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# GREAT LAKE ISLAND BIOGEOGRAPHY OF PLETHODON CINEREUS: EFFECT OF ISLAND ELEVATION ON COLOR POLYMORPHISM

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GREAT LAKE ISLAND BIOGEOGRAPHY OF *PLETHODON CINEREUS*: EFFECT OF  
ISLAND ELEVATION ON COLOR POLYMORPHISM

A Thesis Submitted to the  
Office of Graduate Studies  
College of Arts and Sciences of  
John Carroll University  
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for the Degree of  
Master of Science

By  
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2015

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## **Abstract**

Islands have long fascinated ecologists and evolutionary biologists because they provide replicated systems with which to test hypotheses regarding evolutionary and ecological theory. Because islands are relatively simple systems, they enable researchers to isolate specific factors responsible for observed phenomena. Many current questions in ecology and evolutionary biology have been addressed in island systems. For example, factors involved in the genetic divergence of populations are easier to discern in island populations because confounding effects of gene flow from adjacent populations are limited. This study focuses on isolated island populations of the color polymorphic Eastern Red-backed Salamander (*Plethodon cinereus*) in the Great Lakes. Morph frequencies of *P. cinereus* populations on these islands are largely unknown. In addition, it is unknown whether selection or drift may be acting on these populations to drive them toward fixation of one morph or the other. Reports of monomorphic unstriped populations on islands and peninsulas suggest that unstriped individuals may be selected for on islands or in isolated populations. The goal of this project was first to census island populations throughout the Great Lakes to determine morph frequencies on the islands, and second to explore whether island attributes are related to phenotypic differences among island and mainland populations. I compiled morph frequency records from 30 islands and 14 paired mainland sites occurring in each of the five Great Lakes via literature records, museum specimens, and field surveys. In the census, I found that nine islands are polymorphic, two are monomorphic unstriped, and 15 are monomorphic

striped. Island elevation above lake level was correlated with morph frequencies. Morph frequencies on taller islands tended to differ more from their mainland source population than on shorter islands. This may be because shorter islands have been more susceptible to flooding post-glaciation and have likely been re-colonized more recently than taller islands. Although it is unclear whether drift or selection are acting on island populations, I detected no evidence that selection is favoring the unstriped morph across all islands.

## Introduction

The means by which lineages diverge and species emerge has fascinated scientists for many years. Although it is a relatively common practice for evolutionary biologists to create phylogenetic trees of relatedness among multiple species, it is more difficult to uncover the mechanisms involved in the process of speciation (Schluter 2001).

Polymorphic populations provide a unique opportunity to observe the initial stages of divergence in a lineage that may eventually separate into multiple species (Corl et al. 2010; Huggall and Stuart-Fox 2012). Polymorphic species have been hypothesized to diverge into separate lineages in two ways. The process of sympatric divergence predicts that differences within populations might emerge via divergent selection on morphs (Smith 1966; Kondrashov and Mina 1986; Dieckmann and Doebeli 1999). Alternatively, morphic speciation predicts that populations are free to evolve in a novel direction only after a morph has been lost (West-Eberhart 1986; Corl et al. 2010). When considering these different modes of speciation for polymorphic populations, examination of isolated polymorphic and monomorphic populations where a morph has been lost should enable biologists to more clearly elucidate these two speciation mechanisms. Further, using a study organism that is abundant and easily observed in the field enables biologists to use larger sample sizes and yield more robust results.

The polymorphic salamander *Plethodon cinereus* (Eastern Red-backed Salamander) serves as a model organism in which to examine potential niche partitioning of morphs, owing to its widespread geographic distribution, abundance, and well-studied ecology (Mathis et al. 1995; Jaeger et al. 1995; Petranka 1998; Anthony and Pflingsten



2013). *Plethodon cinereus* is a small, terrestrial salamander that inhabits much of eastern North America, ranging from North Carolina west to Minnesota, and north to Quebec (Petranka 1998). These salamanders are the most abundant vertebrates in North America, with densities ranging from 0.03 to 18.5 individuals / m<sup>2</sup> (Burton and Likens 1975; Jung et al. 2000; Casper 2005). *Plethodon cinereus* exhibits color polymorphism of the dorsal stripe. The two common color morphs are the striped (red dorsal stripe present, redback) and unstriped (no dorsal stripe, leadback).

The proportions of the two morphs in any given population vary geographically. In populations where the striped and unstriped morphs co-occur, the striped morph generally occurs in frequencies of 80% or more (Anthony and Pfingsten 2013). This increased frequency of the striped morph reaches an extreme in the western portion of the range (Illinois, Wisconsin, Minnesota, and the upper peninsula of Michigan) where only the striped morph occurs (Petranka 1998). It was previously thought that only monomorphic striped populations occurred in the far northern portion of the range as well, but unstriped populations have been recently documented in these northern reaches of Canada (Moore and Oullet 2014). The trend of stripe-dominated populations is reversed in northwestern Ohio, where the unstriped morph comprises 70-99% of populations (Pfingsten and Walker 1978). Island populations in this region (Rattlesnake and South Bass) are almost entirely composed of unstriped individuals (Highton 1962; Pfingsten and Walker 1978; Reichenbach 1981; King et al. 1997).

Within polymorphic populations, evidence for sympatric ecological separation has begun to emerge. In studies investigating territoriality and diet, the striped morph has

been found to be more territorial and able to secure higher quality prey items (Anthony et al. 2008; Reiter et al. 2014). Recently, positive assortative mating has been documented in one population of *P. cinereus* (Anthony et al. 2008; Acord et al. 2013). When compared to individuals of the striped morph, unstriped individuals have increased stress hormones (Davis and Milanovich 2010), increased tail breakage (Moreno 1989; Venesky and Anthony 2007), and are more susceptible to *Batrachochytrium dendrobatidis* (Bd) fungus (Venesky et al. 2015). Throughout the range of the species, studies have suggested that the unstriped morph may be less cold tolerant, occurring more frequently at lower latitudes and elevations, and exhibiting earlier burrow retreat in the fall (Lotter and Scott 1977; Moreno 1989; Gibbs and Karraker 2006). While there is a growing body of evidence suggesting that morphs differ in polymorphic populations, the use of isolated island populations may elucidate which specific selective forces are acting on individual populations to maintain the color polymorphism.

The process of morphic speciation occurs in monomorphic populations that were originally polymorphic (Corl et al. 2010). This process occurs when a polymorphic population loses a morph and opens niche space, which can subsequently be filled by the remaining morph. The loss of the second morph allows for differentiation by eliminating tradeoffs where traits that increase fitness in one morph would decrease fitness in the other (West-Eberhard 1986). Very little is known about morphic speciation and the model could be tested using isolated island populations. Morph frequency among populations of *Plethodon cinereus* varies from monomorphic striped to polymorphic to monomorphic unstriped (reviewed in Anthony and Pflingsten 2013), so a first step in

examining morphic speciation in this species is to document localities where monomorphic striped and monomorphic unstriped populations exist.

Island populations may provide the conditions (via founder events, drift, and natural selection) where morphs might be eliminated through population differentiation (Berry 1992). In this study, I assumed that monomorphic islands with a polymorphic source population have lost a morph. The islands of interest for this study occur within the Great Lakes of North America. The Great Lakes lie within the range of *P. cinereus* and contain isolated populations on many of the islands throughout the system. Due to the glacial history of the Great Lakes, the islands have been exposed for no more than 13,500 years, with some being younger than 6,000 years, emerging as the glaciers receded or water levels dropped (Eschman and Karrow 1985; Larson and Schaetzl 2001; Appendix).

Island populations of *P. cinereus* have been largely unexplored, especially with respect to color morph. Bois Blanc Island (Lake Michigan) and South Bass Island (Lake Erie) are the only islands in the Great Lakes where color morph frequencies are known from samples of at least 75 individuals. Bois Blanc was found to be composed of 75% striped individuals (Test 1952), and South Bass was 0.2% striped (Highton 1962; Pfingsten and Walker 1978; King et al. 1997). The monomorphic unstriped population on South Bass Island suggests that islands may promote the loss of polymorphism in a population. This loss may occur on other islands as well. For example, Long Island, NY has a striped and unstriped population of *P. cinereus* living parapatrically on opposite ends of the island (Fisher-Reid et al. 2013). Another example is present on Cove Island,

ON, where a polymorphic population occurs on the north end of the island and shifts to a completely unstriped population on the south end (F. Schueler pers comm). These morph frequency patterns suggest that, at least in some cases, drift, selection, or both may eliminate a morph from an island or peninsula. This elimination sets the stage for the possibility of morphic speciation.

The Great Lakes have roughly 30,000 islands, and of those occurring in the United States, only 31 have confirmed populations of *P. cinereus* (Test 1952; Vogt 1981; King et al. 1997; Holman 2012). The low number of documented populations is most likely a combination of the young age of the islands and the poor dispersal ability of *P. cinereus* (Schmiegelow and Nudds 1987; Cabe et al. 2007). An additional possibility for the low number of documented *P. cinereus* populations on islands is undersampling. In a study that surveyed 107 islands throughout the Great Lakes, *P. cinereus* was reported on ~30% (Hecnar et al. 2002). Most of the islands in the Great Lakes have yet to be sampled, and if this pattern persists for the remaining 30,000 islands, there may be upwards of 9,000 islands with *P. cinereus* populations. Additionally, Werner (1959) suggests that *P. cinereus* may inhabit all islands of the Thousand Islands region, though he only documents populations on two. *Plethodon cinereus* most likely colonized the islands via floating debris or land bridges, since they are completely terrestrial (Hatt et al. 1948; Petranka 1998). The weak dispersal ability of these terrestrial salamanders most likely contributes to very low gene flow among populations (Slatkin 1987).

Low gene flow among populations allows them to drift to fixation for one morph or another (Wright 1943). Gene flow can be determined by calculating the Fixation Index

( $F_{ST}$ ), and values range from 0 to 1. Wright (1943; 1951) suggested a guideline for interpreting  $F_{ST}$  values, where populations with values less than 0.05 have little differentiation and those with values greater than 0.30 have very great differentiation. In a study comparing differentiation among islands in the Georgian Bay of Lake Huron, average  $F_{ST}$  for *P. cinereus* was 0.108. This was significantly higher than that of *Lithobates clamitans* ( $F_{ST} = 0.075$ ), a species that has a greater dispersal ability (Britton 1998). Other studies on differentiation among populations of *P. cinereus* report  $F_{ST} = 0.019$  (continuous forest; Cabe et al. 2007), and  $F_{ST} = 0.13$  (fragmented forests post-glaciation; Larson et al. 1984). Low dispersal abilities and moderate differentiation between mainland and island populations may facilitate the detection of phenotypic differences between mainland and island populations of this polymorphic species.

Selection may also drive differences among populations. Multiple studies on *P. cinereus* have suggested that selection favors the unstriped morph on islands and peninsulas (Highton 1977; King et al. 1997; Fisher-Reid et al. 2013). The unusually high number of reported unstriped populations on these islands and peninsulas suggests that there is potentially some selective advantage for the unstriped morph in isolated populations. Given the relatively few islands where morph frequency is known, it is likely that many more of the islands are monomorphic unstriped, or that the populations are more unstriped than their source population on the mainland.

The purpose of this study was two-fold. The first objective was to document morph frequencies on all but two US islands in the Great Lakes with a documented population of *P. cinereus*. This census provides future researchers studying speciation

mechanisms in *P. cinereus* morph frequency data for islands where the mechanisms of morphic and sympatric speciation can be tested in isolated populations. The second objective was to explore whether monomorphic island populations might be the result of selection or drift. I predicted that populations on islands would be more likely to be fixed for the unstriped morph if selection were acting, and that it would be more likely that monomorphic island populations would be the same morph as the dominant morph in the mainland population if genetic drift were acting. Additionally, I wanted to test for differences in morph proportion among island populations. To test this hypothesis, I compared morph frequencies of island populations to predicted values (based on morph frequencies from the mainland sites, see methods for example). Finally, island size, distance to mainland, distance to other islands, and elevation above the lake should affect the likelihood of fixation with farther, smaller, or taller islands more likely to be monomorphic. I predicted that increasing distances should decrease the likelihood of gene flow “rescuing” the loss of a morph (MacArthur and Wilson 1963), whereas small islands should support smaller populations, thus increasing the rate of fixation of one morph. Islands with higher elevation would have a lower chance of flooding and should harbor older populations, providing more time for the population to drift to fixation. The overriding null hypothesis is that no morph differences between paired island and mainland populations, or among islands of varying size, distance, and elevation, would be found.

## **Methods**

### Study Sites

I examined morph distribution of *Plethodon cinereus* on 30 islands and at 14 corresponding mainland sites (Table 1; Figures 1-4). At least two islands were surveyed in each of the Great Lakes, and each island had a mainland reference point. Since some islands are spatially distributed in archipelagos, those sites share the same mainland reference point (Table 2). Mainland reference points are located on the mainland nearest the island. High Island and Isle Royale are the only islands with reported *P. cinereus* populations that were not sampled, due to logistical and budgetary constraints.

### Morph Identification

Striped individuals were defined as having any dorsal stripe pattern and unstriped individuals were those where the dorsal stripe was completely absent. Specimens were not sexed or aged, because gender and morph have not been found to be correlated (Test and Bingham 1948; Highton 1959; Brown 1965; Lotter 1978), number of juveniles found does not differ by color morph (Test and Bingham 1948), and juveniles hatch with adult coloration (Highton 1975; Anthony and Pfingsten 2013). When comparing museum morph frequencies and field morph frequencies, time between collections was not corrected. While Gibbs and Karraker (2006) found a shift toward the unstriped morph at a rate of 0.06%/yr over the last 100 years over the species range, Brown (1965); Williams et al. (1968); Fisher-Reid et al. (2013); Moore and Ouellet (2014) have found no difference in morph frequencies of populations over time.

### Census Protocol- Museum and Literature

Morph frequencies were initially determined using literature records and museum specimens and remaining populations were directly sampled during field censuses (19 island and 13 mainland sites; Table 1). For color morph frequency estimation, I used all existing museum specimens and literature records from island sites that could be found (Table 1). Robust morph frequency estimates exist in the literature for two islands (Bois Blanc and South Bass) and small sample size literature records exist for one island (Rattlesnake). Approximately 3,500 museum specimens exist for 22 islands. Of those, nine islands have >100 specimens each (Table 1). Specimens are located at the Cleveland Museum of Natural History, University of Michigan Museum of Vertebrate Zoology, Michigan State University Museum, Central Michigan University Museum, Carnegie Museum of Natural History, Milwaukee Public Museum, Illinois Natural History Survey, Cornell University Museum of Vertebrate Zoology, and University of Wisconsin-Steven's Point Museum.

### Census Protocol- Field

After consideration of the museum specimens, 13 mainland sites and 19 islands remained for field study. Study populations that had fewer than 100 museum specimens were censused during the field season. These field sites were distributed throughout the Great Lakes (Table 2). St. Martin and Squaw Islands (Lake Michigan) were not sampled during the field season. These islands have <100 specimens in museum collections (St. Martin, n = 3; Squaw, n = 93), but were excluded from the field census due to budget and logistical challenges associated with sampling the islands.



During field collection, the first 100 individuals discovered were identified as striped or unstriped. At study sites where 100 individuals could not be found, the number of individuals found in 18 search hours was added to the number of museum specimens (if any) for that site to estimate morph frequency. One hundred individuals were desired from each sample site to increase sample accuracy (Test 1952), but in some locations that was not possible. Permits and permission were secured before the start of field data collection.

### Permits

Scientific Collection Permits were secured from Apostle Islands National Lakeshore, Michigan DNR, New York DNR, and Ohio DNR. A Public Land Use Permit was secured from Michigan as well and a Temporary Revocable Permit from New York. A dispersed camping permit was used when camping in Michigan State Forests and camping permits were secured when camping in Apostle Islands National Lakeshore.

### Statistical Analyses

#### *Are islands more likely to become fixed for either morph?*

If genetic drift is the primary factor driving fixation, then island populations should be more likely than mainland populations to be monomorphic. To test this hypothesis, I categorized all island and mainland sites as either monomorphic (irrespective of morph) or polymorphic. I then employed a  $2 \times 2 \chi^2$  Contingency Analysis to determine whether, among all sample sites, a greater number of island populations were monomorphic than mainland populations.

*Are there more monomorphic unstriped islands than expected?*

A primary goal of the research was to determine whether more islands had monomorphic unstriped populations, as would be expected if selection were the primary factor driving fixation. For this analysis, I excluded islands with monomorphic source populations, because these populations lack the variation required for either drift or selection to operate. This resulted in the exclusion of 11 islands, all of which were monomorphic striped. I used a  $\chi^2$  Goodness of Fit test, in which the remaining monomorphic islands were categorized as either unstriped or striped, to test the hypothesis that more than one half of the monomorphic islands were unstriped. I assumed that under random genetic drift, a population was equally likely to become fixed for either the striped or unstriped morph.

*Does mainland morph frequency predict island morph frequency?*

I used a  $\chi^2$  Goodness of Fit test to determine whether island morph frequency differed from expected values generated from mainland frequencies. I calculated test values in the following manner: The observed island values were the number of individuals of each morph that were observed and the expected values were derived from mainland proportions. For example, Mackinac Island (MI) was 70% striped based on 127 individuals observed. The paired mainland was 98% striped. For the calculation, the observed island values were 89 (70%) striped and 38 (30%) unstriped. The expected island values based on the mainland morph frequencies were 124 (98%) and 3 (2%).

*Do island attributes predict the amount of difference in morph frequencies between islands and their paired mainland populations?*

I used  $\chi^2$  test statistics for each island-mainland pair as the dependent variable in a multiple linear regression model in SPSS v22 (IBM Corp 2013). I used the  $\chi^2$  test as a way to calculate a phenotypic proxy of the  $F_{ST}$  values used when comparing genetic differentiation among populations. In a similar manner as  $F_{ST}$  values, a higher  $\chi^2$  value in these tests suggests greater ‘differentiation’ between island and source populations. The goal of the regression model was to determine whether greater differences in morph frequencies between island and mainland populations was related to any of the island attributes measured (elevation, distance, etc). I used a backward elimination for model selection to determine which island attribute(s) significantly predicted the magnitude of morph frequency differences. The dependent variable ( $\chi^2$  value) for the multiple regression was not normally distributed, so I log-transformed the data to fit the assumptions of the test.

An additional method for exploring the influence of an island attribute on morph frequency differences between paired island and mainland sites is to employ a Weighted Least Squares (WLS) regression model. This model uses raw differences in morph proportions (the proportion of striped individuals on the mainland minus the proportion of striped individuals on the island). Because some of these differences in morph proportions are necessarily negative (e.g., if there was a higher proportion of striped salamanders on the mainland compared to the adjacent island), these values cannot be used as a proxy for  $F_{ST}$ . However, negative values are informative in that they indicate cases where one or the other morph may have increased frequency on an island. Based on

the results of the regression using  $\chi^2$  values, I used the WLS regression to test whether increased island height favored populations that were more striped or unstriped than their paired mainland. The slope of the model indicated whether the striped or unstriped morph was favored on taller islands (positive slope = striped favored, negative slope = unstriped favored).

### Island Attributes

The island attributes that were used in the multiple regression were geographic distance to the mainland, stepping stone index, island area, island perimeter, and max elevation of the island above the water level. All island attributes were measured using ArcMap 10.2 and Google Earth 7.1.2.2041 (ESRI 2014; Google Earth 2014). The distance of each island from the mainland was calculated as the straight-line distance from the nearest shore of each island to the shore of the mainland. The stepping stone index applies to islands that have neighboring islands between themselves and a mainland population. The method divides the area of a neighboring island by its distance to calculate an isolation index (Timoney 1983). When the shoreline of an island was traced in GIS, area and perimeter of the polygon were calculated. The maximum elevation of the island above lake water level was calculated as the maximum elevation of each island above sea level minus the current elevation of the water level of the respective lake above sea level.

## **Results**

### Island Census

I investigated 30 US islands in the Great Lakes with potential populations of *Plethodon cinereus* for polymorphism, and found 26 to have confirmed populations of *P. cinereus* (total= 4,832 salamanders from islands, 509 previously documented in literature; Table 1; Figure 1). This study provides robust morph estimates (>75 individuals) for 19 islands that were not previously reported in the literature (Table 3). No specimens could be obtained, nor field census be conducted on High Island and Isle Royale, so the presence and color morph frequency of *P. cinereus* on those islands remains unconfirmed. Of the 26 confirmed island populations, I report 15 with records or observations of the unstriped morph (Table 3). Nine of the 15 islands have polymorphic populations (uncommon morph comprises >1% of the population; Hedrick 2011; Table 3). These polymorphic islands occur in Lake Erie, Lake Huron, and Lake Michigan. I found no polymorphic populations on islands in Lake Superior or Lake Ontario. None of the polymorphic islands had an associated mainland site that was monomorphic, though overall five of the 14 mainland sites were monomorphic striped.

### Are Islands More Likely to Become Fixed for Either Morph?

The initial objective of this study was to determine whether more islands were monomorphic than mainland sites, as would be expected if genetic drift is the primary factor driving fixation. I found that overall there were not more monomorphic island populations (17 of 26) than monomorphic mainland populations (5 of 13). However, the

data did reflect a non-significant trend towards more monomorphic populations on islands than mainland sites ( $\chi^2$  Contingency Analysis;  $\chi^2=2.786$ ;  $p=0.095$ ; Figure 5).

#### Are There More Monomorphic Unstriped Islands Than Expected?

Alternatively, I wanted to know whether more islands had monomorphic unstriped populations than would be expected, suggesting that selection is the primary factor driving fixation. I categorized the islands in my study as monomorphic unstriped and monomorphic striped to determine if more than half of the monomorphic islands were unstriped. I predicted that if the unstriped morph were selected on islands, then more than half of the islands with monomorphic populations would be unstriped. There were not significantly more monomorphic unstriped populations than would be expected ( $\chi^2$  Goodness of Fit;  $\chi^2=0.667$ ;  $p=0.716$ ; Figure 6).

#### Does Mainland Morph Frequency Predict Island Morph Frequency?

An additional objective of the study was determining how well island morph frequencies fit the predicted frequencies derived from their mainland counterparts. I tested this with a  $\chi^2$  Goodness of Fit analysis. Given the assumptions of the test, I could only analyze island-mainland pairs where the mainland population was polymorphic. Of the 26 islands with confirmed populations, 15 have a paired mainland site that is polymorphic (Table 3). When I compared the island morph frequency to the mainland morph frequency, I found that 9 of the 15 sites differed significantly (Figure 7; Table 4).

## Do Island Attributes Predict Amount of Difference in Morph Frequencies Between Islands and Their Paired Mainland Populations?

I used the  $\chi^2$  values from my island to mainland comparisons as a proxy for the degree of phenotypic difference between island and mainland populations. The  $\chi^2$  value was used to estimate 'differentiation' similar to an  $F_{ST}$  value. Like  $F_{ST}$  values, I predicted that larger  $\chi^2$  values indicated greater difference and would be predicted by island attributes that isolated island populations from the mainland. The backward multiple regression model isolated only one variable that was significantly related to the  $\chi^2$  difference between island and mainland sites. Elevation above the lake significantly predicted the  $\chi^2$  difference between island and mainland sites (Multiple Linear Regression;  $y=0.050x-0.20$ ;  $F_{1,14}=10.244$ ;  $p=0.007$ ;  $R^2=0.441$ ; Figure 8). While the regression using  $\chi^2$  values indicated that taller islands were more different from the mainland than shorter islands were, the Weighted Least Squares regression indicated that the difference does not favor one morph over the other. The WLS regression failed to detect a significant correlation between island elevation above the lake and difference in morph proportion (WLS Regression;  $y=-0.001x+0.064$ ;  $F_{1,13}=0.321$ ;  $p=0.581$ ;  $R^2=0.024$ ; Figure 9).

## **Discussion**

### Interpreting Census Results in the Context of Drift and Selection

I expected to find more monomorphic unstriped populations and overall a higher percentage of unstriped individuals on islands than expected by chance alone. Previous literature describes parapatric striped and unstriped populations on Long Island, NY

(Fisher-Reid et al. 2013), a north to south cline from striped to unstriped populations on Cove Island, ON (F. Schueler pers comm) and the Del-Mar-Va peninsula (Highton 1977). Additionally, unstriped populations on Stockton Island, WI (Vogt 1981), South Bass and Rattlesnake islands, OH (King et al. 1997) and Middle Sister Island, ON (Oldham 1996) have been reported. These observations suggest that some attributes of islands or peninsulas favor the unstriped morph. Contrary to my hypothesis, I did not find many monomorphic unstriped island populations. The vast majority of islands that I sampled were either monomorphic striped or >80% striped. Only the Lake Erie islands were less than 70% striped. The most interesting observation about these islands is that while South Bass and Rattlesnake are monomorphic unstriped (King et al. 1997), I found nearby Kelley's Island to be ~ 50% striped.

In addition, I predicted that more than half of the monomorphic populations with polymorphic mainland populations would be unstriped. The  $\chi^2$  Goodness of fit analysis failed to detect a significant nonrandom pattern, providing no evidence that selection is acting in a similar way across all islands. Instead, it appears that the populations are being influenced by genetic drift. If isolated populations drift to fixation for one morph or the other (Wright 1943), we should expect to see that island populations are more monomorphic than mainland populations. I found that, while not statistically significant, there was a trend of more monomorphic island populations than mainland populations.

Since not all island populations are monomorphic, they may be subject to genetic drift. To test this, I used the  $\chi^2$  difference in morph frequencies between the islands and mainland sites to determine how different the two populations are. Larger  $\chi^2$  values can



be interpreted as analogous to high  $F_{ST}$  values, as high  $F_{ST}$  values indicate populations with low gene flow and longer separation time (Wright 1943; 1951). If isolated island populations are experiencing low gene flow and are subject to drift, then morph frequencies should differ more between populations that have been separated for longer periods. My findings of an increased difference in morph frequency between tall islands and their paired mainland suggests that drift may play a role in determining morph frequency.

Lake level variation, specifically the frequency and magnitude of water level changes, are factors that influence island occupancy by fauna in the Great Lakes (Hatt et al. 1948). Taller islands should be more resistant to periodic flooding and thus harbor older populations. Island populations that are isolated from the mainland for longer periods are expected to show evidence of genetic drift (Wright 1951). Populations on shorter islands have a higher chance of becoming extirpated as water levels rise. Rising water shrinks the area of the island and populations on smaller islands are more susceptible to extinction (MacArthur and Wilson 1963). In extreme cases, some of the islands were completely flooded and any population occurring before flooding was likely extirpated from the island. These islands may have populations today because of recent colonization events following extinctions, and as such, have had less time to differentiate from the mainland population (Wright 1943; 1951).

My results suggest that selection is not acting in the same direction across these islands, but rather that genetic drift plays a stronger role in determining morph frequencies. If selection were acting similarly on these populations, and one morph was

avored over the other on taller islands, then one might argue that some factor, intrinsic to taller islands, favors that morph. This is not what I found, as the WLS regression model failed to detect a trend toward one morph or the other ( $p > 0.05$ ). Thus, variations between island and mainland morph frequencies are likely explained by random drift.

Different selective pressures operating on individual islands may explain my results as well. For example, selection might favor one morph on one island, another morph on a different island, and a balanced polymorphism on a third island. The resulting pattern would be difficult to distinguish from genetic drift. In allopatric *Oophaga pumilio* populations in Bocas del Toro, Panama, each island exhibits a different color morph that has arisen by selection acting in different directions (Lande 1980; Wang and Shaffer 2008). These populations have been separated from the mainland for roughly the same amount of time as the Great Lakes islands (~10,000 years; Anderson and Handley 2002), but show much greater phenotypic variation among populations. In the Great Lakes, polymorphic island populations of garter snakes (*Thamnophis sirtalis*) in Lake Erie have highly variable morph frequencies. These variable morph frequencies are thought to be influenced by the confluence of selection and drift (Lawson and King 1996).

Additionally, populations of Lake Erie Watersnake (*Nerodia sipedon insularium*) exhibit a polymorphism on the Lake Erie islands. This is thought to be maintained by selection for a novel morph on the islands, balanced by gene flow from the mainland (King and Lawson 1997). It is possible, though unlikely, that some populations of *P. cinereus* have higher gene flow with the mainland as well. Unlike the highly aquatic water snake, *P. cinereus* are completely terrestrial and likely have poor dispersal over

water (Hatt et al. 1948; Schmiegelow and Nudds 1987). Additional support for the inability of *P. cinereus* to disperse over water comes from a study by Britton (1998) showing that island populations of *P. cinereus* were more genetically isolated from one another than similar populations of the highly aquatic green frog.

### Colonization

*Plethodon cinereus* was found on islands that were at one point connected to the nearby mainland as well as on islands that have not been connected to the mainland or other populated islands since they were last flooded. This suggests that the salamanders likely colonized over both land and water. However, water may be a greater limitation for these completely terrestrial salamanders. Of four completely terrestrial reptile and amphibian species found on islands in the Great Lakes, two (*P. cinereus* and *Lampropeltis triangulum*) “differ slightly from the mainland forms”, while the remaining 11 semi-aquatic species did not. The differentiation suggests these terrestrial species reached the island via a land bridge and have been isolated from the mainland population since (Hatt et al. 1948). Additionally, garter snake populations on the Lake Erie islands have had reduced dispersal abilities since water levels isolated the islands (Lawson and King 1996). Thus, populations that have been separated by water for long periods may have very low gene flow from mainland source populations.

Additional support for isolation was observed in the Apostle Islands archipelago. On these islands, I found moderate numbers of individuals for my study sites (discovery rate 2-5 ind/ workhour) immediately adjacent to islands with no confirmed populations (Table 6). This is similar to survey results from the Beaver Island Archipelago. *Plethodon*

*cinereus* was found on Beaver and Squaw islands, but not on Trout or Gull islands, despite similar sampling effort and suitable habitat (Hatt et al. 1948). In the Apostle Islands, I systematically sampled islands over a 10 day period, and recorded islands with no *P. cinereus* interspersed among those with *P. cinereus*. Lake Superior appears to have had no low water periods since the retreat of the glaciers, so it seems unlikely that the islands were colonized via land bridges. Oak Island is the tallest (150m acl), and was likely never flooded by the high water level 10-8,000 ybp (Lake Minong; Breckenridge et al. 2010). The other inhabited islands in the Apostle Islands (Basswood, Stockton, Otter, and Bear) were likely exposed 7-8,000 ybp (Lake Houghton; Breckenridge et al. 2010; Table 5). Populations found in the Apostle islands were likely colonized over water, which may explain the limited occurrence of *P. cinereus* on the islands.

#### Lake History and Island Age

The history of the Great Lakes islands (see Appendix) matches closely to the results of the linear regression model. Based on the results of the multiple regression, one would expect that the taller islands would have older populations than shorter islands, and thus younger islands should be less different from their paired mainland population. Squaw, Garden, North, Rattlesnake, Hog, and Marion islands did not differ in morph proportion from their mainland source. Squaw and North islands have the youngest earliest possible colonization date, 5,500 ybp and 4,500 ybp respectively (Table 5; Kelley and Farrand 1967; Larson and Schaetzel 2001), and Hog, Squaw, and Garden are the shortest inhabited islands in the Beaver Archipelago (Michigan). None are >20 m acl. It is possible that a shrinking of island area during the high water period 5,500 ybp may

have led to an extirpation of any population that may have been on those islands. A population found there today may be the result of an over-water colonization event <5,500 ybp and thus would be similar to the mainland source population. Beaver Island is the largest and tallest island in the Beaver Archipelago and may have harbored a population during the high water period 5,500 ybp. The population on this island may be as old as 11,800 years, leaving more time for the population to drift to fixation. Marion Island is a seeming outlier in the multiple regression analysis. While Marion Island is a tall island, it is one of the closest islands to the mainland. The close proximity to the mainland may provide less of a barrier to gene flow than farther islands.

#### Census Oddities

On Stockton Island (Superior), only one unstriped *P. cinereus* has been documented out of 99 total individuals. This suggests that there is not an unstriped population on Stockton Island, contrary to the Vogt (1981) description of an entire unstriped population on the island. On Sugar Island (Superior), I documented one unstriped individual out of 106 total salamanders, while no unstriped individuals have been documented in the mainland population. As I did not sample on the Canadian side of the island, it is possible that the source population is Canadian, or that individuals came from the mainland on both sides. The two nearest known Canadian populations of *P. cinereus* are 70-85% striped (Moore and Ouellet 2014), so it is possible that the nearest mainland population on the Canadian side is polymorphic. The final oddity that I noted is the size and location of the *P. cinereus* population on North Island (Huron). On this island, only 15 individuals were found, and they were concentrated near the shore on

the north side of the island. This is suggestive of a recent colonization event where individuals or eggs rafted to the island and were washed up on the shoreline (as suggested by Hatt et al. 1948).

A further incongruence arises when comparing the morph frequencies from my study to a recent morph distribution map (Moore and Ouellet 2014). This map indicates a population comprised of 6-15% unstriped individuals in the Apostle Islands. All of the island or mainland sites I surveyed were monomorphic striped populations. This is an interesting discordance, as I have found no record of a polymorphic population within at least 300 kilometers of those sites. Another interesting site is located on the mainland adjacent to Drummond Island. I sampled Drummond Island in four different localities and found no *P. cinereus*. Since I had no records from the island, I did not census the mainland site. Moore and Ouellet's (2014) distribution map indicates an additional population with 6-15% unstriped individuals on the mainland. The nearest other record of polymorphic populations are in the southern tip of the Upper Peninsula of Michigan, near St. Ignace (Test 1952). This is the approximately 50 km west of the new location near Drummond Island. This may be reasonable, however, as I documented a polymorphic population (98% striped) approximately 70 km west of St. Ignace. The final point of interest on the distribution map occurs on the Ontario side of the St. Lawrence River in the Thousand Islands region. The distribution map indicates a population that is >50% unstriped immediately across the river from two of my study sites. My census indicated that both populations were monomorphic striped, though one unstriped individual was noted on the mainland site. This locality would benefit from increased sampling, as that is

a very large morph difference over a very short geographic distance. This would not be the first time large morph differences have been documented over short geographic distances. In the Del Mar Va peninsula, changes in morph frequency over short distances may be attributed to historical flooding events that isolated populations on islands that are now exposed in a continuous tract of land (Highton 1977).

### Future Studies of Speciation Mechanisms

My census identified nine islands that are polymorphic. All of these polymorphic islands have paired mainland sites that are also polymorphic. This suggests that the uncommon morph likely dispersed from the mainland population and did not arise on its own. Populations on these islands could provide an excellent system in which to study speciation mechanisms, especially in sympatry. They provide multiple isolated populations with varying frequencies of striped individuals in which many interesting questions about sympatric speciation could be addressed.

In addition to the nine polymorphic islands, six islands have monomorphic populations, while their paired mainland site had polymorphic populations (Table 3). This suggests that the uncommon morph may have been present at some point following colonization, but has since gone extinct. These islands provide interesting populations where morphic speciation could be explored. Alternatively, individuals carrying the genes for the uncommon morph may not have been among the individuals that colonized the island. This may be true for Beaver Island and Squaw Island, given that their paired mainland population is comprised of 2% uncommon morph (Table 3). This seems unlikely, however, because many of the other polymorphic islands have a mainland

population where the uncommon morph makes up 2% of the population. Of these, North Manitou, South Manitou, and Mackinac are significantly more unstriped than the mainland. Additionally, individual clutches of *P. cinereus* have been shown to contain individuals of both morphs (Highton 1975), so a single colonization event by a gravid female could establish both morphs on an island.

Finally, comparisons between the nine polymorphic islands and six monomorphic islands with paired polymorphic mainland sites would be a good place to try to tease apart questions about morphic speciation. For these studies, I would recommend all but North Island and Marion Island because of the low number of individuals on those islands (Table 3). Ultimately these 15 islands with paired polymorphic mainland sites would likely be the most interesting sites in which to focus efforts on further understanding speciation mechanisms within *P. cinereus*.

#### Further Research

A follow-up study using genetic material to confirm the mainland source population for each island would be interesting to pursue. The assumption that individuals colonized from the nearest mainland may be false, and genetic methods would help to either confirm or disprove this assumption. Additionally, a follow up study using genetic material could determine the age of populations on each of the islands and whether my conclusion that taller islands harbor older populations is correct. This method could also be used to confirm whether populations on taller islands are indeed more differentiated from mainland populations. These island populations may also be of



interest to conservation geneticists to compare differentiation among populations in fragmented forest habitats to differentiation between mainland and island populations.

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**Table 1.** Source and number of specimens for morph frequency estimation for each mainland, (denoted MNLD) and island population. Museum specimens came from the University of Michigan Museum of Zoology (UMMZ), Michigan State University Museum (MSUM), Central Michigan University Museum (CMU), Illinois Natural History Survey (INHS), University of Wisconsin- Stevens Point (UWSP), Carnegie Museum of Natural History (CM), Milwaukee Public Museum (MPM), Cornell University Museum of Vertebrate Zoology (CUMV), and Cleveland Museum of Natural History (CMNH).

State	Island	Museum	Source	Literature	Source	Field
MI	Beaver	876	UMMZ, MSUM, CMU	-	-	-
MI	Garden	192	UMMZ, MSUM, CMU	-	-	-
MI	Hog	247	UMMZ	-	-	-
MI	Squaw	93	UMMZ	-	-	-
MI	MNLD	-	-	-	-	51
MI	Bois Blanc	314	UMMZ	-	-	-
MI	MNLD	-	-	133	Test 1952	10
MI	Mackinac	127	UMMZ	-	-	-
MI	MNLD	-	-	846	Test 1952	30
MI	Sugar	6	UMMZ	-	-	100
MI	MNLD	31	UMMZ	-	-	25
MI	Marion	1	UMMZ	-	-	87
MI	MNLD	-	-	-	-	100
MI	N Fox	314	UMMZ	-	-	-
MI	S Fox	491	UMMZ	-	-	-
MI	MNLD	-	-	-	-	100
MI	N Manitou	317	UMMZ	-	-	-
MI	S Manitou	368	UMMZ	-	-	-
MI	MNLD	-	-	-	-	62
MI	Au Train	4	INHS	-	-	100
MI	MNLD	-	-	-	-	100
MI	St. Martin	3	UWSP	-	-	-

MI	Summer	4	UWSP	-	-	100
MI	MNLD	3	UMMZ	-	-	23
MI	Drummond	0	-	-	-	0
MI	MNLD	X	-	-	-	X
MI	North	0	-	-	-	16
MI	MNLD	5	UMMZ	-	-	-
WI	Stockton	72	CM, MPM	27	Casper 1996	100
WI	Basswood	2	MPM	55	Casper 1996	37
WI	Bear	1	MPM	31	Casper 1996	48
WI	Oak	1	MPM	15	Casper 1996	72
WI	Otter	1	MPM	-	-	100
WI	Hermit	0	-	-	-	0
WI	Manitou	0	-	-	-	0
WI	Raspberry	0	-	-	-	0
WI	MNLD		-	-	-	54
NY	Wellesley	2	CUMV	-	-	32
NY	Douglas	2	CUMV	-	-	-
NY	MNLD	-	-	-	-	78
OH	S Bass	-	-	376	Pfingsten and Walker 1978; Reichenbach 1981; King et al. 1997	-
OH	Kelley's	2	CMNH	-	-	100
OH	Rattlesnake	-	-	5	King et al. 1997	-
OH	MNLD	78	CMNH	-	-	-
-	Total	3557	-	1488	-	1525

**Table 2.** Sampling localities for island and mainland populations of *Plethodon cinereus*.

Sites with one asterisk were surveyed with only museum specimens and literature

records. Sites with two asterisks were sampled and no *P. cinereus* were found.

Lake	Island	Paired Mainland Reference Site	State
Superior	Stockton, Basswood, Bear, Oak, Otter, Hermit**, Manitou**, Raspberry**	Apostle Islands Nat'l Lakeshore	WI
Superior	Au Train	Gwinn State Forest Area	MI
Michigan	Beaver*, Garden*, Hog*, Squaw*	Sault St. Marie State Forest	MI
Michigan	N. Fox*, S. Fox*	Leelanau State Park	MI
Michigan	N. Manitou*, S. Manitou*	Traverse City State Forest Area	MI
Michigan	Summer, St. Martin*	Shingleton State Forest Area	MI
Michigan	Marion	Pyatt Lake	MI
Huron	Mackinac*	Straits State Park	MI
Huron	Bois Blanc*	Gaylord State Forest Area	MI
Huron	Sugar	Methodist Mission Reserve	MI
Huron	Drummond**	N/A	MI
Huron	North	Albert E. Sleeper State Park*, Weale Rd State Game Area**	MI
Erie	South Bass*, Kelley's, Rattlesnake*	Catawba Island*	OH
Ontario	Wellesley, Douglas*	Keewaydin Point State Park	NY

**Table 3.** Island and paired mainland morph frequencies for all localities sampled. Islands in the first group are polymorphic, those in the second and third group are not considered polymorphic. Islands in the fourth group consist of those islands where *P. cinereus* could not be found. Within each subgroup, islands with polymorphic mainland sites are listed first. A site is considered polymorphic if >1% of the uncommon morph was found.

Island	Site Number	Basin	State	N	Percent Striped	Paired Mainland Site	Site Number	N	Percent Striped
Kelley's	38	Erie	OH	102	47	Catawba	39	78	8
Mackinac	15	Huron	MI	127	70	Straits State Park	16	876	98
S Manitou	31	Michigan	MI	368	71	Traverse City SFA	32	62	98
Bois Blanc	17	Huron	MI	314	83	Gaylord SFA	18	143	89
North	34	Huron	MI	16	88	Albert E. Sleeper SP	35	5	80
N Manitou	30	Michigan	MI	317	93	Traverse City SFA	32	62	98
Garden	21	Michigan	MI	192	97	Sault Ste. Marie SFA	19	51	98
Hog	22	Michigan	MI	247	98	Sault Ste. Marie SFA	19	51	98
Marion	33	Michigan	MI	88	98	Pyatt Lake	34	100	98
Beaver	23	Michigan	MI	876	99	Sault Ste. Marie SFA	19	51	98
Sugar	13	Huron	MI	106	99	Methodist Mission Reserve	12	56	100
Basswood	5	Superior	WI	94	99	Apostle Islands NL	1	54	100
Oak	4	Superior	WI	88	99	Apostle Islands NL	1	54	100
Stockton	8	Superior	WI	199	99	Apostle Islands NL	1	54	100
S Bass	37	Erie	OH	376	0	Catawba	39	78	8
Rattlesnake	36	Erie	OH	5	0	Catawba	39	78	8
N Fox	27	Michigan	MI	314	100	Leelanau SP	29	100	72



S Fox	28	Michigan	MI	491	100	Leelanau SP	29	100	72
Squaw	20	Michigan	MI	93	100	Sault Ste. Marie SFA	19	51	98
Big Summer	25	Michigan	MI	104	100	Shingleton SFA	24	26	100
St. Martin	26	Michigan	MI	3	100	Shingleton SFA	24	26	100
Douglas	42	Ontario	NY	2	100	Keewaydin Point SP	41	78	99
Wellesley	40	Ontario	NY	34	100	Keewaydin Point SP	41	78	99
Au Train	11	Superior	MI	104	100	Gwinn SFA	10	100	100
Bear	3	Superior	WI	79	100	Apostle Islands NL	1	54	100
Otter	6	Superior	WI	101	100	Apostle Islands NL	1	54	100
Drummond	14	Huron	MI	0	N/A	N/A	N/A	N/A	N/A
Hermit	9	Superior	WI	0	N/A	Apostle Islands NL	1	54	100
Manitou	7	Superior	WI	0	N/A	Apostle Islands NL	1	54	100
Raspberry	2	Superior	WI	0	N/A	Apostle Islands NL	1	54	100

**Table 4.**  $\chi^2$  Goodness of Fit values for each paired island mainland analysis. The expected value for each pair was based on the proportion of striped individuals in the nearest mainland (assumed source) population. Islands whose paired mainland was not polymorphic were excluded from the analysis. Nine islands differed significantly from their paired mainland site and six did not vary significantly from their paired mainland locality.

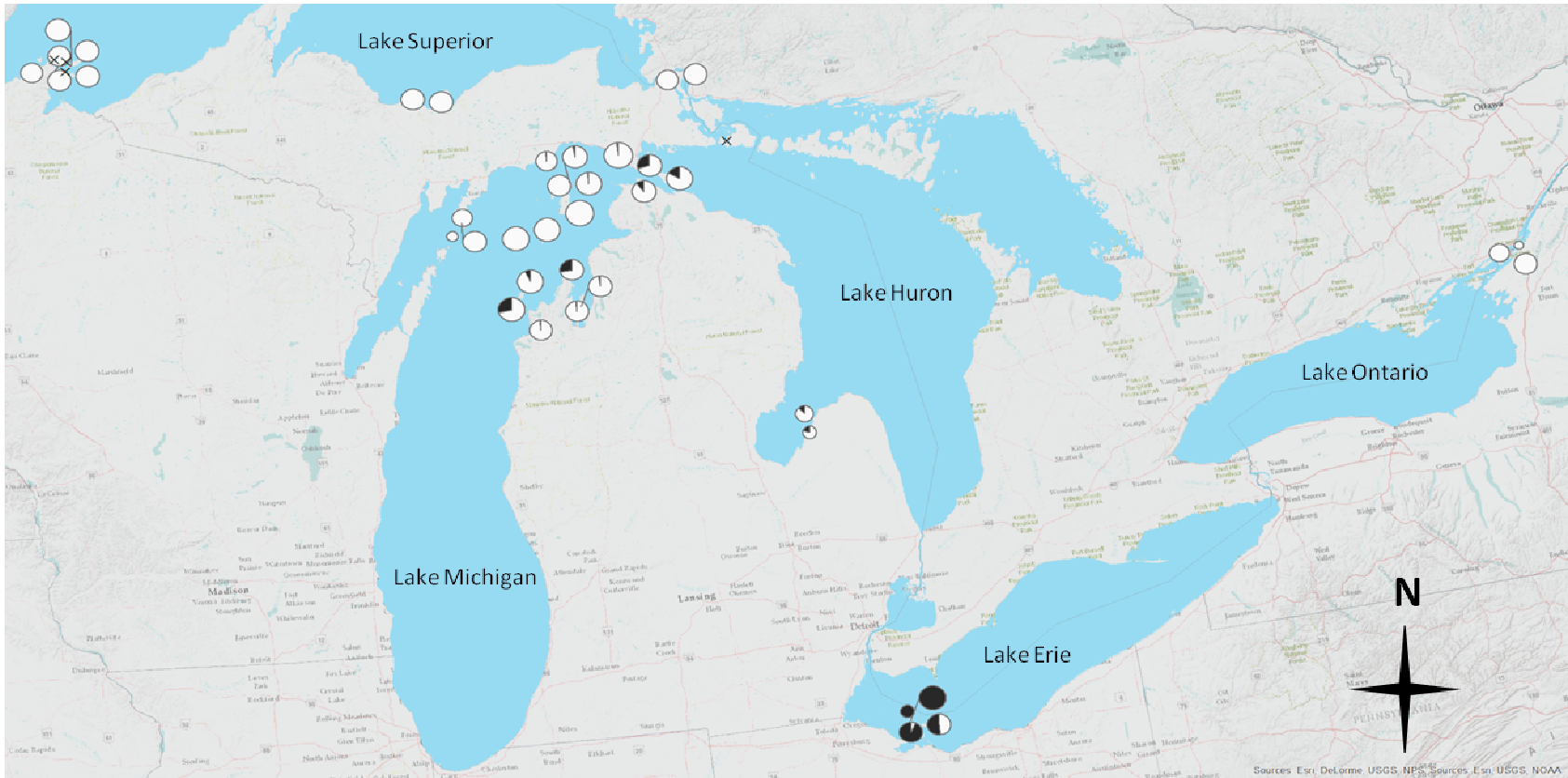
Island	$\chi^2$ value	p Value
South Manitou	1714.61	p<0.0001
Mackinac	559.04	p<0.0001
Kelley's	222.62	p<0.0001
South Fox	190.94	p<0.0001
North Fox	122.11	p<0.0001
North Manitou	50.17	p<0.0001
South Bass	29.20	p<0.0001
Bois Blanc	10.23	p=0.001
Beaver	6.15	p=0.013
Squaw	1.86	p>0.05
North	0.56	p>0.05
Garden	0.41	p>0.05
Rattlesnake	0.41	p>0.05
Hog	0.15	p>0.05
Marion	0.03	p>0.05

**Table 5.** Land bridge timeline based on current lake bathymetry and history of Post-Pleistocene lake water level fluctuation values (NGDC 2014). Earliest over land columns describes earliest time post-glacial retreat that water levels may have been low enough to create a land bridge between island and mainland sites. Latest over land column is based on the latest low water levels that may have exposed a land bridge between island and mainland sites before waters reached current levels. Islands with a dash in these columns have not had water levels low enough to form a land bridge since their last exposure.

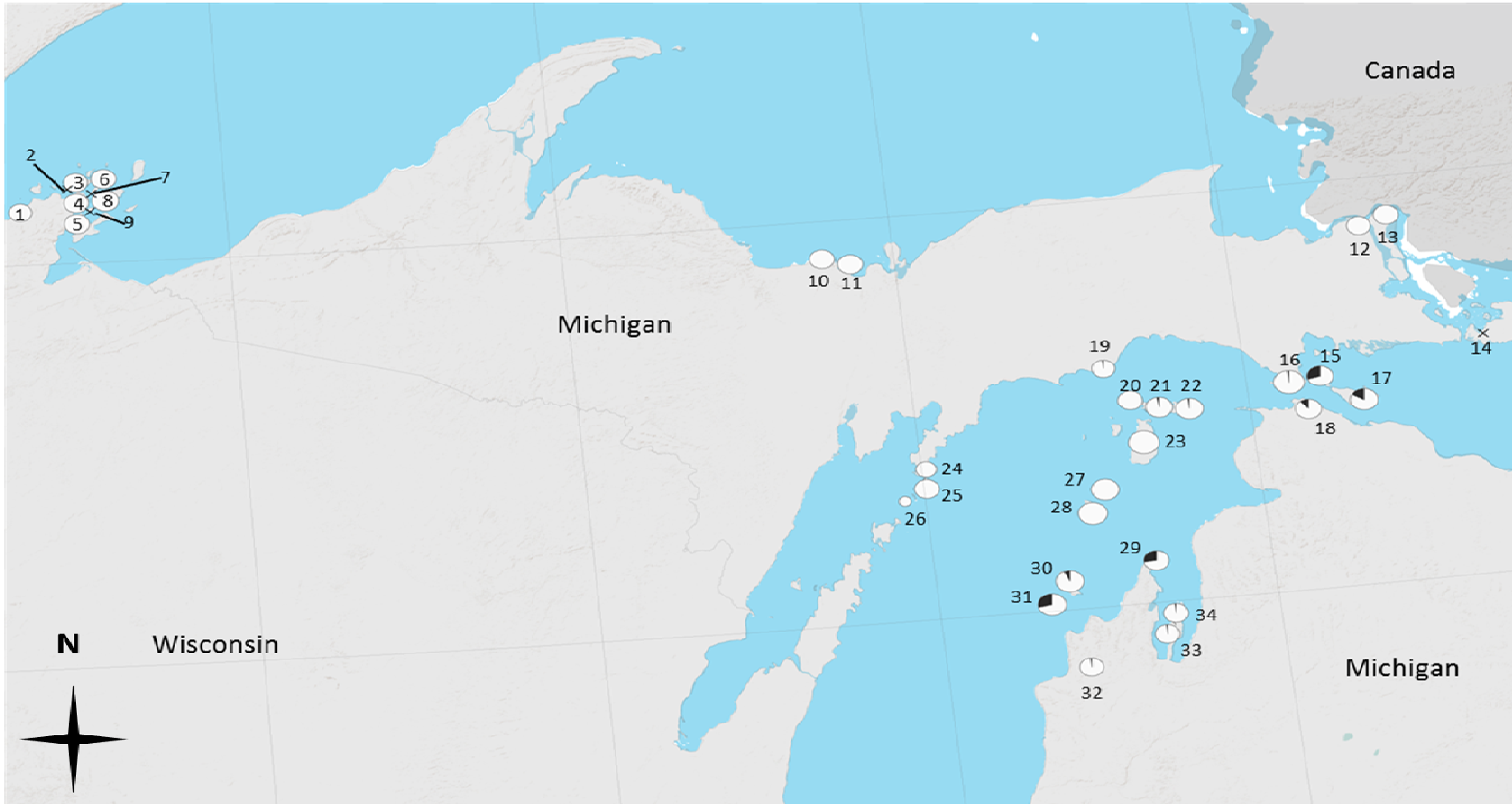
Island	Basin	Elevation Above Lake (m)	Min Depth for Land Bridge (m)	Earliest	Earliest Over Land	Latest Over Land
Kelley's	Erie	18.9	10	12,000	12,000	4,000
S Bass	Erie	21.1	10	12,000	12,000	4,000
Rattlesnake	Erie	10.1	15	12,000	12,000	4,000
Mackinac	Huron	98	75	11,800	10,000	5,000
Bois Blanc	Huron	30.3	25	11,800	10,000	5,000
North	Huron	2.8	5	4,500	-	-
Sugar	Huron	94.8	-	8,000	-	-
S Manitou	Michigan	126.9	25	11,800	10,000	9,500
N Manitou	Michigan	124.5	40	11,800	10,000	9,500
Garden	Michigan	20	40	11,800	10,000	9,500
Hog	Michigan	11.5	40	11,000	10,000	9,500
Marion	Michigan	47.4	15	11,800	10,000	9,500
Beaver	Michigan	64.7	40	11,800	10,000	9,500
N Fox	Michigan	52.6	120	11,800	10,000	9,500
S Fox	Michigan	103.5	120	11,800	10,000	9,500
Squaw	Michigan	7.2	40	5,500	-	-
Big Summer	Michigan	36.4	5	11,800	10,000	6,000
St. Martin	Michigan	25.2	30	11,800	10,000	9,500
Douglas	Ontario	11.6	5-10	14,000	13,500	4-5,000
Wellesley	Ontario	45.2	5-10	14,000	13,500	4-5,000
Basswood	Superior	61	15	8,000	-	-
Oak	Superior	150.4	15	10,000	-	-
Stockton	Superior	63.8	30	8,000	-	-
Au Train	Superior	35.5	25	7,000	-	-
Bear	Superior	74.5	15	8,000	-	-
Otter	Superior	46.5	15	8,000	-	-

**Table 6.** Rate of discovery for sites sampled during the 2014 field season. Sites are separated by state. Within each state, sites are sorted by rate of discovery.

State	Site	Date Sampled	Field n	Work Hours	Rate of Discovery (Ind/ Work HR)
WI	Stockton	6/27/14	100	18	5.6
WI	Otter	6/21/14	100	18	5.6
WI	Oak	6/22/14	72	18	4.0
WI	AINL MNLD	6/24/14	54	18	3.0
WI	Bear	6/20/14	48	18	2.7
WI	Basswood	6/29/14	37	18	2.1
WI	Hermit	6/28/14	0	18	0.0
WI	Manitou	6/26/14	0	18	0.0
WI	Raspberry	6/19/14	0	18	0.0
MI	Au Train	7/7/14	100	7.5	13.3
MI	Marion MNLD	6/4/14	100	7.5	13.3
MI	Au Train MNLD	7/5/14	100	9.75	10.3
MI	Sugar	7/10/14	100	14.25	7.0
MI	Big Summer	6/11/14	100	16.5	6.1
MI	NS Fox MNLD	6/1/14	100	18	5.6
MI	Marion	5/31/14	87	18	4.8
MI	NS Manitou MNLD	6/3/14	62	18	3.4
MI	Beaver Archipelago MNLD	6/9/14	51	18	2.8
MI	Mackinac MNLD	6/7/14	30	18	1.7
MI	Big Summer MNLD	6/13/14	23	18	1.3
MI	Sugar MNLD	7/9/14	25	27	0.9
MI	North	5/29/14	16	18	0.9
MI	Bois Blanc MNLD	6/6/14	10	18	0.6
MI	Drummond	7/12/14	0	18	0.0
MI	North Is MNLD	5/28/14	-	18	0.0
OH	Kelley's	5/5/14	100	10	10.0
NY	Wellesley MNLD	8/30/14	78	18	4.3
NY	Wellesley	8/29/14	32	18	1.8



**Figure 1.** Map of sites surveyed for *Plethodon cinereus*. Pie charts represent morph frequencies. Unfilled circles represent monomorphic striped populations and filled circles represent monomorphic unstriped populations. Partially filled circles represent polymorphic populations, with black representing unstriped proportion and white representing striped proportion. The sizes of the circles are scaled by log sample size. X's indicate sites where no *P. cinereus* have been found (based on field census and museum specimens).



**Figure 2.** Map of sites surveyed for *Plethodon cinereus* in Lake Superior, Lake Michigan, and northern Lake Huron. Pie charts represent morph frequencies. Numbers indicate individual sites (Table 3). X's indicate sites where no *P. cinereus* have been found (based on field census and museum specimens).

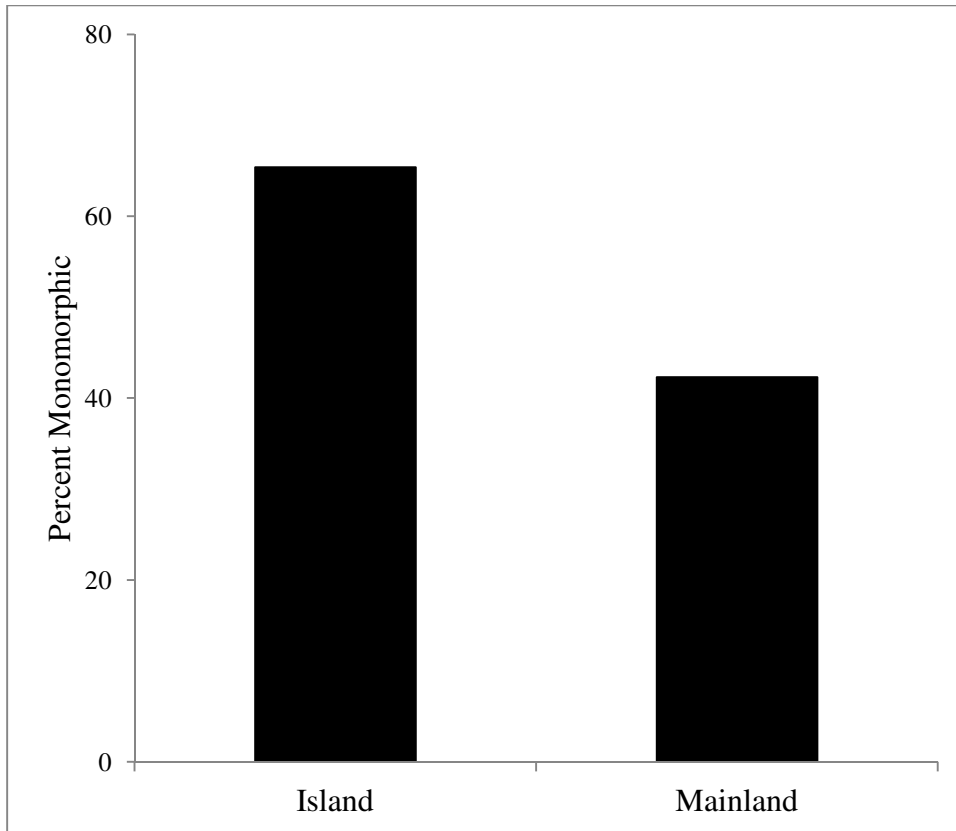


**Figure 3.** Map of sites surveyed for *Plethodon cinereus* in lower Lake Huron and Lake Erie. Pie charts represent morph frequencies. Numbers indicate individual sites (Table 3). X's indicate sites where no *P. cinereus* have been found (based on field census and museum specimens).



**Figure 4.** Map of sites surveyed for *Plethodon cinereus* in Lake Ontario. Numbers indicate individual sites (Table 3). Unfilled circles represent monomorphic striped populations and filled circles represent monomorphic unstriped populations. The sizes of the circles are scaled by log sample size.

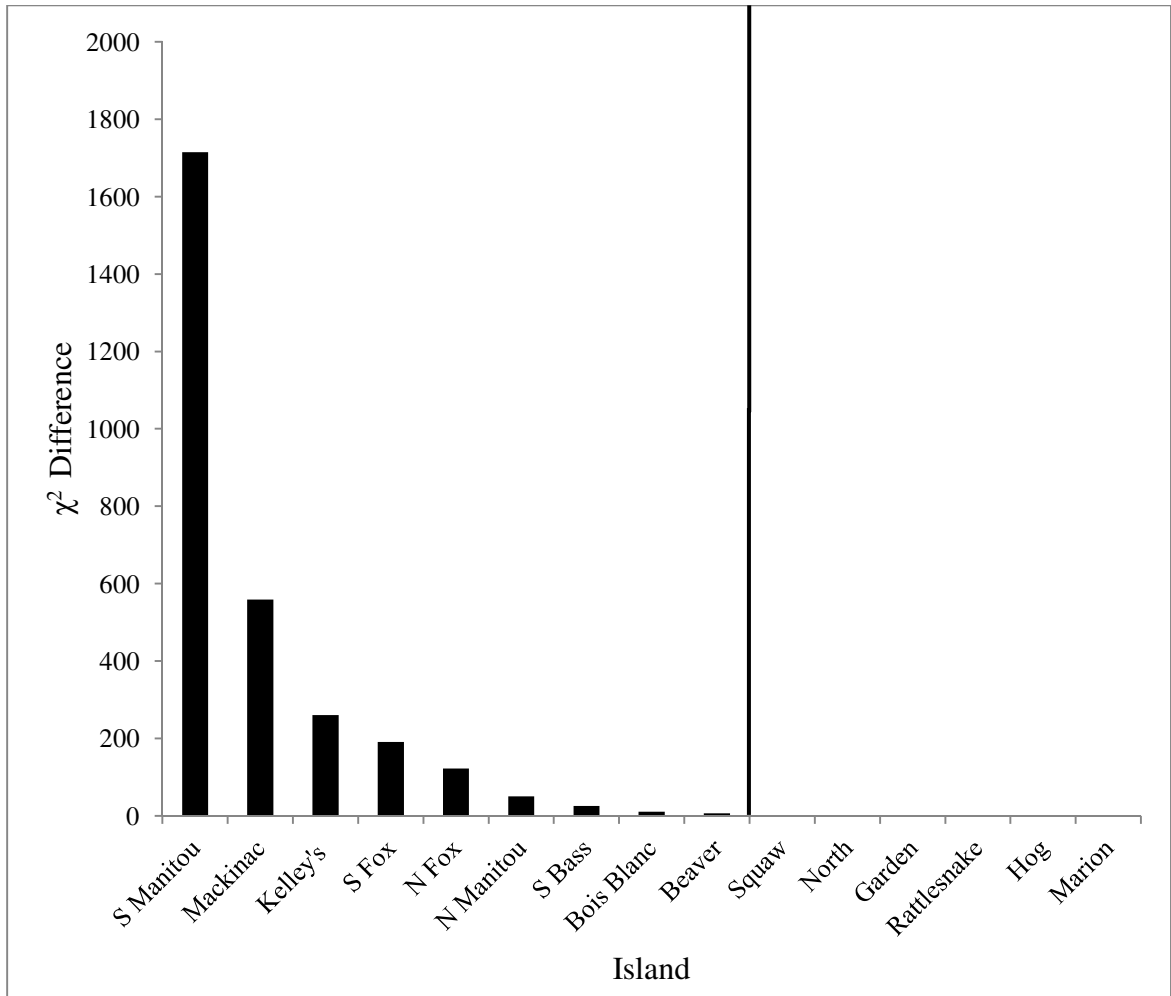




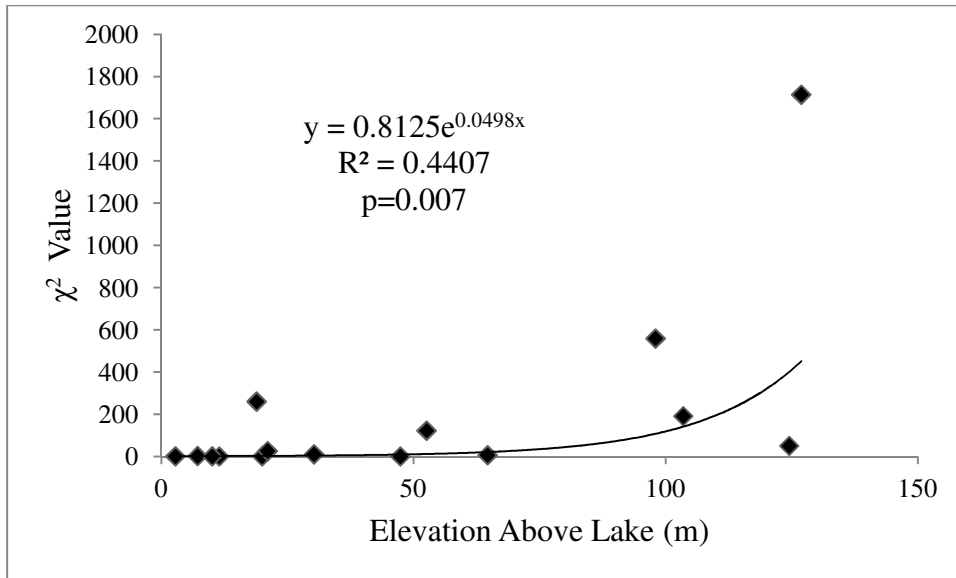
**Figure 5.** Percent of populations from mainland sites and island sites that were monomorphic. While the analysis used raw numbers, the data are portrayed as percentages to account for the uneven sample sizes. I found that island populations were not significantly more monomorphic than mainland populations ( $\chi^2$  Contingency Analysis;  $\chi^2=2.786$ ;  $p=0.095$ )



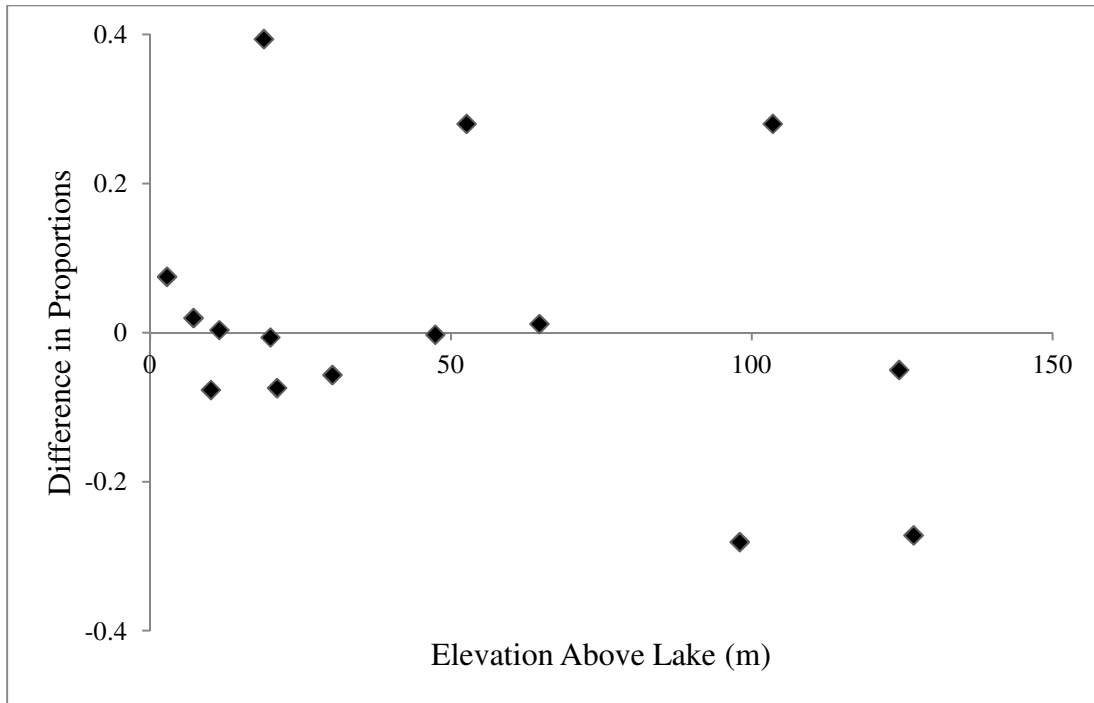
**Figure 6.** Number of monomorphic striped and unstriped populations occurring in the Great Lakes with an adjacent polymorphic mainland population. Contrary to my hypothesis, there were not more unstriped monomorphic populations than would be expected ( $\chi^2$  Goodness of Fit analysis;  $\chi^2=0.667$ ;  $p>>0.05$ )



**Figure 7.**  $\chi^2$  differences between island and paired mainland sites. Islands whose paired mainland sites were monomorphic were excluded from the analyses. Numbers of striped and unstriped individuals on islands were used as observed values and proportion of striped individuals at mainland sites were used to calculate expected frequencies in  $\chi^2$  tests. Values to the left of the vertical line indicate significant deviations from expected values ( $p < 0.05$ ), while values to the right of the line were not significant ( $p > 0.05$ ). A significant result indicated that island morph frequency was more different from the expected (adjusted using mainland morph frequency) than would be expected by random chance.



**Figure 8.** The maximum elevation of islands above lake level significantly predicts  $\chi^2$  difference between island and adjacent mainland populations for 15 islands with polymorphic mainland sites throughout the Great Lakes (Linear Regression;  $y=0.050x-0.0207$ ;  $F_{1,14}=10.244$ ;  $p=0.007$ ;  $R^2=0.441$ ). The  $\chi^2$  data were LN transformed to fit the assumptions of normality during analysis, so the figure shows the back-transformed data with an exponential trendline to represent the relationship between the two linear variables.



**Figure 9.** Difference in proportion of striped individuals between paired islands and their adjacent polymorphic mainland sites. A positive difference corresponds to a higher proportion of striped individuals on an island relative to its paired mainland site. The WLS regression detected no relationship between island elevation and proportional differences between islands and their corresponding mainland sites (Linear Regression;  $y = -0.001x + 0.064$ ;  $F_{1,14} = 0.321$ ;  $p \gg 0.05$ ;  $R^2 = 0.024$ ).

## **Appendix**

### Post-Pleistocene Geologic History of Great Lakes

The entire Great Lakes region was covered by glaciers as little as 15,000 years ago (Larson and Schaetzl 2001). As the glaciers retreated from the region, they underwent a series of re-advances, with each re-advancement smaller than the last. After the glaciers receded, water levels fluctuated due to the creation and breaking of ice dams, isostatic uplift, and downcutting (Larson and Schaetzl 2001). The islands in this study were exposed on a different timeline that corresponds to the lake basin and the elevation of the island.

Lake Erie was the first to be exposed by the receding glaciers. The last glacier retreated from the southwestern region of the lake roughly 14,500-14,000 ybp (Kelley and Farrand 1967; Larson and Schaetzl 2001). After the retreat of the glaciers, water levels remained about 20 m above current level (acl) until ~12,000 ybp (Kelley and Farrand 1967). The tallest of the Lake Erie island sites is 21 m acl and would have been flooded until this time. The lake then mostly dried up and refilled slowly to remain at or below current levels (bcl) for the next 11,800 years (Kelley and Farrand 1967). It reached a level that would have isolated the Lake Erie islands from the mainland roughly 4-5,000 ybp (Holcombe et al. 2003).

Lake Ontario was the next to be exposed, along with southern Lake Huron. Wellesley and Douglas islands (Lake Ontario) were likely exposed 13,000 ybp (Larson and Schaetzl 2001). Around 11,800 ybp, water levels dropped sharply and remained low until 4-6,000 ybp (Larson and Schaetzl 2001; Anderson and Lewis 2012). Around 4,000-

5,000 ybp, water levels reached current levels again (Anderson and Lewis 2012). After the glaciers retreated from southern Lake Huron 13,000 ybp (Kelley and Farrand 1967), the water levels fluctuated greatly until 4,500 ybp, when they rose to 7m acl for the last time (Karrow et al. 1975; Ellis and Deller 1986). Since then, water levels in lower Lake Huron have been at or below current levels.

The northern portion of Lake Huron and Lake Michigan were likely exposed no more than 11,800 years ago, as the glacier retreated from the lake basins (Mickelson et al. 1982; Schaetzl 2001; Larson and Schaetzl 2001). Water levels were 13 m acl in the Lake Michigan basin (Lake Chicago; Kelley and Farrand 1967; Table 5). The Lake Huron and Lake Michigan basins joined 11,000 ybp to create Lake Algonquin, and water levels evened out at 7-8 m acl (Kelley and Farrand 1967; Larson and Schaetzl 2001). Lake Algonquin drained about 10,000-9,500 and hit lows of 100 m bcl (Kelley and Farrand 1967). The water level peaked around 5,500 ybp, when it was ~8 m acl (Kelley and Farrand 1967; Larson and Schaetzl 2001). Lake levels returned to the current level 4,500 years ago and have had minor fluctuations since.

Glaciers began leaving the western portion of Lake Superior 10,000 ybp (Clayton 1983), and the remainder of Lake Superior was likely exposed as the glacier continued to retreat from the basin for the last time 9,500 ybp (Barnett 1992; Karrow et al. 2000; Larson and Schaetzl 2001). As the glacier retreated, the basin was filled and reached >100 m acl 8,000 ybp, but fell to ~45 m acl by 7,500-7,000 ybp (Breckenridge et al. 2010). Water levels continued to fluctuate and slowly fell to current levels (Kelley and Farrand 1967).