for the preservation of the species.

Keywords: American mink, domestic, introgression, *Neovison vison*, Nova Scotia, mitochondrial DNA, Ontario

INTRODUCTION

The domestication of a species is a continuous and complex process (Trut 1999) that for some species began thousands of years ago (Cole and Ronning 1974, Hansen 1996, Wiseman et al. 2000). Domestic animals originated by being bred to fulfill specific purposes for human civilizations (Hafez 1968). The process of domestication involves changes in behavior, morphology, and physiology (Trut 1999). These changes occur across all domesticated mammals and include body size, coat color, reproductive cycle (Trut 1999), and reductions in total brain size (Kruska 1996). Domesticated animals undergo intense artificial selection and line breeding that have reduced genetic variation in domesticated populations (Price 1984). Domestication is a combination of genetic changes over generations, and non-genetic environmental influences on individuals during their lifetime (Price 1984).

The recent domestication of the American mink (*Neovison vison*) began in 1866 when the first mink fur farms were established by fur trappers in Canada (Hensen 1996, Kruska 1996). Since then, mink farms have spread from Canada to the rest of North America, Europe, South America, and Asia (Bowman et al. 2007, Zalewski et al 2010, Hau and Xu 2016). The mink fur industry is global in scope and produces approximately 30 million mink pelts annually (Mason et al. 2001). These domestic mink are actively selected for size, fur quality, color uniformity (Belliveau et al. 1999), high productivity, behavior, as well as other morphological characteristics (Kidd et al. 2009). The intense selection for desirable traits under uniform environmental and nutritional conditions has led to morphological changes of traits that were not under direct selection (Belliveau et al. 1999). Traits include reduced sexual dimorphism in skull size and shape (Lynch and Hayden 1995), differences in baculum size and shape (Schulte-Hostedde and Bowman unpub. data), and reduced brain size (Kruska 1996). The artificial

selection of captive populations can result in evolutionary divergence between wild and domestic individuals (Norén et al. 2005). Domestic mink populations have lower genetic variation than natural populations because of the accumulation of inbreeding but also because of the intense directional selection, line breeding, and relaxed natural selection (Price 1984).

The color of mink fur is selected by individual farms and the market (Joergensen 1985, Belliveau et al. 1999). Certain colors such as iris are line bred because of their recessive nature (Joergensen 1985, Kidd et al. 2009). The black fur color is a result of intense selection from darker brown fur (Joergensen 1985, Belliveau et al. 1999). Brown is the wild type fur color that can range from light to dark (Joergensen 1985). To explore new colors and mink traits, the mink are exposed to line breeding and positive assortative mating (Belliveau et al. 1999). This intense selection for color phases has caused a decline in reproductive performance, various mutations (Belliveau et al. 1999), and likely affects other fitness traits.

Domesticated animals readily escape from farm environments (Naylor et al. 2005, Kidd et al. 2009, Norén et al. 2009) and large-scale introduction of domesticated animals has affected natural populations around the world through hybridization (Wiseman et al. 2000, Norén et al. 2009, Randi 2008, Beauclerc et al. 2013), disease transmission (Naylor et al. 2005, Nituch et al. 2011, Beauclerc et al. 2013), competition (Naylor et al. 2005, Kidd et al. 2009, Beauclerc et al. 2013), and through other stressors caused by these domesticated animals (Naylor et al. 2005). Interactions between domestic animals and their wild relatives are a threat to the survival of wild populations (Naylor et al. 2005, Randi 2007, Norén et al. 2009). Escaped domestic mink in North America are not as adapted to survival in the wild (Price 1984) and are expected to have low fitness (Beauclerc et al. 2013). Animals of albino nature do not have the natural camouflage of their species and tend not to survive. Light colored mink have been shown to be more susceptible

to diseases, particularly to Aleutian disease (Thompson and Aliferis 1964). It is difficult to prevent escapes or releases of domestic mink from farms (Bowman et al. 2017) and mink become feral soon after they escape the farm environment (Hua and Xu 2016).

Hybridization is the mating of individuals that are (Cabria et al. 2011) either different species or from distinct populations of the same species (Vähä and Primmer 2006). Hybridization is known to occur when a given species population is relatively small because of the lack of mates available in the given species (Vilà et al. 1999, Cabria et al. 2011). The process of hybridization can cause the introgression of alien alleles, which may alter evolutionary processes (Wirtz 1999, Lodé et al. 2005) and lead to an increased risk of population decline or extinction (Lodé et al. 2005, Norén et al. 2009, Cabria et al. 2011, Godinho et al. 2011). Hybridization between domestic and wild animals can disrupt local adaptation causing outbreeding depression and reduced fitness that may have detrimental effects on the wild population (Kidd et al. 2009, Norén et al. 2009, Zalewski et al. 2010, Godinho et al. 2011). In their native range of North America, domestic American mink hybridize with wild American mink (Kidd et al. 2009, Beauclerc et al. 2013). This hybridization and the subsequent backcrossing have led to the transfer of domestic genes into the wild population. The decline of the wild mink may be due in part to the effects of feral mink hybridization and outbreeding depression (Bowman et al. 2007, Kidd et al. 2009). Theoretical models suggest that permanent inflow of ranch mink decreases the fitness of the feral mink populations in Europe and may lead to population declines (Zalewski et al. 2010).

The directionality of introgression between domestic and wild mink is still uncertain. Unidirectional flow occurs when hybrids backcross primarily into one of the parental populations and bi-directional flow occurs when hybrids backcross into either parental population (Wirtz

1999, Wiseman et al. 2000). The asymmetric directional introgression of mtDNA is not unusual (Good et al. 2003) and has been shown in many carnivore species including Ethiopian wolves (*Canis simensis*) and dogs (*Canis familiaris*: Gottelli et al. 1994), wolves (*Canis lupus*) and dogs (*Canis familiaris*; Vilá et al. 2003), wolves (*Canis lupus*) and coyotes (*Canis latrans*; Lehmen et al. 1991), African wild cat (*Felis lybica*) and domestic cat (*Felis catus*; Wiseman et al. 2000), European mink (*Mustela lutreola*) and polecats (*Mustela putotius*; Cabria et al. 2011), and the farmed and wild arctic fox (*Alopex lagopus*; Norén et al. 2005), just to name a few. Unidirectional hybridization between species usually arises when the males of the larger species mate with females of the smaller species (Pilgrim 1998, Wirtz 1999, Rutledge et al. 2010). Domestic mink are much larger than wild mink but the qualities acquired from domestication may limit their ability to survive and breed in the wild (Davison et al. 1999). This theory is further supported by the differences in baculum morphology between domestic and wild mink, suggesting that domestic males have sub-optimal bacula (Schulte-Hostedde and Bowman unpub.). For instance, in the hybridization of foxes, domestic females mate with wild males (Norén et al. 2005). The hybrid females backcrossing into the parental populations facilitate the movement of mitochondrial DNA (mtDNA) in hybrid zones (Good et al. 2003).

Biparentally inherited microsatellite markers can be used to identify parental groups and to classify hybrids (Cabria et al. 2011). The direction of hybridization in populations of the same species can be distinguished by their mtDNA and/or the Y-chromosome (Wirtz 1999, Cabria et al. 2011). Mitochondrial DNA may be unable to differentiate between mustelid species (Davison et al. 1999, Lodé et al 2005) but more recently it has been found that the mtDNA control region works well for distinguishing hybrids and the direction of flow in their prospective mustelid species (Cabria et al. 2011, Zalewski et al. 2011). The combined use of microsatellites, and

mitochondrial DNA yields the best results for the direction of hybridization (Jaarola et al. 1997, Vilá et al. 2003, Cabria et al. 2011).

I hypothesized that bi-directional hybridization is occurring and the hybrids would backcross into both of the parental populations because the American mink microsatellite DNA data have shown bi-directional introgression in areas of Ontario (Kidd et al. 2009, Beauclerc et al. 2013). I also hypothesized the more natural domestic color phases will have higher fitness in the wild than lighter colored domestic mink. In particular, I predicted that: (1) the domestic population will have low genetic diversity because of the intense artificial selection and line breeding domestic mink are exposed to (Price 1984); (2) mink will have mtDNA haplotypes specific to the source population (wild or farm) from lack of gene flow between groups and populations; (3) domestic and wild mink will have unbiased gene flow with hybrids breeding into either parental population; and (4) the natural color phases (brown, mahogany) of domestic mink will be related to the hybrids because other color phases have lower fitness in the wild environment.

METHODS

Sample Collection and Extraction

I used samples that had been previously collected from museums, live trapping, road kill, farming and trapper collaborations from 2005-2012. From those samples, I used 319 samples that have previously been genotyped with a panel of microsatellites that could distinguish between wild, hybrid (F2+), and farmed samples from Ontario and Nova Scotia using Bayesian assignment tests (Kidd et al. 2009, Beauclerc et al. 2013, Bowman et al. 2017). The q-value of these tests determined what group they were in $q<0.2$ is domestic, $0.2<q<0.4$ and $0.6<q<0.08$ are the introgressed mink (F2+), $q>0.8$ were wild. F1 hybrids with $0.4<q<0.6$ were excluded from

the analysis. We also incorporated several historical samples including a "sea mink" from museum collections. Hereinafter, I refer to the introgressed group of mink (where $0.2<q<0.4$ or $0.6<q<0.8$) as 'hybrids'.

DNA was extracted using the DNeasy Tissue Kit (Qiagen; Poulakakis et al. 2008). Extracted DNA quantity was calculated by Quant-iT Picogreen® dsDNA Assay Kit (Invitrogen TM). One hybrid sample had extremely low concentrations of DNA and was concentrated using Amicon Centrifugal Filter Device as per Amicon protocol.

mtDNA Amplification and Sequencing

I amplified approximately 300bp of the D-Loop at the beginning of the Mitochondrial Control Region (Cabria et al. 2011, Zalewski et al. 2011, Cabria et al. 2015) using primers: CTRL-L 5′-CAC YWT YAACWC CCA AAG CT (Bidlack and Cook 2001) and TDKD 5′–CCT GAA GTA GGA ACC AGA TG (Kocher et al. 1993, Bidlack and Cook 2001). This region was selected because it has shown to provide genetic variation (Bidlack and Cook 2001) in mustelids (Pertoldi et al. 2006, Pertoldi et al. 2008, Cabria et al. 2011, Zalewski et al. 2011, Cabria et al. 2015).

Amplifications were done in 20uL total volume containing 12.7uL ddH_2O, 2uL 1X PCR buffer, .8 $MgCl_2$, 2uL dNTPs, .2uL TDKD, .2uL CTRL-L, .10uL Taq Polymerase, and 2uL of 2.5 ng DNA or 2uL of 1/10 diluted DNA. PCR was conducted under the following thermocycler conditions: The initial denaturation of 5 min at 94^{o} C followed by 34 cycles of 30 sec at 94^{o} C, 1 min at 55^{o} C, 1 min at 72^{o} C, with a 1.30 min final extension at 72^{o} C. The historical samples were amplified separately using the same protocol with filtered pipette tips and fresh reagents.

5uL amplified DNA of each sample was placed on a 96 well plate with 3uL of Orange G. These samples were then loaded onto a 1.5 concentration Agarose gel for visualization of the

quantity for dilution. These samples were submitted to the Natural Resource Department (NRD) at Trent University for sequencing. The submitted amplified products were diluted. The diluted products were purified with ExoSap (New England Biolabs) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The Sequencing products were ethanol precipitated. The precipitated product was suspended in HiDi and sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems). Sequences were aligned using default parameters of CLUSTAL W (Cabria et al. 2015, Larkin et al. 2007) in MEGA 7 (Tamura et al. 2013) with manual revisions.

mtDNA Statistical Analysis

As in Zalewski et al. (2011) I used single base pair changes between haplotypes. This method provides a higher resolution when examining the recent divergence and introgression of domestic and wild mink using the mtDNA control region. Relationships among haplotypes were determined by collapsing and creating a haplotype network using the statistical parsimony method in TCS version 1.21 (Clement et al. 2000, Poulakakis et al. 2008, Zalewski et al. 2011). Haplotype frequencies and haplotype diversity (*h*) were calculated using Arlequin version 3.5.2.2 (Excoffier et al. 1992, Excoffier and Lischer 2010). Genetic structure between the populations was estimated using pairwise distance Φ st in Arlequin version 3.5.2.2. The significance of the Φ st values was determined with 3000 permutations. Mitochondrial DNA variation among the population groups was determined using an Analysis of Molecular Variance (AMOVA) in Arlequin version 3.5.2.2 to determine the relatedness of the populations/groups (Excoffier et al. 1992, Excoffier and Lischer 2010).

I examined different grouping structures of the American mink. AMOVA 1 had 4 groups excluding the hybrids since hybrids should be related to multiple groups: Nova Scotia wild,

Nova Scotia farm, Ontario wild, and Ontario farm. AMOVA 2 excluded the hybrids and combined the farms because farms share stock (Canadian Fur Breeders Association, personal Communication). AMOVA 2 had three groups: Nova Scotia wild, Ontario wild, and Farm. Directional introgression was tested using pairwise distance ϕ st in Arlequin version 3.5.2.2. The significance was determined with 3000 permutations.

RESULTS

Mitochondrial Analysis

Of the 373 American mink samples amplified, only 319 produced useable sequences. Samples were taken from separate populations of wild, hybrid, and farmed individuals from both Ontario and Nova Scotia, and yielded 63 haplotypes (Figure 2.1), 20 of which were also found in mink from Poland (Zalewski et al. 2011). Three haplotypes were only found in wild Nova Scotia mink while 27 haplotypes were only found in wild Ontario mink (Figure 2.2). Two haplotypes were shared between the Nova Scotia wild and the Ontario wild populations. Thirteen haplotypes were only found on farms and 13 haplotypes were shared by all of the populations studied. Six haplotypes only had hybrid individuals so we were unable to determine origin of these haplotypes as wild, farm, or both. The "sea mink" had its own haplotype (hap73) but was consistent with American mink according to its mtDNA sequence (Figure 2.1). Haplotypes 1, 13, and 31 had the highest frequencies among the populations with 26, 28, and 24 individuals respectively. The haplotype diversity (*h*) value for the total sample was 0.96% +/- 0.004. Individual population haplotype diversity (*h*) varied from 0.7 to 1.0 (Table 2.1).

Phylogeographic Difference-Population Pairwise ϕ st

Genetic differentiation of American mink among the census divisions (populations) was determined using pairwise ϕ st. The pairwise ϕ st between populations was moderate ranging

from 0 to 0.268 (Table 2.2). Most of the Φ st values were significant (p <0.05), suggesting that these populations are distinct. The Nova Scotia wild populations were significantly different from the Ontario wild populations (Φ st= 0.110- 0.203, p <0.001). The only exceptions were the populations of Pictou (Nova Scotia) and Wellington (Ontario), which had a Φ st of 0.049 (p=0.084) indicating no significant difference. The farm populations (Ontario and Nova Scotia) were significantly different from all populations except for two. The Nova Scotia farm was not significantly different from Wellington, Ontario (Φ st=0.02, p=0.21) and the Ontario farms were not significantly different from the Nova Scotia hybrids (Φ st=0.072, p=0.075). The Ontario hybrids were significantly different from all populations (0.039-0.107, p<0.02) except for four: Nova Scotia hybrids (Φ st=0.062, p=0.075), Wellington, Ontario (Φ st=0.004, p=0.39), Leeds and Grenville, Ontario (Φ st=0.017, p=0.210), and Essex, Ontario (Φ st=0.020, p=0.182). The Wellington population was significantly different from only very few populations suggesting that this population has a high level of gene flow and possible genetic introgression.

Color Pairwise Φ st

Mink color (Black, Pastel, Iris, Mahogany, and Demi (standard brown)) was added to the analysis of pairwise Φ st in place of farms to determine if color had an effect on the population to which it was related (Table 2.3). The pairwise Φ st between populations was moderate, ranging from 0-0.297. Black mink were significantly different from most of the populations except for Wellington, Ontario (Φ st=0.012, p=0.324), Demi (Φ st=-0.005, p=0.477), and Mahogany (Φ st=0.050, p=0.144). Pastel mink were significantly different from most of the populations but not significant from 3 populations: Nova Scotia hybrids (Φ st=0.044, p=0.288), Mahogany (Φ st=0.111, p=0.189), and Demi (Φ st=-0.005, p=0.550). Iris was only different from two populations, Wellington Ontario (Φ st=0.058, p=0.063) and Demi (Φ st=0.009, p=0.514).

Mahogany was different from 7 populations (Φ st=0.092-0.231, p=0.00-0.01), but is not significantly different from the other 9 populations (Φ st=0.006-0.111, p=0.135 - 0.590). Demi was related to all populations.

AMOVA

Two AMOVAs were performed in order to determine group differentiation and population genetic variation (Tables 2.4-2.5). I found that 89% of the total genetic variation in American mink mtDNA control region was attributed to differences among individuals. Genetic differentiation among populations was significant at 8.35%-9.08% of the overall genetic variance. The first AMOVA between Ontario wild, Ontario farms, Nova Scotia wild, and Nova Scotia farms attributed 1.26% of the genetic variance among the groups (Table 2.4) and was not significant (p=0.181), suggesting that mink do not differ among farms. The second AMOVA (combined farms) was run based on this conclusion and attributed 2.38% of the total genetic variation among groups (Table 2.5) and supported the genetic differentiation among Nova Scotia wild, Ontario wild, and farmed mink (p=0.040).

Direction of Introgression

When Ontario and Nova Scotia hybrids (F2+) were grouped together, they were not distinguishable from the wild population (Φ st=0.006, p=0.130) but were distinguishable from the farm population (Φ st=0.024, p=0.004; Table 2.6). I analyzed the Nova Scotia hybrid population separately and found that the Nova Scotia hybrid population was not related to either of the wild (Φ st=0.227-0.258, p=0.003) or farm (Φ st=0.063, p=0.035) populations in Nova Scotia. The Nova Scotia hybrid population and the Ontario hybrid population were not significantly different (Φ st=0.062, p=0.076).

DISCUSSION

The results indicated that hybrid mink are different from all groups except the Ontario wild group which indicates that hybrid mink have unidirectional introgression. This is contrary to the hypothesis that bi-directional introgression is occurring, and thus, hybrids (F2+) are primarily backcrossing into both of the parental populations. The results mostly supported the hypothesis that more natural domestic color phases, such as demi and mahogany, will have higher fitness in the wild, with the exception of Nova Scotia where it was found that pastel (light brown) was also related to the hybrids.

I found higher than expected levels of mitochondrial DNA diversity in all populations and groups and this is supported by the Poland mink study which found higher than expected mitochondrial diversity (Zalewski et al. 2011). The high mitochondrial diversity of the farm population was unexpected and contrary to my predictions. We expected domestic mink to have lower genetic variation as they are exposed to inbreeding and intense artificial selection (Price 1984). High levels of genetic variability found on farms in black and brown mink in Ontario (Kidd et al. 2009), domestic mink in Poland (Zalewski et al. 2011) and in the farms of my study, may be a result of diverse origins of the domestic mink (Belliveau et al 1999) and/or the continuous trading of mink between farms (Belliveau et al. 1999, Canadian Mink Breeders Association).

I was able to detect color phase relationships as expected. Most domestic mink fur colors are linebred because all of the mink color phases are recessive to the brown color phase (Joergensen 1985). Mahogany is the mix of black and brown phases (Joergensen 1985) and not surprisingly, I found that mahogany is related to both the black and demi (standard brown). Demi was found to be related to all colors and populations. Demi is crossed with many other fur color phases to produce new colors and to increase genetic variability (Belliveau 1999, Joergensen

1985). Demi is the only color phase that is related to the iris color phase in this study. Iris is almost exclusively linebred due to its recessive nature (Joergensen 1985). Black, demi, and mahogany were more closely related to the wild populations than the other color phases (Belliveau et al. 1999). I found this also to be the case using the mtDNA for the wild Ontario group and only demi for the wild Nova Scotia group. The hybrids are more closely related to the brown colors phases of demi and mahogany suggesting that these color phases may have a higher fitness than the other color phases in the wild at least in terms of surviving long enough to produce offspring.

The pairwise Φ_{st} and AMOVA supported the mitochondrial DNA differentiation between the Nova Scotia wild, Ontario wild, and domestic mink groups. The pairwise differences were low between several populations of Ontario wild mink and the farms. This suggests that gene flow is occurring between these genetically different groups. Several studies have found high numbers of domestic and hybrid mink in the wild (Beauclerc et al. 2013, Bowman et al. 2007, Bowman et al. 2017, Kidd et al. 2009). In southwestern Ontario 36% of the free ranging mink captured were of domestic origin and another 28% were domestic-wild hybrids (Beauclerc et al. 2013). In Nova Scotia, 51% of free ranging mink captured were domestic and another 8% were domestic-wild hybrids (Bowman et al. 2017). Although these escaped domestic mink tend to stay around the farm area (Beauclerc et al. 2013, Bowman et al. 2007, Bowman et al. 2017, Kidd et al. 2009) and are not spreading extensively as they do in Europe (Beauclerc et al. 2013, Zalewski et al. 2011), they may still be doing damage through the "cryptic invasion"(Kidd et al. 2009) of their native range by hybridizing with the wild mink (Beauclerc et al. 2013, Bowman et al. 2007, Bowman et al. 2017, Kidd et al. 2009). This hybridization may have fitness costs for the wild mink and lead to the loss of local adaptation (Beauclerc et al. 2013).

I found that directional introgression is occurring which was demonstrated by an extremely low hybrid Φ_{st} value. This result was contrary to previous studies using microsatellite data where a bi-directional introgression was found to occur (Kidd et al. 2009, Beauclerc et al. 2013). The finding of a uni-directional introgression using mtDNA suggests that females are primarily mating into the wild population, whereas, the males could be mating into both of the parental populations.

The direction of the introgression is a conservation concern because if the hybrids breed back into the domestic population, the domestic traits would not be transferred to the wild population (Wiseman et al. 2000). However, the direction of the introgression in our population is towards the wild population in Ontario, which makes the passing of undesirable traits and outbreeding depression a major concern which likely has detrimental effects on the wild mink (Norén et al. 2005, Wiseman et al. 2000) such as reducing wild mink genetic diversity (Bowman et al. 2017). The directional introgression of the Nova Scotia Hybrid population could not be determined because the hybrids were not significantly related to the wild or farm population in Nova Scotia but were related to the Ontario hybrids, Ontario farm, and the Ontario wild population. Over the last 40 or so years there has been a transfer of Ontario farm minks to the Nova Scotia farms (Canadian Mink Breeders Association pers. comm.), which could explain the Nova Scotia hybrid relatedness to Ontario mink.

Introgression between wild and domestic individuals has been seen in several other species such as wolves (Gottelli et al. 1994), wild cats (Wiseman et al. 2000), polecats (Davison et al 1998), salmon (Naylor et al. 2005), and foxes (Norén et al. 2009) with similar consequences of domestic alleles being transferred to the wild population or getting out competed by the domestics conspecific (Randi 2007). The introgression can lead to rapid declines in some species

(Cabria et al. 2011) and makes prevention of escapees an important priority. The amount of escaped mink caught in the wild suggests that the current biosecurity measures are ineffective (Kidd et al. 2009, Beauclerc et al. 2013, Bowman et al 2017). In fact, farm biosecurity in Ontario is not equal amongst farms (Beauclerc et al. 2013) and biosecurity improvements need to be made in order to mitigate the introgression of domestic mink into the wild populations.

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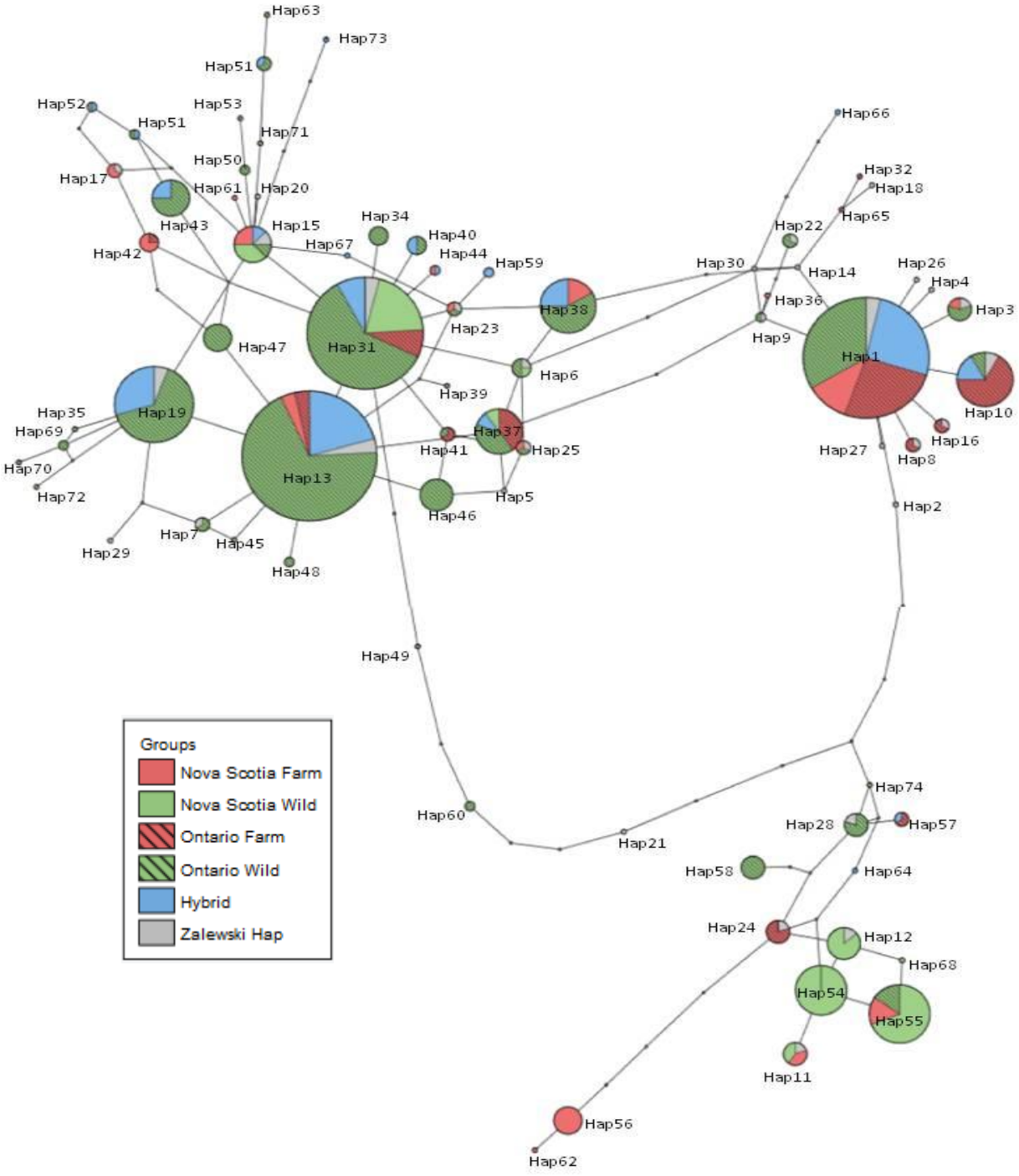


Figure 2.1 Haplotype network of wild and domestic American mink *Neovison vison* found in Ontario and Nova Scotia. This network contains74 mtDNA control region haplotypes, 63 of which are found in Ontario and Nova Scotia. The circles are proportionate to the number of mink in which the haplotype occurred and the colors are proportionate to the number of mink in the group that contains the haplotype.

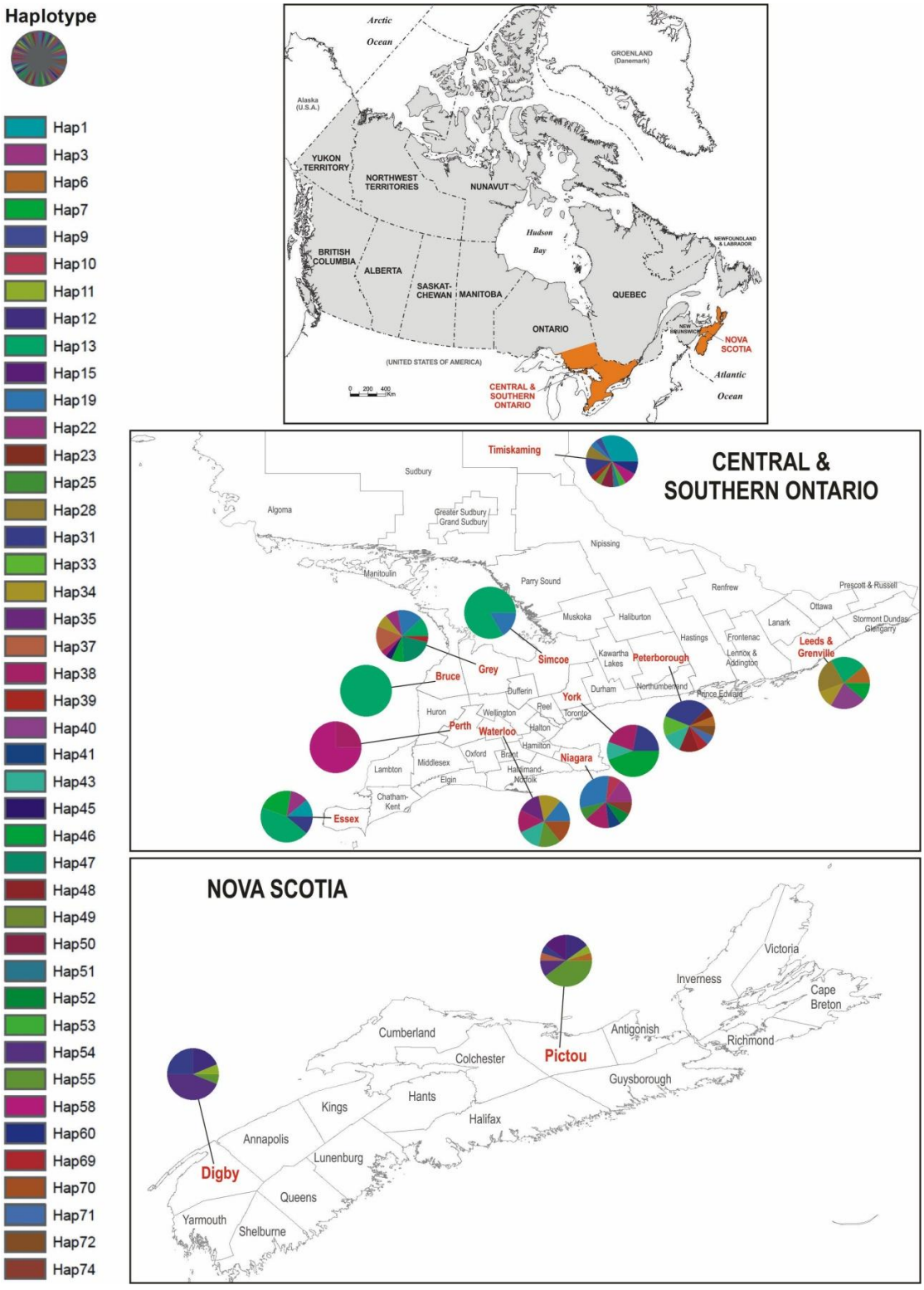


Figure 2.2. Distribution of wild American mink (*Neovison vison*) haplotypes across Ontario and Nova Scotia. Highlighted in red are the census divisions in which the mink are located. The circles are proportionate to the number of mink of each haplotype that occur in each census division.

Table 2.1. Mitochondrial DNA haplotype (h) diversity of American mink (*Neovison vison*) by population and group.

Population	Number of Haplotypes	Sum of Squares	*h*
Digby NS	5	0.297	0.750 +/- 0.078
Farm NS	16	0.094	0.938 +/- 0.027
Pictou NS	8	0.225	0.816 +/- 0.071
Hybrids NS	3	0.440	0.700 +/- 0.218
Hybrids ON	17	0.092	0.938 +/- 0.024
Farm ON	13	0.123	0.901 +/- 0.026
Niagara ON	8	0.174	0.890 +/- 0.060
Timiskaming ON	12	0.152	0.883 +/- 0.051
Essex ON	5	0.284	0.806 +/- 0.120
Peterborough ON	9	0.164	0.892 +/- 0.060
Grenville ON	6	0.185	0.917 +/- 0.073
Grey ON	10	0.130	0.907 +/- 0.029
Wellington ON	7	0.143	1.000 +/- 0.076
York ON	4	0.309	0.778 +/- 0.110
Combined population groups			
Ontario Wild	41	0.059	0.948 +/- 0.008
Nova Scotia Wild	8	0.184	0.840 +/- 0.029
Hybrids	18	0.1	0.925 +/- 0.024
Farm	26	0.068	0.946 +/- 0.013
Total	74	0.042	0.961 +/- 0.004

Table 2.2. Pairwise Φ st values between populations of American mink *Neovison vison* from Ontario, Nova Scotia, and Farms.

	Nova Scotia				Ontario									
Location	Digby	Pictou	NS Farm	NS Hybrids	ON Hybrids	ON Farm	Niagara	Timiskaming	Essex	Peterborough	Grenville	Grey	Wellington	York
Digby	0													
Pictou	**0.117**	0												
NS Farm	**0.142**	**0.083**	0											
NS Hybrids	**0.268**	**0.227**	**0.098**	0										
ON Hybrids	**0.136**	**0.114**	**0.039**	0.062	0									
ON Farm	**0.156**	**0.132**	**0.059**	0.072	**0.042**	0								
Niagara	**0.181**	**0.149**	**0.071**	**0.138**	**0.042**	**0.086**	0							
Timiskaming	**0.154**	**0.144**	**0.058**	-0.008	**0.037**	**0.044**	**0.103**	0						
Essex	**0.203**	**0.184**	**0.092**	**0.186**	0.020	**0.105**	**0.129**	**0.107**	0					
Peterborough	**0.110**	**0.131**	**0.082**	**0.183**	**0.055**	**0.088**	**0.109**	**0.078**	**0.117**	0				
Grenville	**0.175**	**0.134**	**0.064**	**0.177**	0.017	**0.087**	**0.097**	**0.086**	0.045	**0.090**	0			
Grey	**0.167**	**0.131**	**0.072**	**0.144**	**0.036**	**0.077**	**0.053**	**0.099**	**0.090**	**0.098**	0.046	0		
Wellington	**0.134**	0.049	0.020	0.113	0.004	**0.057**	-0.002	**0.061**	**0.102**	0.042	0.028	0.012	0	
York	**0.193**	**0.192**	**0.124**	**0.255**	**0.107**	**0.140**	**0.135**	**0.139**	**0.188**	**0.085**	**0.109**	**0.110**	0.072	0

Significant values are in bold ($P < 0.05$).

Table 2.3.Pairwise ϕ st values between populations of wild American mink (*Neovison vison*) from Ontario, Nova Scotia, and farmed domestic mink which are separated by color type.

	Nova Scotia			Farm Color					Ontario								
Location	Digby	Pictou	NS Hybrids	Black	Pastel	Iris	Mahogany	Demi	ON Hybrids	Niagara	Timiskaming	Essex	Peterborough	Leeds and Grenville	Grey	Wellington	York
Digby	0																
Pictou	**0.117**	0															
NS Hybrids	**0.268**	**0.227**	0														
Black	**0.136**	**0.080**	**0.099**	0													
Pastel	**0.297**	**0.259**	0.044	**0.144**	0												
Iris	**0.164**	**0.147**	**0.192**	**0.077**	**0.161**	0											
Mahogany	**0.231**	**0.174**	0.013	0.050	0.111	**0.150**	0										
Demi	0.159	0.081	0.010	-0.005	-0.063	0.009	0.006	0									
ON Hybrids	**0.136**	**0.114**	0.062	**0.037**	**0.124**	**0.065**	0.033	-0.009	0								
Niagara	**0.181**	**0.149**	**0.138**	**0.063**	**0.202**	**0.081**	**0.146**	0.050	**0.042**	0							
Timiskaming	**0.154**	**0.144**	-0.008	**0.057**	**0.077**	**0.102**	0.026	-0.010	**0.037**	**0.103**	0						
Essex	**0.203**	**0.184**	**0.186**	**0.090**	**0.234**	**0.142**	0.073	0.089	0.020	**0.129**	**0.107**	0					
Peterborough	**0.110**	**0.131**	**0.183**	**0.076**	**0.221**	**0.078**	**0.145**	0.067	**0.055**	**0.109**	**0.078**	**0.117**	0				
Leeds and Grenville	**0.175**	**0.134**	**0.177**	**0.059**	**0.222**	**0.097**	0.095	0.049	0.017	**0.097**	**0.086**	0.045	**0.090**	0			
Grey	**0.167**	**0.131**	**0.144**	**0.057**	**0.206**	**0.101**	0.053	0.058	**0.036**	**0.053**	**0.099**	**0.090**	**0.098**	0.046	0		
Wellington	**0.134**	0.049	0.113	0.012	**0.191**	0.058	**0.092**	0.000	0.004	-0.002	**0.061**	**0.102**	0.042	0.028	0.012	0	
York	**0.193**	**0.192**	**0.255**	**0.113**	**0.291**	**0.146**	**0.213**	0.132	**0.107**	**0.135**	**0.139**	**0.188**	**0.085**	**0.109**	**0.110**	0.072	0

Significant values are in bold (P<0.05).

Table 2.4. Analysis of Molecular Variance (AMOVA) between 4 groups of American mink (*Neovison vison*): Ontario farm, Ontario wild, Nova Scotia farm, and Nova Scotia wild.

Source of Variation	D.F.	Sum of Squares	Percentage of Variation	Fixation Index	*P*
Among groups	3	5.71	1.26	*Fct*= 0.013	0.181
Among populations within groups	8	8.6	9.08	*Fsc*= 0.092	<0.001
Within populations	204	89.68	89.66	*Fst*= 0.103	<0.001

Table 2.5. Analysis of Molecular Variance (AMOVA) between 3 groups of American mink (*Neovison vison*): Ontario wild, farm mink and Nova Scotia wild.

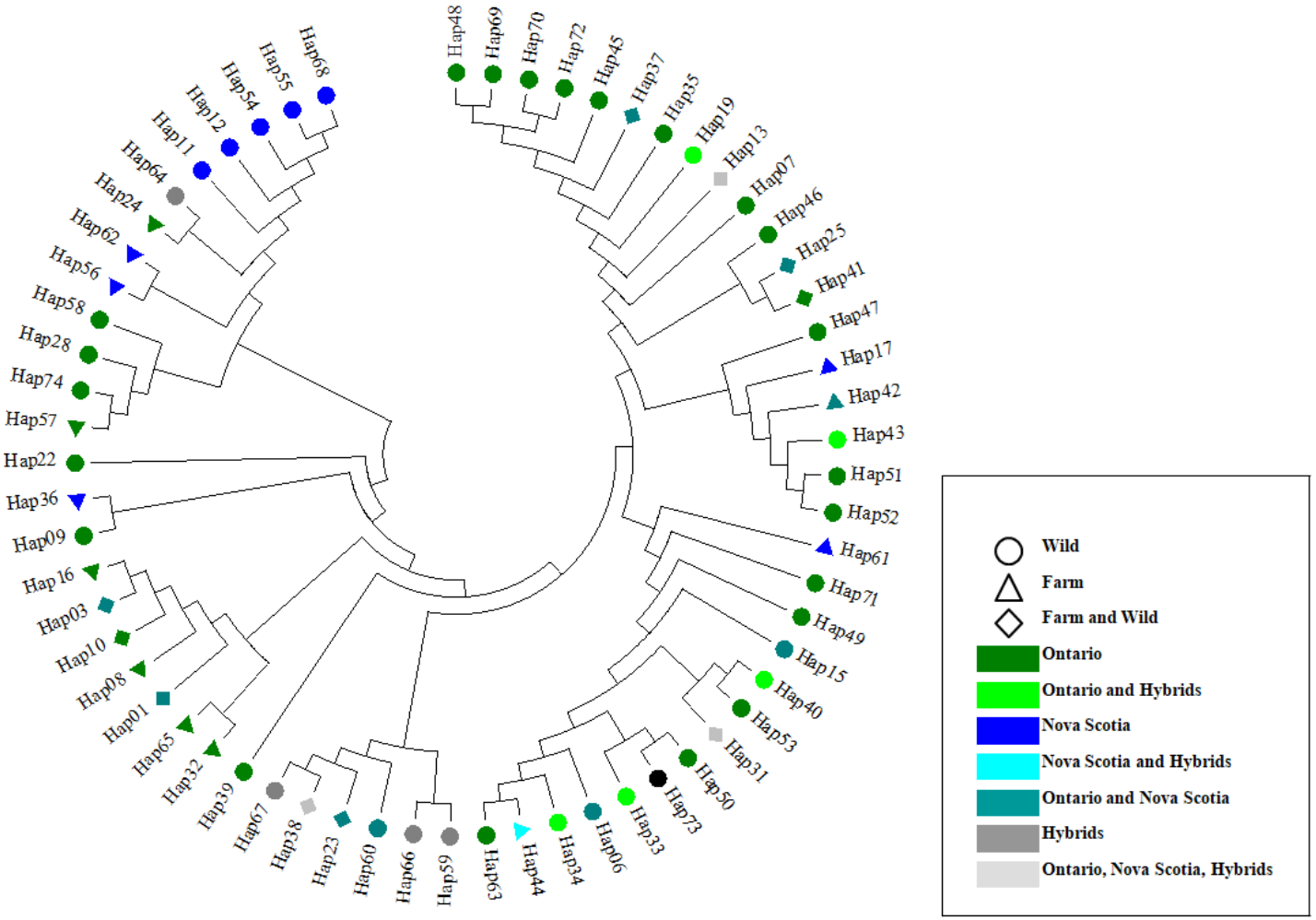
Source of Variation	D.F.	Sum of Squares	Percentage of Variation	Fixation Index	*P*
Among groups	2	4.32	2.38	*Fct*= 0.024	0.04
Among populations within groups	9	9.99	8.35	*Fsc*= 0.086	<0.001
Within populations	204	89.68	89.27	*Fst*= 0.107	<0.001

Table 2.6. Pairwise Φ st values between groups of American mink (*Neovison vison)* from Ontario, Nova Scotia, and Farms to determine directional introgression of the hybrids.

Groups	Nova Scotia Wild	Hybrids	Farm	Ontario Wild
Nova Scotia Wild	0			
Hybrids	**0.109**	0		
Farm	**0.089**	**0.024**	0	
Ontario Wild	**0.086**	0.006	**0.032**	0

Significant values are in bold (P<0.05).

APPENDIX



Appendix Figure 2.1. Neighbor-joining haplotype tree for American mink (*Neovison vison*) in Ontario and Nova Scotia. Farm/Wild groups are defined by shapes. Location is defined by color (Ontario/Nova Scotia/Hybrid). The so called "sea mink" from the New Brunswick museum is included in the tree and is designated by a black circle.

GENERAL DISCUSSION

Domestic-wild interactions are occurring in many species (Wiseman et al. 2000). These widespread introductions of domestic species are a threat to the survival of the wild populations (Wiseman et al. 2000). Negative effects of domestic animals can occur through a variety of different mechanisms including hybridization (Wiseman et al. 2000, Norén et al. 2009, Randi 2008, Beauclerc et al. 2013), disease transmission (Naylor et al. 2005, Nituch et al. 2011, Beauclerc et al. 2013), competition (Naylor et al. 2005, Kidd et al. 2009, Beauclerc et al. 2013), and through other stressors caused by these domestic animals (Naylor et al. 2005). This thesis aimed to provide a better understanding of the American mink (*Neovison vison*) harvest decline and to better understand the effects of past and current hybridization.

Mink are in decline in Ontario and across Canada (Bowman et al. 2007). The decline in the American mink harvest can occur though a variety of different factors. In the first chapter, I looked at several factors that could cause the mink harvest to decline. I used two statistical models to determine the variables affecting mink harvest. The multiple linear regression showed two variables that effected the mink harvest growth rate, the muskrat harvest growth rate and road density. The tree regression model selected muskrat harvest growth rate, road density, and annual precipitation. The tree regression showed that the lowest rates of decline are in areas with low road density and a high annual precipitation. This occurred in central Ontario where mink farms do not occur. Though the density of mink farms did not affect the mink harvest in either model this still may be an important factor in the conservation of wild American mink.

In the second chapter I examined mitochondrial DNA to find what haplotypes occur in this region and to determine if directional introgression between wild and domestic mink is occurring. I found 63 haplotypes in this region containing both wild and domestic mink from

Nova Scotia and Ontario. There was a moderate amount of haplotype overlap between the wild and farm populations. The F1 hybrids mostly contained these overlapped haplotypes, and because of this the original thesis topic of sex-bias hybridization was unable to be determined. Unidirectional introgression however was found and the hybrid population is indistinguishable from the wild population with a pairwise Φ st value of 0.006. This result is associated with unidirectional introgression where the female hybrids are primarily mating into the wild population and few are mating into the domestic parental population. These introgression results are contrary to a study done by Kidd et al. (2009) who found that hybrids backcrossed in both directions, suggesting a bidirectional introgression in the Niagara Ontario region. The unidirectional introgression pattern found in this thesis could be explained by the abundance of one parental population (Cabria et al. 2011) or sex-biased introgression where the females mate primarily into the wild population and the males mate into both parental populations or into the domestic population. The unidirectional introgression result is a cause for concern. The International Union for the Conservation of Nature (IUCN) stated that actions should be taken to prevent hybridization between domestic escapees and wild populations because of the potential of outbreeding depression (Norén et al. 2009). Unidirectional introgression makes outbreeding depression in this population a major possibility.

Outbreeding depression of the wild mink population may be why the mink harvest is in decline along with the muskrat harvest growth rate, high density of roads, and low annual precipitation. Hybridization alone can have large detrimental effect that can cause a decline in the wild population (Wiseman et al. 2000, Norén et al. 2005) but with the muskrat harvest in a 75% decline over the last 30 years (Roberts and Crimmins 2010) this could also have major effects on the mink populations that rely on muskrats as a primary food source in winter in

northern Canada. Mink tend to stay away from roads and places where humans are active (Previtali et al. 1998). A small increase in development has been shown to cause a rapid decline in mink activity (Racey and Euler 1985) and a single road could impact populations of up to 2 km away (Findlay and Bourdages 2000).

Future Directions

In researching American mink populations there is the confounding variable of domestic mink. To understand this confounding variable, future research should determine if feral domestic mink behave differently than their wild counterparts (i.e. remain closer to human disturbed areas, differences in diet, etc). Many papers have been published studying habitat use of invasive mink around the world and their findings have been applied to the native American mink (Hodder et al. 2017). There is a lack of research studying American mink in their native range and this has resulted in mink being poorly understood in North America. Research is needed to allow us to understand the behavioral differences between domestic and wild mink and perhaps also the survival of domestic mink in the wild.

Further research should also be conducted on the abundance of domestic-wild parental groups in a population and if that affects mating success of feral individuals and the direction of introgression. The Y chromosome should also be characterized to give more certainty in the total direction of introgression and to determine if sex-biased introgression is occurring. This will greater enhance our knowledge of the domestic wild introgression that is taking place.

If I started this thesis today with my current knowledge there are a couple of things I would change. For the first chapter I would have included stream level, snowpack and other water related data for across Ontario into the statistical analysis to provide a greater

understanding on how different changes in water affects mink. For the second chapter I would have included more colored mink samples from farms, more F1 and F2+ samples, and more wild samples for the mtDNA analysis. I used all of the hybrid and wild samples that were available to me at the time. I also would have liked to include more mink color phases into the analysis.

This research as a whole provides us with a better understanding of the mink harvest decline and a greater understanding of current domestic-wild introgression. This research also provides a greater understanding of domestic and wild conspecific introgression within a native range.

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