

**Assessing habitat suitability and connectivity for an endangered salamander
complex**

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Abstract

Assessing habitat suitability and connectivity for an endangered salamander complex

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Habitat loss and fragmentation have significantly contributed to amphibian population declines, globally. Evaluating the state of remaining habitat patches can prove to be beneficial in identifying areas to prioritize in conservation efforts. Pelee Island, Ontario is home to a complex of salamanders including small-mouthed salamanders (*Ambystoma texanum*), blue-spotted salamanders (*A. laterale*) and unisexual *Ambystoma* (small-mouthed salamander dependent population). These populations have declined from intense landscape changes since the late 1800s, particularly from the historical drainage of wetlands. In this thesis, I evaluated the suitability and connectivity of habitat patches occupied by these salamanders to assess the size of, and dispersal capabilities between, remaining habitat patches. I found that there was a low amount of suitable terrestrial habitat available for this complex of salamanders, and existing habitat patches were small and isolated. Forested areas and non-breeding wetlands were considered to be suitable habitat when adjacent to existing breeding locations, suggesting that these habitats should be a focus for conservation efforts. Notably, intervention may be necessary to maintain this amphibian complex as many assemblages are isolated from one another and potential corridors currently consist of primarily unsuitable habitat. Given that much of the salamander complex is reliant on one species for reproduction, the long-term viability of this population of *Ambystoma* salamanders may rely on the enhancement of suitable habitat near current breeding sites by conservation organizations

and local stakeholders. Ultimately, the approach used in this thesis emphasizes the value of evaluating habitat within a fragmented landscape to focus conservation efforts on imperilled species.

Keywords: Amphibians, landscape fragmentation, habitat suitability, connectivity, landscape resistance, unisexual

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Chapter 1: General Introduction

Habitat loss and fragmentation are among the main causes of ecosystem disruption, including eliciting changes in the distribution and abundance of native plants and animals (Mantyka-Pringle et al. 2012; Haddad et al. 2015). Loss of available habitat, particularly through the fragmentation of larger habitat patches into small areas with low connectivity to other suitable areas, is detrimental for the persistence of many populations and metapopulations (Debinski and Holt 2000). Contemporary habitat loss and fragmentation are primarily caused by anthropogenic disturbances, including the conversion of natural ecosystems to agricultural and urban development and the construction of roadways and other geographic barriers that limit dispersal and genetic exchange (Hoekstra et al. 2005; Cristine and Kerr 2011). Impacts of habitat loss and fragmentation are aggravated in species with restrictive habitat requirements or that have low mobility or dispersal ability (Vos et al. 2002), and it follows that modelling the potential spatial distribution of such species across a range of habitat loss and fragmentation scenarios is crucial for prioritizing conservation planning efforts targeting habitat protection and restoration.

Effects of Habitat Fragmentation and Loss on Species Populations

Fragmentation refers not only to habitat loss but habitat reconfiguration, where patches of suitable habitat become smaller, more numerous, and increasingly isolated (Fahrig 2003). It is debatable whether the complete loss of habitat is substantially more detrimental to the persistence of species than is habitat fragmentation per se, as fragmentation does not always lead to an immediate decline in distribution and abundance depending on species' habitat requirements and the intensity of habitat change

(Jackson and Fahrig 2013; Fletcher et al. 2018). However, habitat fragmentation is still a threat for species whose populations are small and isolated, and these responses may be compounded in light of edge effects that further erode the integrity of marginal habitat (Laurance et al. 2007; Jackson and Fahrig 2013). Accordingly, there can be both direct and indirect effects of habitat change on species, and it remains challenging to disentangle which factors are driving variation, especially when responses are gradual and difficult to track.

Reduced inter-patch movement is characteristic of species exposed to high fragmentation (Debinski and Holt 2000; Haddad et al. 2015), but such impacts can be mitigated when traversable habitat remains available between patches (Vos et al. 2002; Hilty et al. 2019). Continuous corridors are one type of pathway that provides an unbroken stretch of habitat between patches; vegetated overpasses and riparian corridors are examples of continuous corridors that may be used by a variety of wild animal species ranging from mammals to amphibians (Hilty et al. 2019). As a rule of thumb, corridors should constitute the shortest length possible between suitable habitat patches and be comprised of high-quality habitat. However, it is not always possible to establish short, continuous high-quality corridors between habitat patches, meaning that stepping-stone corridors comprised of lesser-quality habitat may be necessary (Lindenmayer and Fischer 2006). These corridors would primarily benefit species that can move rapidly through intermediate habitat (Doer et al. 2010), and are known to have successfully linked habitat patches for species such as kiwis (e.g., *Apteryx mantelli*; Potter 1990) and some butterflies (e.g., *Proclassiana eunomia*; Neve et al. 1996). Yet, for many species with restricted movement or high vulnerability to extirpation when in poor habitat conditions,

lower-quality corridors may be of limited use and populations may become functionally isolated (Henein and Merriam 1990; Gustafsson and Hansson 1997). Given the crucial role that corridors play in maintaining dispersal and breeding opportunities and thus promoting genetic diversity and favourable community dynamics (Schlaepfer et al. 2018), their suitability and functionality should be assessed (Hilty et al. 2019).

Habitat and Connectivity Modelling

Evaluating habitat availability and suitability for species can help support robust conservation planning decisions, including identifying areas that should be prioritized for protection to ensure species persistence (Lindenmayer and Fischer 2006; Hilty et al. 2019). Habitat suitability models support the development of maps outlining the geographic extent of favourable and unfavourable abiotic and biotic variables, using prior observations across similar environmental conditions as the basis for model training (Phillips et al. 2006). In general, habitat suitability models can be designed specifically to assess and identify priority environmental features playing a crucial role in current and potentially future species occurrence and persistence (Elith et al. 2006); the role of such modelling exercises may be especially important for rare or endangered species, those with specific habitat requirements, or identifying possible areas to protect and conserve for the benefit of declining populations (Sattler et al. 2007; Crawford et al. 2020). Numerous studies have modelled future habitat suitability and species distribution, such as evaluating changes in distributions for the Chinese giant salamander (*Andrias davidianus*) – a loss of at least two-thirds of their suitable habitat by 2070 – and the wood turtle (*Glyptemys insculpta*) – a loss of over half of their suitable habitat in the northeastern US by 2070 (Zhang et al. 2020; Mothes et al. 2020). During habitat

suitability model development, variables should be chosen based on factors relevant to the environmental niche of the species, which refers to the environmental factors promoting population persistence (Hirzel and Le Lay 2008). There are many recent technological developments that have improved habitat suitability modelling, including remote sensing datasets that provide spatial and temporal information on environmental features relevant to organisms, such as temperature/precipitation, land cover type, and urban activity (Hirzel and Le Lay 2008). It follows that integrating such sources of information into habitat suitability models for at-risk species may play an indispensable role in supporting an informed assessment of the abundance, spatial configuration, and composition of occupiable habitat in an area. Likewise, habitat connectivity or landscape resistance models are valuable tools for assessing existing and potential future linkages between suitable habitat patches; these models evaluate how continuous or resistant an environment is to dispersal based on land cover types (Rudnick et al. 2012). Measuring connectivity allows conservation planners to make informed decisions about whether sub-populations are likely to be facing isolation and what measures may remedy weak linkages to core populations (Bennett 2003).

Study System: Ambystoma salamanders on Pelee Island

I evaluated habitat suitability and connectivity of *Ambystoma* salamanders on Pelee Island, Ontario in the extreme southwestern portion of the province of Ontario, in Lake Erie. Salamanders on Pelee Island are currently reliant on a small number of habitat patches, with apparently limited connectivity between suitable patches (Hossie 2018). This group of salamanders consists of small-mouthed salamanders (*Ambystoma texanum*), blue-spotted salamanders (*A. laterale*) and Unisexual *Ambystoma* (COSEWIC

2017). Unisexual *Ambystoma* are an all-female lineage, who use sperm packets laid by parental *Ambystoma* species to stimulate egg development; this genetic material can sometimes be incorporated in egg fertilization but more often eggs are clones of their mother and sperm is not incorporated into the egg (Bogart et al. 2007). Five *Ambystoma* salamander species throughout the Great Lakes region in eastern North America can promote egg development in unisexuals, but only *A. texanum* and *A. laterale* are present on Pelee Island (Bogart et al. 2007). *Ambystoma* salamanders rely on fishless wetlands to lay eggs in early spring, but while larvae require wetlands for survival, they metamorphose and occupy more terrestrial habitat during mid-late summer (Harding and Mifsud 2017).

Historically, Pelee Island was mostly forested wetlands and included several distinct land bodies separated by sandbars that likely served as barriers to salamander dispersal (Forbes et al. 2000). In the 1880s, these wetlands were deforested and drained to support agriculture, thereby decreasing the size of habitat areas and potentially altering habitat availability for salamanders, as well as the connectivity between terrestrial habitat patches (Forbes et al. 2000; Hossie 2018). In the past 2 decades, conservation organizations and local landowners working on Pelee Island have initiated protection and restoration of natural areas, but nevertheless, salamander habitat remains largely disjunct across the island. This is a concern for a number of reasons, including that: 1) small-mouthed salamanders are listed as endangered in Canada and Ontario and their distribution is entirely restricted to Pelee Island; 2) blue-spotted salamanders, although widely distributed across eastern Canada, are uncommon on Pelee Island and may have distinct genetic status on Pelee Island and thus require special protection (Hossie 2018;

Hossie 2021, unsolicited report); 3) unisexual *Ambystoma* on Pelee Island, although seemingly common on the island, are also listed as endangered in Canada (under the Committee on the Status of Endangered Wildlife in Canada [COSEWIC]) and Ontario and their sustainability is entirely reliant on the viability of parental species that serve as sperm donors (small-mouthed and blue-spotted salamanders). It follows that there is an urgent need to assess the distribution and quality of both terrestrial and aquatic salamander habitat on Pelee Island, to determine requirements for viability and persistence of this assemblage.

Research Questions

The aim of this thesis is to analyze salamander habitat suitability and connectivity in a fragmented landscape on Pelee Island. I addressed this research objective by modelling habitat suitability and connectivity using location data for Pelee Island salamanders collected over 5 years (2015-2019). The extensive sampling effort yielded larval, juvenile, and adult *Ambystoma* salamanders from across the assemblage of groups, and salamander capture methods included coverboards, minnow traps, and dipnet surveys. In 2019, I added additional coverboards specifically to sample under-surveyed areas, including locations expected to have a low probability of salamander occurrence. Models of salamander habitat suitability and connectivity were supported by geospatial environmental data representing the primary landcover and abiotic factors that were likely to influence salamander distribution and abundance. The research questions addressed in this thesis are:

- How much habitat is available on Pelee Island for the *Ambystoma* complex?

Historical forested regions and populations of salamanders have been fragmented

by increased agricultural and urban development, meaning that suitable habitat is expected to be patchily distributed across the landscape.

- How connected are the remaining patches of suitable salamander habitat?
Salamander assemblages should have some level of connectivity between occupied habitat patches to maintain dispersal and genetic exchange, but patches may be too isolated if they are beyond the normal dispersal distance of *Ambystoma*.
- What is the state of remaining habitat for small-mouthed salamanders specifically, and how connected are their populations? Small-mouthed salamanders are likely the sole sperm donor for unisexuals in many breeding locations, and thus are disproportionately important to the complex. Due to their higher habitat specificity and tendency to remain close to breeding sites compared to unisexuals, small-mouthed salamanders likely have a smaller amount of suitable habitat on Pelee Island as compared to unisexual *Ambystoma*. In contrast, unisexuals are more abundant than small-mouthed salamanders and likely tolerate a wider range of habitats due to the varied composition of their nuclear genome (Mills et al. 2020).

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Chapter 2: Assessing habitat suitability and connectivity for an endangered salamander complex

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Abstract:

Context

Evaluating habitat quality for isolated animal communities is a high priority in landscape ecology and conservation biology. Where possible, such efforts should focus on understanding the needs of species that are disproportionately important for maintaining diversity.

Objectives

We evaluated the habitat of *Ambystoma* salamanders on Pelee Island, ON, Canada, including unisexuales and the bisexual species on which they are reproductively dependent (*A. texanum*). Existing assemblages on the island appear isolated from one another, perhaps reflecting limited habitat suitability and low connectivity.

Methods

Field surveys resulted in 2076 salamander detections in terrestrial and aquatic areas. We modelled habitat suitability using MaxEnt and connectivity with Circuitscape.

Results

Island-wide habitat availability was low (6.9%). Proximity to wetlands was crucial for habitat suitability, with forested areas and non-breeding wetlands being suitable only when found near established breeding locations. *A. texanum*, the critical sperm donor species, had 44.8% less suitable habitat area compared to its unisexual dependents. On average, existing salamander assemblages were separated by 2.1 ± 1.3 (SE) km, which is beyond the typical dispersal distance for *Ambystoma*. Potential dispersal corridors were primarily comprised of unsuitable habitat for salamanders.

Conclusions

The *Ambystoma* salamander complex on Pelee Island hinges on the viability of a sperm donor species with marked habitat and dispersal restrictions. Combined with contemporary anthropogenic land use, this has created isolated assemblages with uncertain long-term viability. Preserving this complex will require habitat protection in proximity to established breeding locations, as well as the enhancement of dispersal corridors to increase connectivity among patches of suitable habitat.

Keywords: Amphibians, landscape fragmentation, habitat suitability, connectivity, landscape resistance, unisexual

Introduction:

Habitat loss and fragmentation are among the primary sources of biodiversity loss globally (Thomas et al. 2004; Hoffmann et al. 2010). Land conversion has reduced the total amount of viable habitat and reduced connectivity between increasingly isolated habitat patches, resulting in population declines, species loss, and disruption of community structure and function (Mantyka-Pringle et al. 2012; Haddad et al. 2015). These impacts are particularly detrimental for species with strong habitat specialization, small areas of occupancy, and low mobility (Fischer and Lindenmayer 2007). It follows that when species have multiple life stages with distinct environmental needs, habitat-related challenges to population persistence may be especially harmful (Becker et al. 2007). In fragmented landscapes, individuals in legacy habitat patches may persist as components of a metapopulation if sub-populations are sufficiently connected to adjacent populations; without such connections, sub-populations experience a higher risk of local extinction (Hanski 1998). Ultimately, metapopulation persistence depends on having functional connectivity among habitat patches and meeting minimum requirements of habitat patch size and suitability (Keymer et al. 2000).

The above principles of metapopulation dynamics extend to ecological communities and assemblages, where persistence at a site requires meeting the habitat needs of a variety of members. Further, the habitat requirements of some species can disproportionately influence the composition and viability of communities or assemblages when their membership is highly inter-dependent and contingent on the presence of other species (Callaway 2007; Hatcher et al. 2012). In such cases, entire communities may be especially vulnerable when key members have restricted habitat

requirements or dispersal ability, and community persistence may critically depend on habitat that favours these species. Further, maintaining metapopulation dynamics requires that habitat patches are well-connected through immigration/emigration and recolonization, especially to preserve genetic diversity (Crooks and Sanjayan 2006). Identifying potential corridors is central to determining whether functional metapopulations exist or whether metapopulation dynamics can be restored in fragmented landscapes (Rudnick et al. 2012; Dondina et al. 2018). Functionally, effective corridors linking suitable habitat patches can range from poor-quality areas with a low resistance to movement, to high-quality habitat patches that constitute suitable habitat (Beier and Noss 1998; Bennett 2003). Importantly, connectivity metrics do not guarantee functional connectivity, and landscape resistance modelling can provide a more holistic view of the potential connectivity within a landscape (Bennett 2003; Pierik et al. 2016).

In contemporary landscapes, habitat loss and fragmentation are particularly impactful to forest and wetland systems (Mantyka-Pringle et al. 2012). Wetlands have experienced especially dramatic declines during the last century (Davidson 2014; Dixon et al. 2016), including in southern Ontario, Canada, where >85% of original wetlands have been lost (Ducks Unlimited Canada 2010; Environmental Commissioner of Ontario 2018). Many amphibians depend on wetlands as critical habitat for egg-laying and larval development. In addition, amphibians often require suitable terrestrial habitat because of their biphasic life history, meaning that comprehensive suitability assessments should consider both wetlands and nearby terrestrial habitat (Hecnar 2004; Scott et al. 2013). Declining landscape connectivity as a result of habitat fragmentation remains an

important cause of amphibian declines and can be especially severe when terrestrial and aquatic habitats become decoupled (Lehtinen et al. 1999).

Canada's only population of small-mouthed salamanders (*Ambystoma texanum*) occurs on Pelee Island, Ontario, where they live alongside blue-spotted salamanders (*A. laterale*) and unisexual *Ambystoma* salamanders (Small-mouthed salamander dependent population) (Bogart et al. 1985; Hossie 2018). Unisexual *Ambystoma* are an all-female lineage that are reproductively-dependent on sperm produced by males of other *Ambystoma* species, although they all possess at least one *A. laterale* genome (Bogart et al. 2007). Unisexuales typically produce clonal offspring, but can also produce ploidy-elevated offspring when the nuclear DNA from the sperm is incorporated into the egg (Bogart and Licht 1986; Bogart 2019b). *A. texanum* are disproportionately important to this *Ambystoma* complex, as the few remaining *A. laterale* have a highly constrained distribution on Pelee Island, and unisexual *Ambystoma* are almost exclusively reliant on *A. texanum* to reproduce (Hossie 2018). To complete their larval phase, all three of these *Ambystoma* salamanders require fishless breeding locations in proximity to forested terrestrial habitat (Porej et al. 2004; Porej and Hetherington 2005). Adult *A. texanum* salamanders do not travel far from their breeding locations (Gopurenko et al. 2007), although they are capable of moving >150 m in a single movement session (Williams 1973; Denton et al. 2017) across suitable habitat. By contrast, unisexuales travel <50 m in a single session (Denton et al. 2017), but may tolerate a wider range of habitats (Greenwald et al. 2016). This can impact the metapopulation structure of the salamander complex if sites are isolated and dispersal is impeded by barriers like agriculture or human development that impact the three salamander taxa differently (Marsh and

Trenham 2001; Greenwald et al. 2016). Further, characterizing differences in habitat suitability between host species and reproductively-dependent unisexuals is essential for conserving this salamander complex.

We assessed suitability and connectivity of *Ambystoma* salamander habitat on Pelee Island in southern Ontario and predicted that limited natural habitat and extensive fragmentation have caused disjunct salamander populations rather than a functional metapopulation. Specifically, we predicted that remaining habitat patches would be structured in relatively small patches that are separated by a distance exceeding the typical dispersal distance for *Ambystoma* salamanders. We expected that *A. texanum* – which are a key member of the *Ambystoma* salamander complex on Pelee Island but have low overall availability relative to unisexuals – would have higher habitat specificity, including restricted proximity to breeding locations and lesser suitability of potential corridors, resulting in lower overall habitat availability compared to unisexuals. On Pelee Island, *A. texanum* are the more abundant and widely distributed host compared to *A. laterale* (Bare et al. *in review*), and therefore they are central to supporting the *Ambystoma* complex in our study area and thus are the focal species for our investigation. Finally, we also predicted that unisexuals should generally tolerate a wider range of habitats and longer dispersal distances than *A. texanum*. However, because unisexuals can take on a variety of genetic forms (i.e., genotypes), their habitat suitability and connectivity requirements should more closely reflect those of *A. texanum* depending on the number of *A. texanum* genomes present in individual animals.

Methods:

Study Area and System

Our study was conducted on Pelee Island, Ontario, Canada (41.77 N, 82.66 W), a 42 km² island in Lake Erie that holds Canada's only population of *A. texanum* (Hossie, 2018). Historically, the island was heavily forested and covered with wetlands, but currently forests and wetlands only cover 16.5% and 5.7% of the island, respectively (G. Smith, unpublished data). Agricultural land is now the primary land cover type (62.1%), with remnant forest and wetlands scattered across the island. Unisexual *Ambystoma* (*Ambystoma laterale* - *texanum*) are reproductive parasites and possess nuclear DNA from both *A. texanum* and *A. laterale* hosts (Bogart et al. 2009; COSEWIC 2017). A unique nomenclature is used to distinguish among the various unisexual forms ("genomotypes") that can arise based on the number of *A. laterale* ('L') and *A. texanum* ('T') chromosome sets they possess (Lowcock et al. 1987; Bogart et al. 2007). For example, a diploid unisexual with a single chromosome set from *A. laterale* and *A. texanum* would be referred to as 'LT' and a *texanum*-dominant triploid would be 'LTT'. Salamander identity was determined by genotyping tissue samples using microsatellite loci (*Atex74*, *Atex102*, *Atex141*, *AjeD75*, *AjeD94*, *AjeD283*, *AjeD346*, *AjeD378*, *AjeD422*) (see also Williams and DeWoody 2004; Peterman et al. 2012), and these methods have been fully detailed elsewhere (Bogart 2019a; Van Drunen et al. 2020).

A. texanum use several types of moist habitats, including tall-grass prairies, dense hardwood forests, and occasionally agricultural land when it is adjacent to suitable breeding locations (Environment and Climate Change Canada 2020). Adults frequently use burrows created by semi-terrestrial crayfish (Williams 1973; Owen and Jutterbock

2013). Microhabitat requirements also differ between groups, with *A. texanum* preferring moist, forested habitat and *A. laterale* being more tolerant of drier soils and open habitat (Jutterbock and Owen 2013; Mills et al. 2020). However, it is not clear whether these different preferences lead to landscape-level differences in habitat requirements across the salamander complex. Unisexual *Ambystoma* with genomes from *A. texanum* may be restricted to areas with clay-based soils (Petranka 1998), but recent work shows that the ‘hybrid’ nuclear genome of unisexual *Ambystoma* gives rise to intermediate habitat requirements compared to their hosts (Mills et al. 2020). It follows that unisexual *Ambystoma* should occupy intermediate habitat to their sperm-donor host species (see Greenwald et al. 2016; Mills et al. 2020).

Fieldwork

During March-August (2015-2019), monthly salamander surveys were conducted at various sites across the island ($n = 10$) (with additional sampling in September 2019). Here, we define ‘site’ as a large area of suitable habitat (including both terrestrial and aquatic landcover) separated from other areas of suitable habitat by more than the typical dispersal distance for *Ambystoma* salamanders (i.e., > 600 m, acknowledging that infrequent dispersal up to 750-1350 m has been reported: Madison 1997; Gamble et al. 2007). These sites were all separated by agricultural land and urban development, and range in size from 16 to 240 ha (and some sites span multiple protected areas) (G. Smith, unpublished data). Sites contain breeding locations, which we define as bodies of water with evidence of salamander breeding; multiple breeding locations can be present at a given site. Over this time from 2015-2019, salamanders were captured through opportunistic searches, systematic searches using coverboards, and minnow-trapping for

adults during the breeding season (March). Coverboard arrays were established between 2015-2017 at all sites across the island with known salamander populations ($n = 304$ boards). We identified breeding locations by minnow-trapping breeding adults, conducting visual egg searches, and dip-netting for larvae. The only confirmed breeding location with *A. laterale* was not sampled as it was on private land. In May and June 2019, an additional 157 coverboards were deployed strategically across the island to improve spatial coverage of survey efforts, including areas predicted to have low salamander occupancy rates. Based on preliminary models using data from 2015-2018, 106 boards were placed in areas predicted to be movement areas and corridors (i.e., with a high current flow as per Circuitscape models, see below) and were separated by at least 50 m; the remaining 51 boards were spaced 300 m apart and placed in random locations with lower predicted flow. For all 461 boards, most (57.1%) were in forested areas, with the rest in wetlands, tallgrass communities and agricultural land within protected areas (Proportion of boards in forested areas: 57.1%; wetlands: 22.3%, tallgrass comm.: 15.2%, agricultural: 5.4%). From June - September 2019, we conducted three surveys of the new boards. We also searched natural cover objects within 5 m of boards, including at the time of deployment, to further increase salamander detection. To supplement these efforts, linear transects that involved overturning natural cover objects were conducted in areas predicted to have low and high habitat connectivity (based on preliminary models), from June - September 2019 (June: $n = 19$, August: $n = 29$, September: $n = 34$). During these transect searches, three observers spaced 2.5 m apart walked 50 m transects. Finally, we also conducted opportunistic searches in areas known to have salamanders. We combined capture data from all sampling periods (i.e., 2015-2019) for our analyses,

although genetic data was not completed for the samples collected in 2019 so genotype-specific analyses were restricted to data from only 2015-2018.

Habitat Suitability

We used Maxent (version 3.4.0) to assess salamander habitat suitability on the island. Maxent combines organism presence data with environmental grids to generate a predicted distribution for the species of interest (Elith et al. 2011). Salamander locations ($n = 1237$), where ‘location’ refers to the specific geographic location where a salamander was found, were used to develop the preliminary Maxent model, with 40% of locations used in model training. Location points were spatially clustered, so we spatially filtered our data to a single observation per 10 x 10 m grid cell, resulting in 476 unique locations (22.9% of the complete dataset). Distance to breeding location, land cover type, soil type, and elevation served as relevant environment variables given that: i) Ambystomatids typically remain close to breeding areas, inhabiting shaded woodland areas with soft, moist soil (Harding and Mifsud 2017); ii) agricultural land is typically avoided by *Ambystoma* spp. (Kolozsvarly and Swihart 1999; Greenwald et al. 2009); and iii) elevation may help distinguish areas likely to remain wet vs. becoming dry by late summer. Land cover and soil type were treated as categorical variables, while distance to breeding location and elevation were continuous. Land cover was based on the Southern Ontario Land Resource Information System (SOLRIS) (SOLRIS Technical Team 2015). For ‘undifferentiated’ land cover types, Google Earth (version 9.2.78.1, accessed February 2019) served to visually classify land cover types. We also collapsed some land cover categories to simplify this variable (see Table S1). Soil data were derived from the Ministry of Natural Resources Land Information Ontario Data Description Soil Survey

Complex (OMAFRA 2019) and elevation data were from the Ministry of Natural Resources Provincial Digital Elevation Model - South (Provincial Mapping Unit 2018). Distance to breeding location was calculated in ArcGIS 10.7.1 based on the distance of each grid cell from nearest margin of a breeding location. Environmental variables were converted from their source file type to rasters, with a cell size of 10 x 10 m.

Probability of suitable habitat values ranged from 0 to 1; here we treated values 0-0.2 as unsuitable habitat, 0.2-0.4 as low, 0.4-0.6 as intermediate, and >0.6 as high suitability (see also Atmoko et al., 2020; Singh et al., 2017; Yuh et al., 2020). Habitat suitability was interpreted using median output value of a specified area to compare suitability across various scenarios. Response curves were generated to assess variable fit (Phillips 2017). In these plots the y-axis is given as a logistic output, which represents probability that a species is present and categories within a specific environmental variable are ranked. Variable contribution to model and permutation importance were tested via jackknife. Additional model scenarios were created by subdividing location points to compare salamander genotypes (LT: $n = 286$, LTT: $n = 156$, and TT: $n = 37$), and we also modelled habitat suitability according to salamander life stage (adults: $n = 369$, juveniles: $n = 179$) and seasonality (March and April: $n = 190$, May-July: $n = 233$, and August and September: $n = 136$) to better understand sources of variability in our results. Genotype and seasonality datasets only consisted of adult capture locations (SVL > 40 mm) and excluded recently-metamorphosed juveniles which emerge as early as late June (all juveniles initially remain close to their natal location). Finally, highly suitable habitat for all *Ambystoma*, LT only, LTT only, and TT only was characterized using Fragstats, a program used to quantify landscape structure, and Patch Analyst, a

Fragstats extension for ArcGIS (Elkie et al. 1999; McGarigal 2015). Variables measured include mean patch area (MPA), perimeter length (PERIM), radius of gyration (GYRATE), perimeter-area fractal dimension (PAFRAC), clumpiness index (CLUMPY), mean nearest neighbour (MNN), number of patches (NP), patch density (PD), and mean proximity index (MPI) (McGarigal 2015).

Habitat Connectivity

Circuitscape (version 4.0) was used to predict ecological connectivity between communities and identify potential movement routes between disconnected areas (McRae et al. 2014). Circuitscape models were created with spatially filtered locations, using the same location points as with Maxent models ($n = 476$). Landscape features are given a resistance value based on animal travel likelihood through that area (Table S1).

Resistance values were based on work by Compton et al. (2007) who conducted a resistant-kernel estimator for *A. opacum* and *A. maculatum* and created resistance values for dispersal from breeding locations and migration (see Table S1). Resistance values were based on land cover types following the same classification used in our previous habitat suitability analyses. Landscape grids were created using ArcGIS 10.7.1 with a cell size of 10 x 10 m to enable the detection of fine-scale movement corridors. Resistance values were interpreted by Circuitscape (in ohms) as electrical resistance, and circuit maps were created between locations points with a 'current' value for each grid cell (in amperes) (McRae et al. 2008). There is no upper range limit for these values, but high output values represent increased likelihood of movement through these areas (McRae et al. 2008). Potential movement corridors linking core sites were identified using Linkage Mapper version 2.0.0 (McRae and Kavanagh 2011), which combines GIS and

Circuitscape to identify potential habitat corridors and pinch-points (where movement is funnelled between core habitat areas) (Gallo and Greene 2018). Linkage Mapper is packaged within ArcGIS and uses a resistance grid and map of core areas. We used the same map of resistance values as with our habitat connectivity models, with breeding locations serving as core areas. Finally, we generated a map of linkages between breeding locations (termed ‘core areas’) illustrating least-cost paths, which represent the shortest and most likely route an animal would take between patches, which were overlaid on the initial map (Sawyer et al. 2011; Gallo and Greene 2018). All parameters in the model were set to their default setting, creating links between all focal areas and dropping links that intersect focal areas or were >50 km.

Results:

During 2015-19, we caught 2076 post-metamorphic salamanders (2015: 4.4%; 2016: 15.6%; 2017: 19.7%; 2018: 19.9%; 2019: 40.4%) from 10 sites across Pelee Island. Most animals (52.2%) were captured during wetland surveys (of breeding and non-breeding wetlands), with others captured during opportunistic searches on land under natural cover objects (25.3%), coverboards (18.4%) or on linear transects (2.9%). Most captured animals (83.5%) were adults (SVL > 40 mm) with the remainder being newly-transformed individuals (SVL 21-40 mm). Most captures occurred during March-April (67.0%), followed by May-July (19.6%) and August-September (13.5%). Fifty-eight percent of our total sample of adults and juvenile salamanders ($n = 1197$) were genotyped (i.e., all samples collected from 2015-18) and we found that 96% were unisexual *Ambystoma* (LT: 65.3%; LTT: 19.6%; LTTT: 3.8%, LTTTT: 0.08%), with parental species being rare (*A. texanum*: 3.5%; *A. laterale*: 0.2%). Evidence of breeding

(salamander egg masses or larvae) was documented at 33 natural and constructed wetlands (i.e., including vernal pools, forested wetlands, marshes, and swamps) across the island.

Overall Habitat Suitability (All Ambystoma)

Our habitat suitability assessment revealed that the majority of Pelee Island is not currently suitable for *Ambystoma* salamanders (93.1% of the island has a Maxent value < 0.2). The island-wide median output value was 0.0016 ± 0.1 (\pm SD) and the island-wide average was 0.04 ± 0.1 (range across the island: 0 - 0.94; Figure 1, Figure 2A). Low, intermediate, and highly suitable habitat is restricted to small areas throughout the island (Low [i.e., 0.2-0.4]: 4.4% of the island; Intermediate [0.4-0.6]: 1.5%; High [>0.6]: 1.0%). Highly suitable habitat patches were relatively small and aggregated (Mean Patch Size = 1.4 ± 3.3 ha; Table 1). The 10×10 m locations where salamanders were detected ($n = 476$) were in highly suitable habitat (median \pm SD: 0.60 ± 0.24 , range = 0.02-0.85; ~ 383 x higher than island-wide average). By comparison, randomly-selected locations across the island ($n = 476$) were largely in unsuitable habitat (0.0017 ± 0.1 , range = 0 - 0.93), indicating that our model captured relevant spatial patterns in salamander habitat suitability. These results were further corroborated by our salamander detections under coverboards: of 106 boards placed in what was deemed suitable habitat, salamanders were detected under 21.7% of these boards, whereas detections were only obtained under 3.9% of boards that were deliberately placed in apparently poor habitat (forested habitat but far from breeding locations; salamanders were only detected under 2 of these 51 boards). Finally, the Maxent model also identified that breeding locations ($n = 33$) were

comprised of highly suitable habitat (0.75 ± 0.13 , range: 0.05 - 0.88; 479x higher than island-wide average).

Distance from breeding location was the strongest predictor of habitat suitability, contributing 59.9% to Maxent model fit, followed by land cover type (29.2%), soil type (8.4%) and elevation (2.5%) (Table 2; Figure S7). Habitat suitability decreased dramatically with distance from a breeding location, with an ~85% decline in predicted suitability upon reaching ~2.1 km from the center of the breeding location (Figure 1A). By ~1.6 km from the center of the breeding location, habitat suitability reached the island-wide average. Landcover types most strongly associated with suitable habitat were forested areas and swamps (predicted probability of suitable conditions were 41.0% and 31.7%, respectively; Figure S7), but forests and swamps were predicted to be unsuitable when they were distant (i.e., >1 km) from known breeding locations (0.004 ± 0.01 , range: 0 - 0.09). Agricultural areas and roads were the least suitable habitat (1.8% and 7.9%, respectively; Figure S7). In general, poorly drained soils and areas with moderate elevation were most suitable (Figure S7). The estimated suitability of habitat within a 300 m buffer zone around a breeding location was surprisingly low (0.14 ± 0.22 , range: 0.0005 - 0.94; 90x higher than island-level average). Even after reducing this buffer zone to 150 m around breeding locations, mean habitat suitability of these areas is lower than expected (0.35 ± 0.24 , range: 0.002 - 0.94; 220x higher than island-level average). That said, nearly all the highly suitable habitat on the island (Maxent value >0.6) is within the 150 m buffer around breeding locations (98.7%).

Overall Habitat Connectivity (All Ambystoma)

Salamander habitat connectivity was low across the island (median output value \pm SD [output values are measured in amperes]: 377.4 ± 326.2 ; range: 0 - 9108; Figure 2B) and current flow across a randomly-selected group of locations ($n = 476$) was only 2.8% lower (388.2 ± 308.8 ; 1.03x higher than island-wide median). Terrestrial habitat surrounding breeding locations (i.e., within a 300 m buffer extending radially from wetland edges, excluding areas extending into Lake Erie) had moderately high current (560.6 ± 639.2 , range: 47 - 9108; 1.5x higher than island-wide average). Spatially-filtered locations where salamanders were detected ($n = 476$) occurred in areas with relatively high habitat connectivity (3307.3 ± 2132.7 , range: 850-9108; 8.8x higher than island-wide average). Forested and tallgrass communities had moderately high current flow (Forest = 599.3 ± 485.2 , 1.6x higher than island-wide average; Tallgrass = 440.0 ± 263.2 , 1.2x higher than island-wide average). Most of the known salamander sites are separated from one another by >600 m (mean \pm SD = 2.1 ± 1.3 km), with the area between them being primarily agricultural land with low current flow (median output value \pm SD: 348.2 ± 123.6 ; 0.9x lower than island-wide average).

The Least-Cost Paths analysis identified potential routes between core areas, with long stretches occurring in forested areas between farmland (42.5%), as well as through agricultural areas (22.5%) (median path length \pm SD = 4564.7 ± 2948.7 m). Paths between breeding locations within the same core area (which were the only pathways <1 km), had high current flow (median output value \pm SD [output values are measured in amperes] = 1092.7 ± 1096.0 ; 2.9x higher than initial habitat connectivity model) and were primarily restricted to wetland and tallgrass habitat (39.2% and 34.6% respectively). Paths between core areas (which were pathways >1 km) had moderate current flow (606.5

± 496.6 ; 1.6x higher than initial habitat connectivity model). We also examined habitat suitability of potential dispersal routes identified by the Least-Costs Path analysis by examining their habitat suitability (i.e., Maxent output values). While these routes appeared to be composed of intermediately suitable habitat (median habitat suitability \pm SD: 0.33 ± 0.19 , range: 0.0-0.87; median is 210x higher than island-level average; Figure 2B), most of the area these routes traversed was unsuitable habitat (Unsuitable [i.e., <0.2]: 81.6% of the total area of paths; Low [0.2-0.4]: 5.6%; Intermediate [0.4-0.6]: 8.2%; High [>0.6]: 4.6%). Routes between breeding locations within the same core area, which were exclusively comprised of pathways <1 km in length, had higher habitat suitability (median \pm SD: 0.58 ± 0.24 , range: 0.05-0.86; 366.4x higher than island-level average). Paths <1 km primarily traversed intermediately and highly suitable habitat (Unsuitable [i.e., <0.2]: 13.2% of the total area of paths; Low [0.2-0.4]: 1.6%; Intermediate [0.4-0.6]: 47.9%; High [>0.6]: 37.2%).

Habitat Suitability and Connectivity across Genotypes

We found important differences in predicted habitat suitability across genotypes (Figure 3), even though habitat suitability of all adult salamanders was mostly governed by the same variables. Distance from breeding location was the best single predictor for all genotypes, although this variable was substantially more important for *A. texanum* than diploid unisexuals (Figure 1B; TT: 86.4%, LT: 54.9%, LTT: 71.8%; Table 2). Habitat suitability decreased markedly, particularly for *A. texanum*, based on distance from breeding location. Specifically, habitat suitability reaches the island-wide average for *A. texanum* at 0.4 km, LT unisexuals at 1.9 km, and at 1.5 km for LTT unisexuals (Figure 1B). Highly suitable habitat (>0.6) patches were

largest for *A. texanum* but were farthest apart, less numerous and less dense compared to patches for unisexuals (see Table 1). Habitat patches for diploid unisexuals were, on average, smallest but were much closer than patches for triploid unisexuals and *A. texanum*, and were denser and more numerous (see Table 1). The amount of highly suitable habitat available in the landscape was greatest for diploid unisexuals and least for *A. texanum* (see Table 1). The second-best predictor for *A. texanum* was soil type (TT: 10.2%, LT: 7.9%, LTT: 8.1%; Table 2), with marsh soil type being identified as the most suitable (Figure S9). In contrast, land cover was the next best predictor for both diploid and triploid unisexual types (TT: 3.4%, LT: 35.1%, LTT: 18.1%; Table 2), with forest cover type being the most suitable for each group (TT: 71.3%, LT: 52.3%, LTT: 42.8%; Table 2). Highly suitable habitat (>0.6) was most isolated for *A. texanum* compared to unisexuals, with highly suitable habitat patches for LT unisexuals being more isolated than LTT unisexuals (Figure 4; Mean Proximity Index: TT = 18.2, LT = 29.8, LTT = 46.6; Mean Nearest Neighbour: TT = 1336.1 m, LT = 84.5 m, LTT = 342.3 m; MPS: TT = 4.1 ha, LT = 1.7 ha, LTT = 2.5 ha; Table 1).

Habitat Suitability and Connectivity across Life Stage and Seasonality

To assess the sensitivity of our observations to salamander life stage and seasonal differences, we generated separate models for adults ($n = 215$) and juveniles ($n = 169$). These models revealed that for both groups, distance to breeding location was the primary predictor (Figure S7; percent contribution to model: Adult = 54.4%, Juveniles = 81.5%, Table 2). Adults occurred in intermediately-suitable to highly-suitable habitat (0.57 ± 0.24 , range: 0.03-0.88), but juveniles were largely restricted to highly-suitable habitat in proximity to breeding locations (0.68 ± 0.26 , range = 0.006-0.89). Soil type

was more influential than land cover type for both groups (Adult soil: 20.3%; Adult land cover: 18.8%; Juvenile soil: 10.0%; Juvenile land cover 8.1%). Forests and wetlands were primary habitat features for adults (Forest: 48.9%, Wetland: 41.5%; Figure S10), but while forests remained important for juveniles, wetland importance was surprisingly lower (Forest: 49.9%, Wetland: 29.6%; Figure S10). Although salamander habitat suitability spatially varied seasonally, distance to breeding location was consistently the primary predictor of suitability (Figure S8; Mar-Apr: 60.9%, May-Jul: 68.1%, Aug-Sep: 71.2%, Table 2) and forest landcover was the primary suitable habitat type (Mar-Apr: 33.3%, May-Jul: 45.4%, Aug-Sep: 29.8%; Figure S7); wetlands were an especially important landcover type in late summer (Mar-Apr: 19.3%, May-Jul: 31.8%, Aug-Sep: 37.2%; Figure S7).

Discussion:

We evaluated suitability and connectivity of *Ambystoma* habitat on Pelee Island and found that the amount of suitable terrestrial habitat currently available for salamanders is small and mostly adjacent to breeding locations. Forested areas and non-breeding wetlands are only important when contiguous with breeding locations or within <1 km. Habitat suitability varied across salamander genotypes, with *A. texanum* having the least available habitat, being most associated with breeding locations and other wetlands, and also having lower habitat connectivity compared to the unisexual *Ambystoma*. LTT triploid unisexuals have a narrower habitat niche than their diploid (LT) counterparts, whereas habitat suitability for both adults and juveniles of all groups becomes less tied to wetlands as the summer progresses. Connectivity among remaining patches of suitable habitat on the island is low, and inter-patch corridors are both

prohibitively long and poor-quality to maintain functional connectivity. Overall, our findings emphasize the need for habitat protection and enhancement (primarily wetland breeding locations and nearby forest) if the long-term viability of this unique salamander complex is to remain a priority. More broadly, our analysis reveals important challenges facing salamanders when they require connectivity across human-dominated landscapes.

Many of the salamander breeding locations on Pelee Island are adjacent to, or surrounded by, unsuitable habitat (e.g., agricultural land), which has created distinct islands of suitable habitat with low connectivity to other suitable areas. Despite this, the occupied areas mostly contain a diversity of salamander genotypes, including parental species (mainly *A. texanum*, occasionally *A. laterale*) and various types of unisexual *Ambystoma*. While additional forested habitat is available on the island, breeding locations are apparently absent from much of these areas and consequently, they do not appear to function as habitat. Indeed, the biphasic life history of most *Ambystoma* salamanders requires both suitable aquatic and terrestrial habitat, which imposes constraints on their habitat availability and suitability. Further, it is recognized that amphibian species, including *A. texanum*, avoid disturbed landscapes like agricultural fields and juveniles may migrate through areas outside the area of primary habitat suitability after emerging from their natal site (Walston and Mullin 2008). Interestingly, Greenwald et al. (2009) found that agricultural land contributed to *Ambystoma* population isolation, reinforcing the challenges faced by salamanders inhabiting human-dominated landscapes.

Paths between salamander breeding locations and surrounding areas were longer than could realistically be traversed by *A. texanum* or unisexual *Ambystoma* (Williams

1973; Denton et al. 2017), implying that there is limited movement among core sites. Most terrestrial *Ambystoma* salamanders can travel ~160 m in a single movement session (Williams 1973; Denton et al. 2017), which suggests that functional connectivity between sites requires suitable habitat at regular intervals. This does not appear to be the case across much of Pelee Island, and metapopulation dynamics may be limited or absent for these *Ambystoma* salamanders. Several of the identified paths between breeding locations traverse hedgerows between agricultural fields and these may be too long and narrow to function as effective habitat corridors for salamanders, particularly for *A. texanum* (e.g., see Vos et al. 2002). We acknowledge that evaluating the maximum useable distance of corridors based on species dispersal capabilities does not constitute a stand-alone connectivity analysis (e.g., see Baguette et al. 2013; Cushman et al. 2013), but our approach provides a crucial first step in understanding population connectivity for Pelee Island *Ambystoma* (e.g., Sawyer et al. 2011).

The observed differences between suitable habitat and connectivity for *A. texanum* and unisexuales emphasize the need for forested wetlands close to breeding locations. Patterns of suitable habitat for *A. texanum* are consistent with previous reports that this species tends to remain particularly close to their breeding sites (Williams 1973). Although some identified habitat patches for *A. texanum* were large and could be considered less fragmented, they are more isolated than patches for unisexuales, and inter-patch movement is unlikely. Importantly, we found that only three of 10 core sites across Pelee Island were highly suitable for *A. texanum* species, and we know of 1-2 other breeding locations that may contain *A. laterale*, the other parental species on the island (COSEWIC 2017). In contrast, the habitat at 7 of the sites where we found salamander

communities was deemed unsuitable for *A. texanum*, although *A. texanum* was still detected in low abundance at these sites (Bare et al., in review). Breeding sites within these 7 sites also continue to produce larvae so we can infer the presence of male bisexual *Ambystoma*, though likely at a low abundance (G. Smith, unpublished data). These sites are at risk of extirpation if low habitat suitability and low connectivity to source populations compromise the viability of the host species population (see Bogart et al. 2017; Bogart 2019b). The restricted habitat requirements of *A. texanum* compared to other members of the salamander complex on Pelee Island (see also Vrijenhoek 1989; Greenwald et al. 2016) translate to a smaller total amount of suitable habitat and lower connectivity between suitable habitat patches. This presents a challenge in maintaining salamander diversity at most sites on the island where *A. texanum* is the only host available. Likewise, the variability in salamander habitat suitability across age classes and seasons indicates that habitat suitability is dynamic through time and this variability should be considered explicitly in conservation efforts.

The identification of suitable habitat and potential corridors in this study provides critical information necessary for identifying strategies and locations for habitat restoration necessary for the persistence of *Ambystoma* salamanders on Pelee Island. Their long-term viability may require conservation strategies similar to those recommended by Semlitsch & Bodie (2003) and Calhoun et al. (2005), which include the protection and enhancement of suitable habitat near current breeding locations. Scott et al. (2013) also emphasize the importance of terrestrial buffers around breeding locations. Recent work on endangered *A. jeffersonianum* advocates for protection of 400-450 m of terrestrial habitat around breeding locations (Van Drunen et al. 2020). Similarly, we find

that most of the suitable habitat for *Ambystoma* salamanders on Pelee Island, as well as most of the individual salamanders, are found within 450 m of a breeding site and protecting a similar amount of habitat around breeding locations would assist the long-term persistence of *A. texanum* and unisexual *Ambystoma*.

Given *A. texanum*'s role as the primary reproductive host in this system and their limited suitable habitat we now consider the possible management implications. To increase the amount of suitable habitat, establishing breeding locations within 1 km of occupied sites, but still away from unusable habitat, should bolster population viability and the persistence of this complex. However, constructed ponds may remain unsuitable for 15-20+ years (Mitsch and Wilson 1996), so site selection and construction of new breeding locations must be strategic and accompanied by additional conservation management efforts (Ward and Hossie 2020). On Pelee Island, re-establishing functional connectivity among patches of suitable habitat remains a major challenge because most land outside core breeding locations and adjacent forest is on private land, meaning that incentives may be necessary to encourage natural landcover restoration and conservation. Regardless, it is important to note that given the current scale of salamander habitat fragmentation on Pelee Island, it may simply not be possible to restore functional metapopulation dynamics. More drastic interventions like head-starting juveniles and assisted dispersal may need to be considered as a means to maintain the viability of the host populations which support this complex (Seddon et al. 2014; O'Donnell et al. 2017). Accordingly, over the longer term it may be more effective to bolster the protection of suitable salamander habitat within currently-occupied sites, which is consistent with the outcome of other habitat suitability and connectivity modelling studies in human-

dominated landscapes (e.g., see Compton et al. 2007; Pereira et al. 2011; Crooks et al. 2011; Rondinini et al. 2011).

Ultimately, these findings showcase the importance of identifying suitable habitat for species existing in a fragmented system. Future studies on this *Ambystoma* complex should focus on environmental monitoring to evaluate the effectiveness of *in situ* conservation efforts, such as the construction of new breeding locations and reforestation. We concur with the recommendations of De Kort et al. (2020), in that the accuracy of habitat suitability models improves when accounting for species dispersal, as well as by validating model results in unsampled areas. Our results also suggest that a targeted focus on the habitat requirements for key members of a community or assemblage in conservation and restoration planning can be beneficial, as their protection can function as a conservation umbrella and protect other species (Johnson et al. 2017). Indeed, given the rapid decline in biodiversity globally (Hoffmann et al. 2010), ecosystem and community-based approaches to identifying and protecting key habitat are increasingly needed to inform and guide conservation efforts.

Tables and Figures

Table 1: Patch-level, class-level and landscape-level metrics measured with Fragstats for highly suitable habitat for *Ambystoma texanum* and unisexual *Ambystoma* on Pelee Island, ON. All metrics were measured at the class-level, aside from AREA and PERIM measured at patch-level and GYRATE measured at landscape-level. See main text for metric descriptions. The model with all locations was created with data from 2015-2019, and genotype-specific models were created with data from 2015-2018.

Fragstats Metric	Definition	Median Value (\pm SD)			
		All Locations	LT	LTT	TT
MPS (ha)	Mean patch area in ha	1.40 \pm 3.31	1.31 \pm 3.08	2.34 \pm 3.72	3.40 \pm 5.15
TA (ha)	Total patch area	41.92	47.25	44.58	20.43
PERIM (m)	Mean patch perimeter (m)	517 \pm 726.14	460 \pm 607.46	714 \pm 650.65	836 \pm 933.59
GYRATE (m)	Mean radius of gyration, refers to the mean distance from the patch centroid to patch edge	35.16 \pm 41.16	34.68 \pm 37.68	54.23 \pm 46.37	61.82 \pm 63.27
PAFRAC	Perimeter-area fractal dimension, reflects shape complexity; $1 \leq \text{PAFRAC} \leq 2$	1.17	1.2	1.19	1.05
CLUMPY	Mean clumpiness index, refers to degree to which habitat patches are aggregated/clumped; -1 (disaggregated) \leq CLUMPY \leq 1 (aggregated)	0.94 \pm 0.07	0.91 \pm 0.12	0.91 \pm 0.08	0.91 \pm 0.01
MNN (m)	Mean Euclidean nearest neighbour distance, measured between all patches	298.78	84.48	342.28	1336.11
NP	Number of patches	30	36	19	6
PD (#/100 ha)	Patch density	71.57	76.18	42.62	29.36
MPI	Mean proximity index, refers to the sum of the ratio of patch size to ENN; reflects the degree of isolation and fragmentation of a patch. MPI increases as patches become less isolated.	14.24	29.84	46.64	18.21

Table 2: Percent contribution and permutation importance of selected environmental variables in Maxent models for *A. texanum* and unisexual *Ambystoma* captured from 2015 – 2019, and from 2015-2018 for genotype-specific models.

Model	Variable	Percent Contribution	Permutation Importance
All Locations	Distance to Pond	59.9	72.8
	Land Cover Type	29.2	18.1
	Soil Type	8.4	5.4
	Elevation	2.5	3.7
Adults	Distance to Pond	54.4	64.3
	Land Cover Type	18.8	18.9
	Soil Type	20.3	12.9
	Elevation	6.5	3.9
Juveniles	Distance to Pond	81.5	89.3
	Land Cover Type	8.1	6.2
	Soil Type	10.0	3.7
	Elevation	0.5	0.8
<i>Ambystoma texanum</i> (TT)	Distance to Pond	86.4	98.4
	Land Cover Type	3.4	0.0
	Soil Type	10.2	1.6
	Elevation	0.0	0.0
Unisexual <i>Ambystoma</i> (LT)	Distance to Pond	54.9	66.5
	Land Cover Type	35.1	23.6
	Soil Type	7.9	6.9
	Elevation	2.1	3.0
Unisexual <i>Ambystoma</i> (LTT)	Distance to Pond	71.8	87.8
	Land Cover Type	18.1	5.1
	Soil Type	8.1	5.3
	Elevation	2.1	1.8
March – April captures	Distance to Pond	60.9	66.7
	Land Cover Type	29.8	20.9
	Soil Type	6.7	8.9
	Elevation	2.5	3.5
May – July captures	Distance to Pond	68.1	77.2
	Land Cover Type	14.2	11.4
	Soil Type	16.1	9.9
	Elevation	1.6	1.4
August – September captures	Distance to Pond	71.2	78.7
	Land Cover Type	15.5	10.2
	Soil Type	13.0	10.5
	Elevation	0.3	0.5

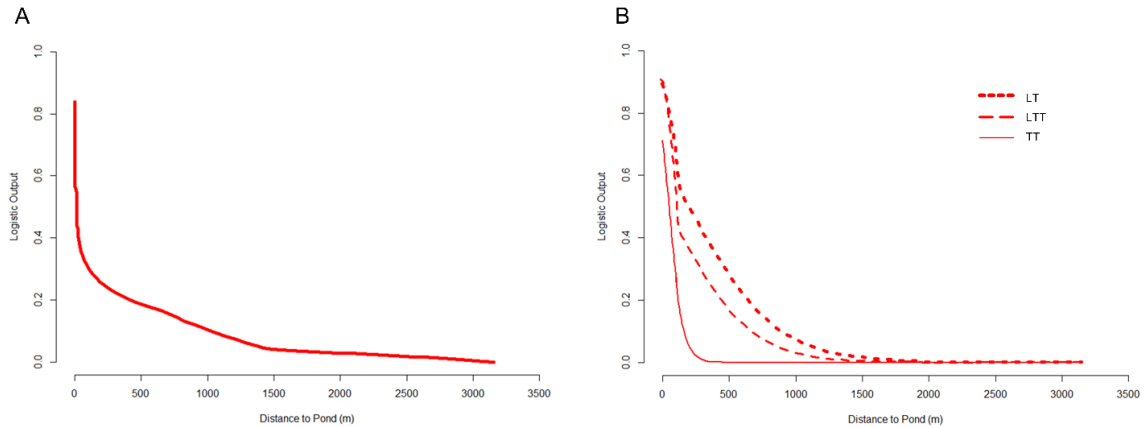


Figure 1: Relationship between distance to breeding location (m) and predicted habitat suitability for *Ambystoma* salamanders on Pelee Island, ON, Canada (based on response curves from Maxent models). A) The relationship between distance to breeding site and predicted habitat suitability based on spatially filtered location data ($n = 476$) for all adults and juvenile salamanders collected from 2015-2019. B) The relationship between distance to breeding site and predicted habitat suitability for three distinct *Ambystoma* genotypes, from data collected from 2015-2018. Dotted line depicts the relationship for diploid (LT) unisexual *Ambystoma* salamanders, the dashed line depicts the relationship for triploid (LTT) unisexual *Ambystoma* salamanders, and the solid line depicts the relationship for *A. texanum* (TT) salamanders. Higher values on y-axis represent higher probability of presence.

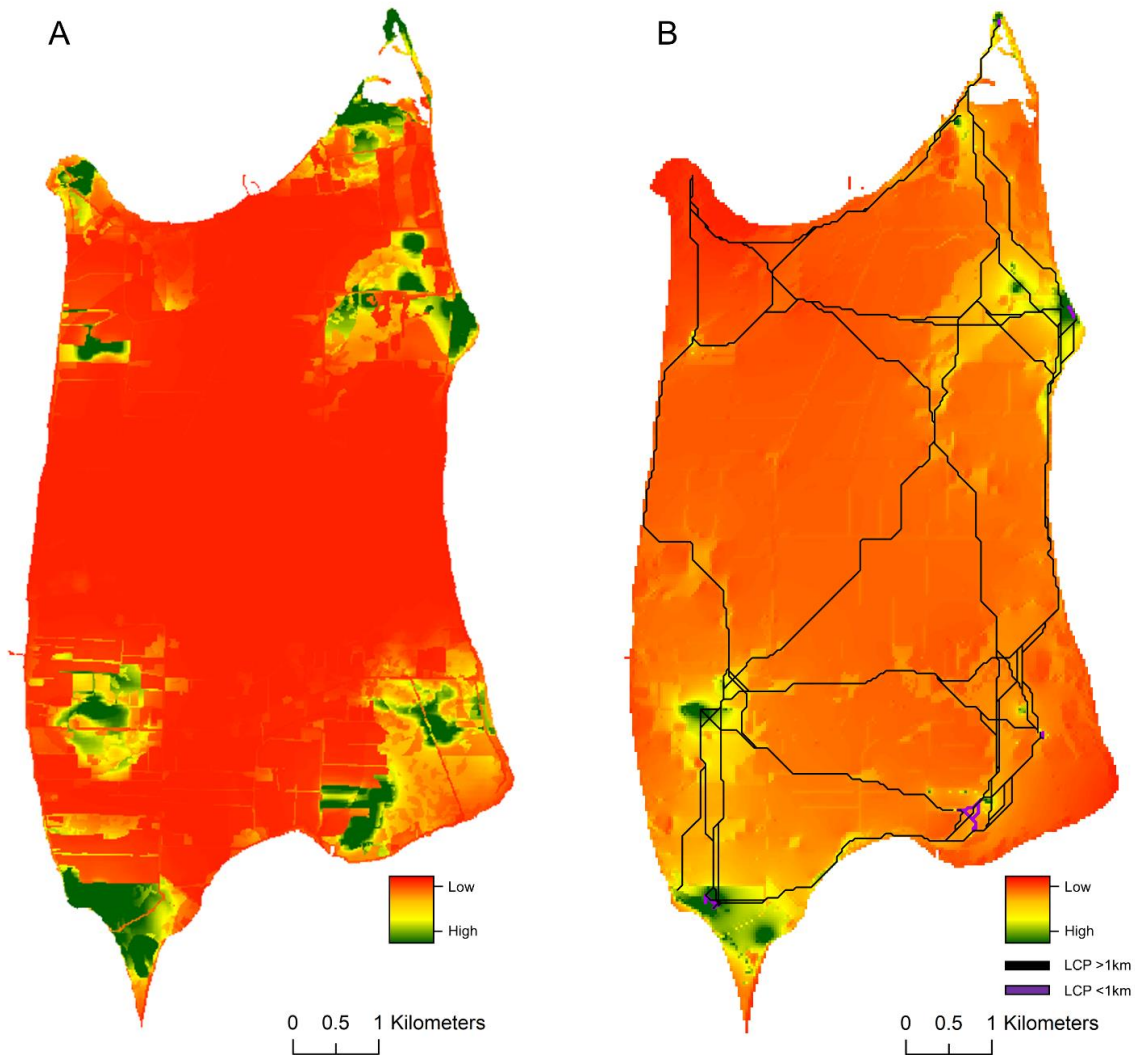


Figure 2: A) Predicted habitat suitability for *Ambystoma* salamanders on Pelee Island, ON, Canada using spatially filtered location data ($n = 476$) collected from 2015-2019 (as modelled in Maxent). Environmental predictor variables include distance to breeding site, land cover type, soil type, and elevation. Green indicates areas with predicted high habitat suitability and red indicates areas with low habitat suitability. B) Predicted habitat connectivity for *Ambystoma* salamanders on Pelee Island, ON, Canada produced using spatially filtered localities ($n = 476$) and a land cover raster grid using resistance values based on Compton et al. 2007 (see Table S1). Green indicates well-connected areas and red indicate areas with low connectivity. Lines depict predicted least-cost paths among known communities, with paths under 1 km in length depicted in purple, and paths are over 1 km in black.

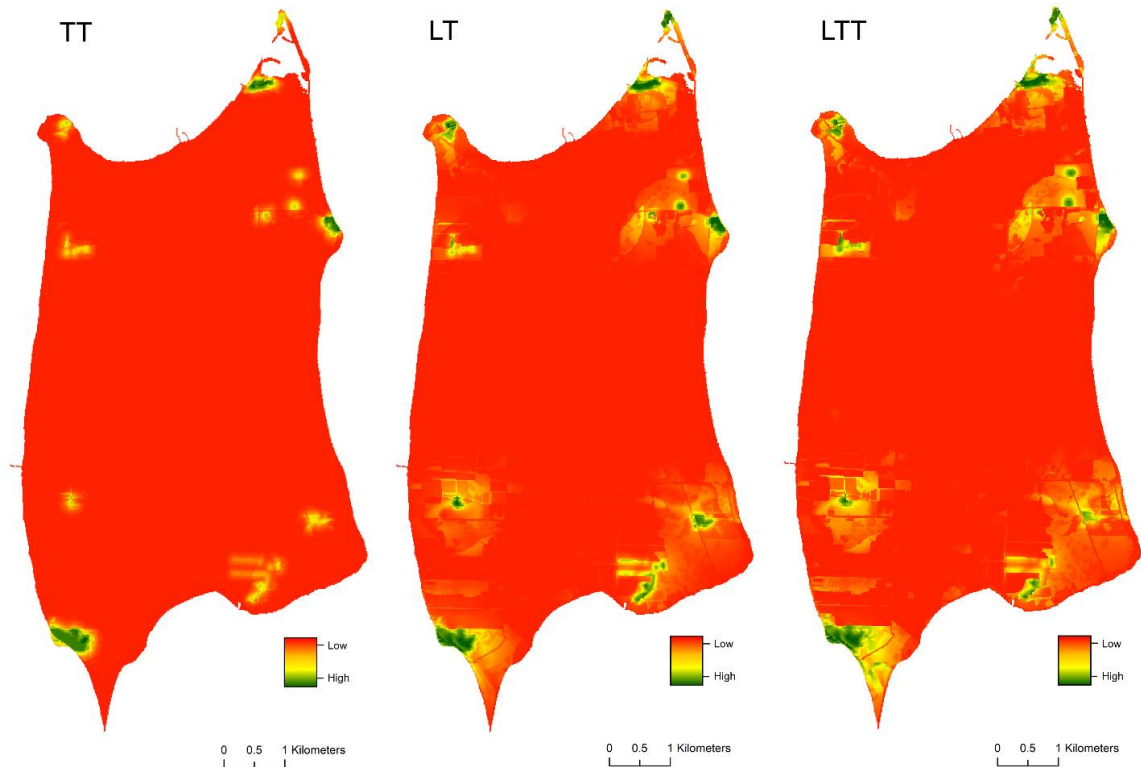


Figure 3: Predicted habitat suitability for three *Ambystoma* salamander genotypes on Pelee Island, ON, Canada, using spatially filtered location data (from 2015-2018) and environmental predictor variables including distance to breeding site, land cover type, soil type, and elevation. Left panel: Predicted habitat suitability for *Ambystoma texanum* (TT) salamanders. Middle panel: Predicted habitat suitability for diploid (LT) unisexual *Ambystoma* salamanders. Right panel: Predicted habitat suitability for triploid (LTT) unisexual *Ambystoma* salamanders. Green indicates areas with predicted high habitat suitability, with orange and red indicating areas with low habitat suitability.

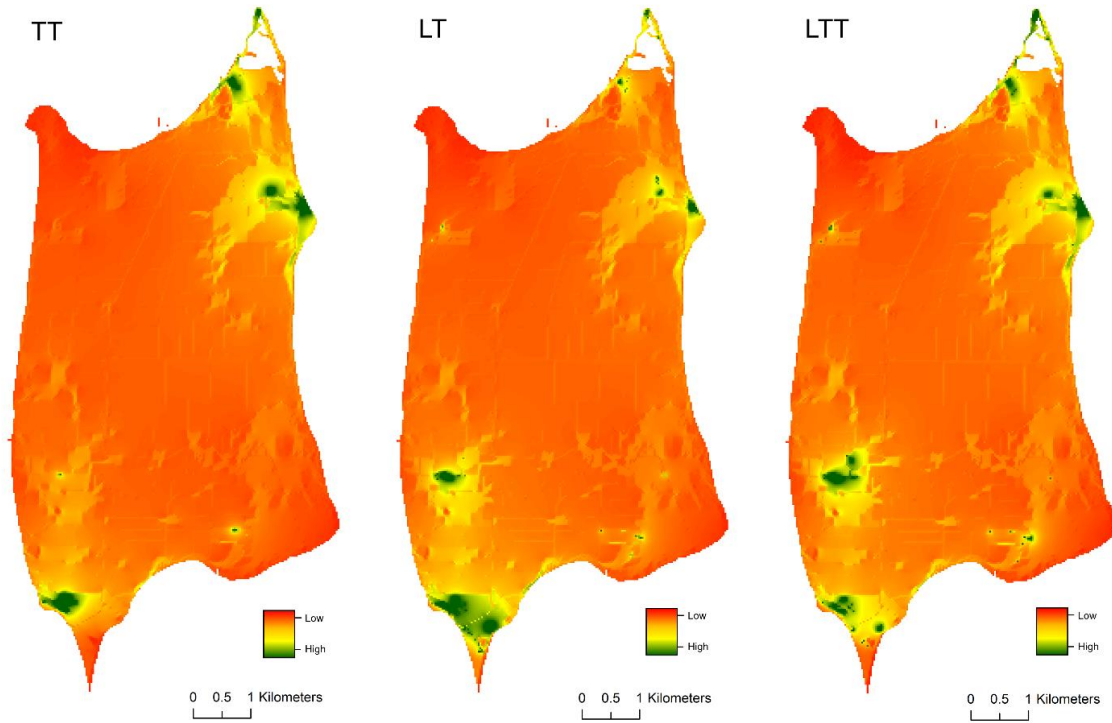


Figure 4: Predicted habitat connectivity for three *Ambystoma* salamander genotypes on Pelee Island, ON, Canada. The models used spatially filtered localities (from 2015-2018) and a land cover raster grid with resistance values based on Compton et al. 2007 (see Table S1). Left panel: Connectivity for *A. texanum* (TT) salamanders. Middle panel: Connectivity for diploid (LT) unisexual *Ambystoma* salamanders. Right panel: Connectivity for triploid (LTT) unisexual *Ambystoma* salamanders. Green indicates well-connected areas, with orange and red indicating areas with low connectivity.

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Chapter 3: General Discussion

Thesis Goals: Revisited

This thesis aimed at identifying suitable habitat and corridors for an imperilled assemblage of salamanders, *Ambystoma* spp., across a fragmented island landscape. I determined that small amounts of suitable habitat remain on Pelee Island, and unsurprisingly, terrestrial habitat was most suitable when close to breeding locations. Habitat patches were poorly connected and most were likely too distant from one another to be realistically accessed by dispersing salamanders without habitat modification inside corridors. Of all salamander genotypes I evaluated, habitat for *A. texanum* was most restricted and isolated whereas diploid (LT) unisexuals had the broadest habitat suitability. From a conservation perspective, increased attention will need to be paid to the status and habitat availability for *A. texanum* on Pelee Island, including through population monitoring and habitat restoration, particularly areas where corridors or suitable breeding ponds could be strategically restored or placed. Overall, our study demonstrates the value of assessing the quality of terrestrial and wetland habitat for imperilled salamanders, especially when habitat patches are small and metapopulation dynamics may be impacted by high fragmentation and limited opportunities for dispersal.

Implications for Ambystoma Conservation Management on Pelee Island

There is much concern over the viability of the *Ambystoma* salamander complex on Pelee Island and whether populations are sustainable over the long term (Hossie 2018). First, the persistence of this complex is especially precarious because of the interconnectedness of each group, including reliance of unisexual *Ambystoma* on the much rarer parental species for sperm parasitism. Results from this study highlight differences

in habitat preferences for different groups, meaning that protection or restoration measures must be directed collectively while also avoiding disruptively favouring one group over another. I recognize that the salamander complex requires both terrestrial and aquatic habitat, meaning that protection of areas surrounding breeding locations is essential. Currently, there is no legislation which defines protected habitat for small-mouthed salamanders or the associated unisexuals, however the provincial recovery strategy recommended protection of “all suitable terrestrial habitat extending radially 300 m from the edge of a known small-mouthed or unisexual *Ambystoma* breeding [location]” (Hossie 2018). Similarly, recent recommendations for protection of the endangered *A. jeffersonianum* salamander in southern Ontario include protecting a 400-450 m buffer around known breeding locations (Van Drunen et al. 2020). My analysis highlights the benefit of a larger buffer size, up to 400-450 m from the breeding location, to accommodate all life stages and critical behaviours like foraging or overwintering. To achieve this goal, it may be necessary to actively restore suitable habitat in protected areas in proximity to sites that are currently occupied by salamanders to bolster the small size of suitable habitat patches (i.e., wetland and forest). Semlitsch and Bodie (2003) advocate for the stratification of protection zones to include both aquatic and terrestrial buffers up to 400 m, where the terrestrial buffer extends beyond the core habitat area. This recommendation highlights the deficiency in Ontario’s current land-use planning approach because wetland protection rarely encompasses accompanying terrestrial buffers of sufficient size to support adult salamanders.

Broadly speaking, current conservation and habitat restoration efforts on Pelee Island are relatively widespread, with roughly 18% of the island being protected (777.6

ha) publicly and by private landowners (including conservation easements, provincial parks, and areas managed by environmental non-governmental organizations); this compares to only 11% protection for mainland Ontario (MNRF 2017; Office of the Auditor General of Ontario 2020). The protected areas on Pelee Island include forested and wetland ecosystems like swamp forests, as well as some historical farmland which has been converted into native meadows as well as small wetlands. Some of these protected areas are relatively large, ranging in size from 9 to 183 hectares (G. Smith, unpublished data). A number of these areas are isolated, but many are well-connected and effectively serve as large conservation areas managed by multiple stakeholders. Pelee Island supports many species listed as threatened both federally and provincially, including over 100 plant species and several unique snails and herpetofauna. Pelee Island also includes several unique ecosystem types which are globally rare, including alvars and oak savannahs (Forbes et al. 2000; NCC 2008).

As a testament to the momentum for active involvement in stewardship and restoration efforts in protected areas on Pelee Island, note that the Nature Conservancy of Canada (NCC) recently constructed a ~25 ha wetland on their property in proximity to an existing population of salamanders. NCC constructed additional ponds in 2021 in areas identified by our team as potential movement corridors for salamanders and is also supporting natural forest succession in several open fields adjacent to existing salamander habitat (J. Crosthwaite, personal communication, 2021). My study showed that forest and non-breeding wetland ecosystems must be close to breeding locations to be considered suitable habitat for *Ambystoma* spp., so the improvement of these areas will be beneficial for the persistence of this population by increasing breeding habitat and overall

connectivity within this area. These efforts will be particularly beneficial for some of the largest populations of salamanders that I studied, as the constructed ponds and expanded forested habitat are directly adjacent. Accordingly, efforts to establish additional protected areas on Pelee Island are likely to help promote the long-term viability of the *Ambystoma* spp. complex. As evidence of this success, three newly-constructed ponds (fall 2020), which were established following recommendations informed by my preliminary research findings and modified based on follow-up recommendations, have already been used by salamanders for breeding purposes (i.e., larvae and juveniles were detected in 2021 in one breeding locations, eggs were detected in spring 2022 in all three breeding locations; J. McDonald, personal communication). This pond is >300 m from the nearest known existing *Ambystoma* spp. breeding location, providing a promising indication that this conservation effort is having tangible benefits. Future restoration efforts and research should continue to evaluate the success of these constructed ponds considering that they may not always be situated in areas that are as suitable for salamanders as natural breeding locations (Ward and Hossie 2020).

Improving HS and Connectivity Modelling Approaches

Habitat suitability and connectivity models such as those used in this thesis are predictive and assume that current conditions are stationary and that species observations used to train the model are representative. It is reasonable to assume that Pelee Island's landcover features and environmental conditions are not rapidly changing, but occurrence data are often recognized as being biased towards areas having received disproportionate survey efforts in the past; this bias can exaggerate the assessed importance of over-represented sampling locations (Fourcade et al. 2014). It follows that such

misrepresentation can lead to severe repercussions for conservation and management (Kramer-Schadt et al. 2013). Yackulic et al. (2013) found that most (87%) of reported studies assessing species occurrence records had not applied appropriate measures to limit potential bias in their sampling methods. Notably, my work included considerable efforts to avoid similar problems by rarefying location data to minimize spatial autocorrelation of the environmental predictors used in my models. Further, because species distribution models are ‘presence-only’ and therefore do not use known absence data but rather generate pseudo-absences, there is a high risk of false-negative detections, especially for species that are cryptic or have seasonally variable distributions (Hirzel and Le Lay 2008; Lobo et al. 2010). However, my efforts to purposefully sample in areas assumed to be unsuitable habitat and in previously unsampled areas aimed to avoid this bias (i.e., avoid ‘methodological absences’; see Lobo et al. 2010). This testing largely confirmed that areas modelled as being poor salamander habitat would not be able to support animals.

Species distribution and connectivity models are associated with a degree of uncertainty and should be interpreted based on the biology of the species of interest. Models are solely based on the input variables, and the output can be sensitive to variation in animal-environment interactions (Thuiller and Münkemüller 2010; Rudnick et al. 2012). These input variables had to be carefully selected in my analyses to only include variables relevant to *Ambystoma* spp., including avoiding the stratification of land cover types into groups that were functionally equivalent to salamanders. Connectivity models, in particular, must account for species biology, including the ability of organisms to travel a given distance in marginal habitat. It follows that field validation of this

assumption is a necessary aspect of developing corridors that are relevant to the species of interest (Rudnick et al. 2012). Particular attention should be paid to geographical barriers to movement, including unusable habitat such as roads or tilled fields (Kramer-Schadt et al. 2004; Kimmig et al. 2020). These land use changes can compound the effects of habitat fragmentation on dispersal capability, but the extent of acceptable disturbance will depend on an animal's ability to occupy and cross through marginal habitat. Given their small size and dependence on moisture for survival, *Ambystoma* salamanders may have limited ability to use marginal habitat unless it provides sufficient structural cover and protection from sun and wind.

Role of Modelling in Research and Conservation Management

Conservation planners and managers must prioritize protection and strategic integration of areas that have high ecological value, as well as identify potential corridors for maintaining metapopulation dynamics and species persistence. Habitat suitability and connectivity models can be used to evaluate the current distribution of species and determine areas for conservation priority or species reintroduction (Larson et al. 2004; Thorn et al. 2009). For *Ambystoma* spp. on Pelee Island, this could potentially involve assisted colonization or *ex situ* head-starting in intermediately- or highly-suitable areas. Such efforts could improve *Ambystoma* spp. resilience to demographic and environmental changes particularly in areas with low numbers of parental species (Semlitsch et al. 2017). Future investigation will be necessary to assess the feasibility of these recovery strategies and whether newly-established animals fully use restored habitats for reproduction or dispersal and whether viable populations can be sustained. Additionally, habitat suitability models, if designed properly, have the potential to predict areas where

unknown populations may be found; this can be particularly beneficial for focusing field surveys and conservation efforts which have finite resources (e.g., see Stratmann et al. 2016; Udyawer et al. 2020). As mentioned previously, my preliminary work identifying potentially suitable habitat along movement corridors on Pelee Island helped inform the establishment of several new *Ambystoma* breeding ponds and my final results help highlight additional candidate sites.

Conclusions and Future Directions

Broadly, my findings emphasize the importance of evaluating remaining habitat features for imperilled species occupying altered landscapes. I identified characteristics of the remaining habitats that are important for the *Ambystoma* salamander complex on Pelee Island, many of which will require increased and continued conservation management and habitat restoration planning to maintain their long-term viability. This approach would be beneficial for other species at risk of extirpation, such as the Blanding's turtle (*Emydoidea blandingii*) and the blue racer (*Coluber constrictor foxii*) on Pelee Island, or others that are threatened as a result of encroaching urban development on natural areas and have limited distributions on the island. More work needs to be done on the Pelee Island salamander complex to evaluate the success of habitat creation efforts and to monitor changes in habitat suitability and connectivity as well as population and distribution. Future investigation should also incorporate population monitoring on groups within smaller and more isolated areas of suitable habitat to assess their long-term viability. Such efforts are especially needed in populations with low abundance of parental *Ambystoma* species, where extinction risk may be high. Finally, I recommend that future studies incorporating habitat suitability and connectivity models for terrestrial

salamanders need to account for biased spatial and input data to improve the reliability of the models. These efforts will ensure the accuracy of model findings and thereby help guide effective conservation efforts in highly fragmented landscapes. In sum, my thesis makes an important contribution in this area and provides a strong foundation for future efforts targeting the conservation of salamanders and other organisms on Pelee Island and beyond.

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Supplementary Information

Table S1: Resistance values created for *Ambystoma* salamanders on Pelee Island, ON, Canada, based on work by Compton et al. (2007). A resistance value of 1 indicates minimal resistance (i.e., most easy to travel through), and a value of 20 indicates maximum resistance (i.e., movement completely impeded). This table also outlines how we grouped land cover types in our analyses to reduce the dimensionality of this variable.

	Resistance Value	Land Cover Type	Description
Wetland	1	Breeding Pond	Based on location data
		Pond; Thicket Swamp	No fish, no record of breeding; Thick growths of tall shrubs (willow, dogwood, alder)
Forest	2	Thicket	<10% tree cover and >25% tall shrub cover
		Treed swamp	>25% tree or shrub cover, seasonally flooded
		Deciduous forest	Tree cover >60%, upland deciduous tree sp, >2m
		Forest	Tree cover >60%, upland tree species >2m in height
		Alvar	Level, unfractured limestone, veg cover <60%
		Marsh	Open, shrub and treed communities
Tall grass Comm	4	Developing forest	Some trees and vegetation
		Meadow	Open, vegetated by grass and non-woody plants
		Converted field	Previously farmland, currently growing grasses and shrub cover
		Adjacent agricultural land	Windbreak
		Hedge row; Culvert	Tree cover >60%, 2m in height, linear, min 10m width, max 30m width; Somewhat open water
		Treed sand dune	Exposed sands, veg cover <60%
		Plantations; Beach	Tree cover >60%, min 2m in height, linear, uniform tree type; <25% veg cover, sand
Agricultural	6	Agricultural Land	Used for various types of crops (soybean, wheat, etc)
		Orchard	Linear, ground cover is grass, grape fields
		Grass	
Transportation	7	Dirt road	On properties or through farmland
		Road; Small building	Paved; Building that takes up <30% of cell
		Airway road	
Human Disturbance	15	Small building	Building that takes up 50% of cell
		Large building	Building that takes up <75% of cell
		Built Up/Barns	Building that takes up 100% of cell
		Dock; Extraction	Boat docks; Pits, quarries
Water	20	Lake	Open water

Table S2: Proportion of land cover types across Pelee Island, ON, according to the classification used in these models.

Land Cover	Area (km ²)	Proportion of Terrestrial Area (%)
Wetland	2.3	5.7
Forest	6.7	16.5
Tallgrass Community	3.1	7.5
Agricultural	25.3	62.1
Transportation	1.6	3.8
Human Disturbance	1.8	4.4

Table S3: AUC values of Maxent models for *Ambystoma texanum* and unisexual *Ambystoma*. Training data AUC is calculated from training data selected from the data set, and test data AUC is the real test of the model's predictive power.

Model	Training data AUC	Test data AUC
All Locations	0.965	0.961
Adults	0.964	0.966
Juveniles	0.980	0.971
Unisexual <i>Ambystoma</i> (LT)	0.968	0.967
Unisexual <i>Ambystoma</i> (LTT)	0.973	0.969
<i>Ambystoma texanum</i> (TT)	0.991	0.992
Mar – Apr captures	0.972	0.969
May – Jul captures	0.968	0.954
Aug – Sep captures	0.983	0.970

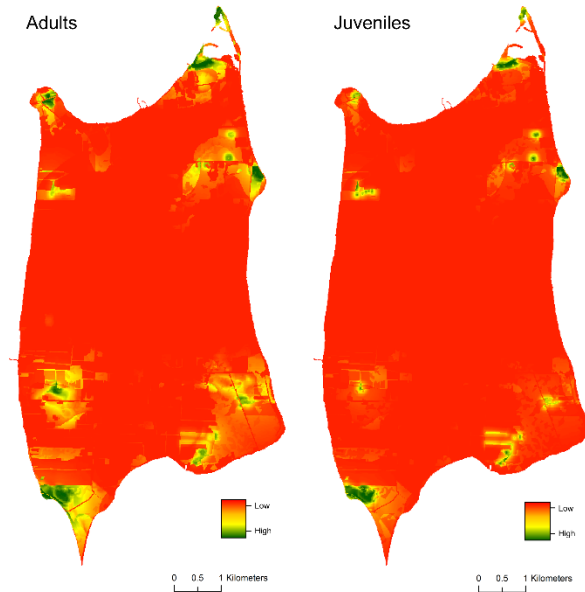


Figure S1: Predicted habitat suitability for adult and juvenile salamanders on Pelee Island, ON, Canada, using spatially filtered localities (from 2015-2019) and environmental predictor variables, including distance to breeding site, land cover type, soil type, and elevation. Left panel: Predicted habitat suitability based on all adult *Ambystoma* salamander locations ($n = 369$). Right panel: Predicted habitat suitability based on all juvenile *Ambystoma* salamander locations ($n = 179$). Green indicates areas with predicted high habitat suitability and red indicate areas with low habitat suitability.

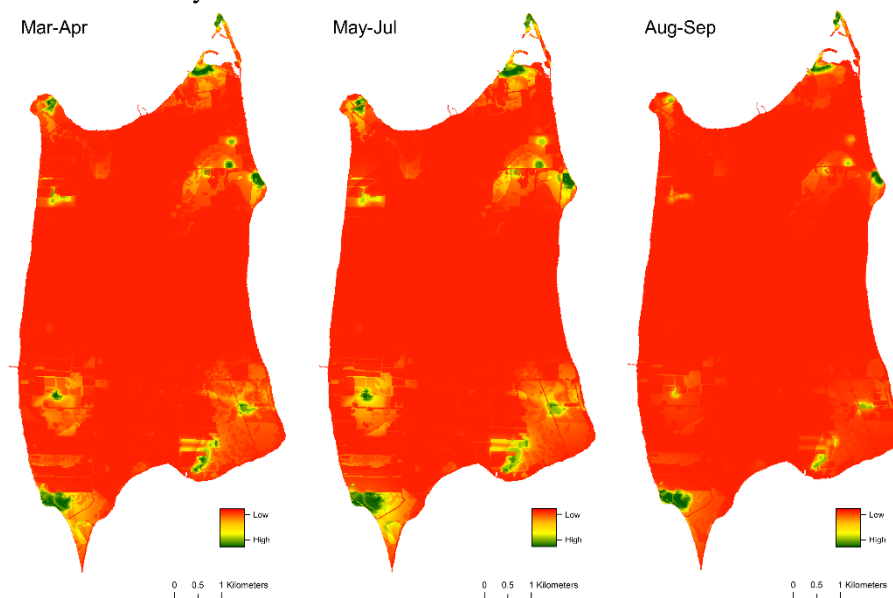


Figure S2: Predicted habitat suitability for three seasons on Pelee Island, ON, Canada, using spatially filtered localities (from 2015-2019) and environmental predictor variables, including distance to breeding site, land cover type, soil type, and elevation. Left panel: Predicted habitat suitability based on *Ambystoma* salamander locations from March and April 2015-2019 ($n = 190$). Center panel: Predicted habitat suitability based on *Ambystoma* salamander locations from May – July 2015-2019 ($n = 233$). Right panel: Predicted habitat suitability based on *Ambystoma* salamander locations from August and September 2015-2019 ($n = 136$). Green indicates areas with predicted high habitat suitability and red indicates areas with low habitat suitability.

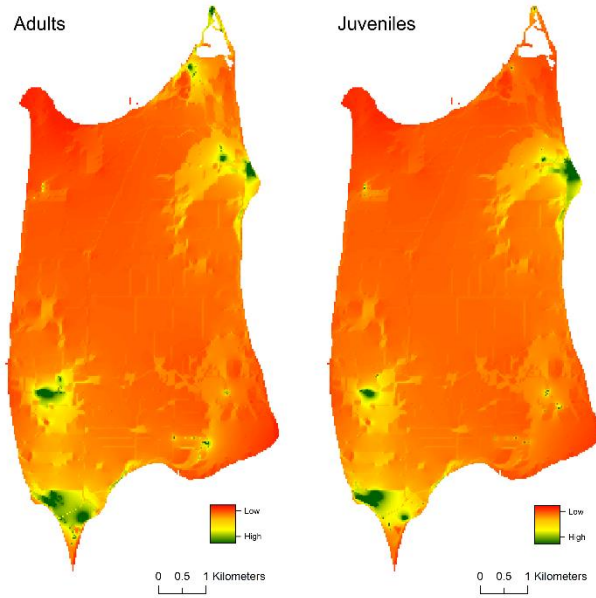


Figure S3: Predicted habitat connectivity for adult and juvenile life stages on Pelee Island, ON, Canada, using spatially filtered localities (from 2015-2019) and a land cover raster grid using resistance values based on Compton et al. 2007 (see Table S1). Left panel: Connectivity based on adult *Ambystoma* salamander locations ($n = 369$). Right panel: Connectivity based on juvenile *Ambystoma* salamander locations ($n = 179$). Green indicates well-connected areas and red indicates areas with low connectivity.

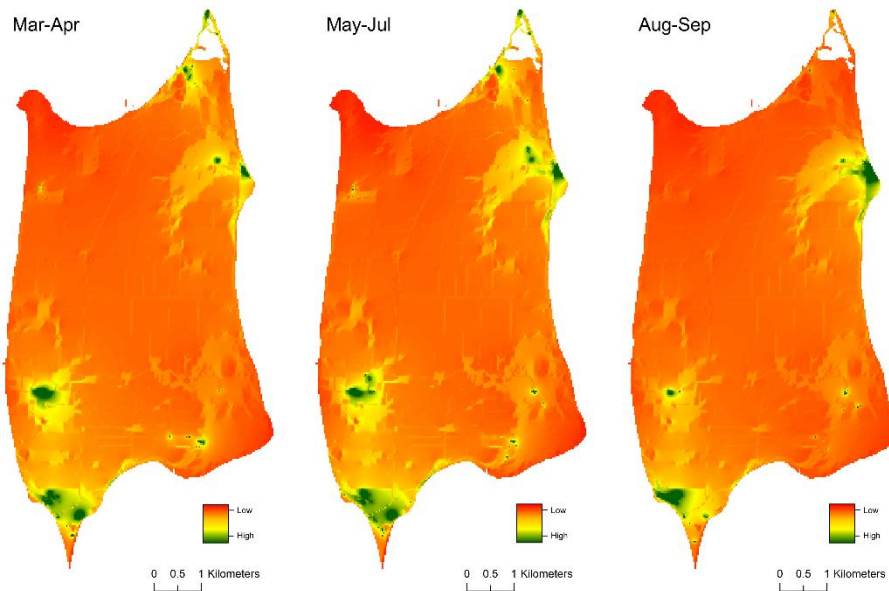


Figure S4: Predicted habitat connectivity comparing seasons on Pelee Island, ON, using spatially filtered localities and a land cover raster grid using resistance values based on Compton et al. 2007 (see Table S1). Left panel: Connectivity based on *Ambystoma* salamander locations from March and April 2015-2019 ($n = 190$). Center panel: Connectivity based on *Ambystoma* salamander locations from May – July 2015-2019 ($n = 233$). Right panel: Connectivity based on *Ambystoma* salamander locations from August and September 2015-2019 ($n = 136$). Green indicates well-connected areas, with orange and red indicating areas with low connectivity.

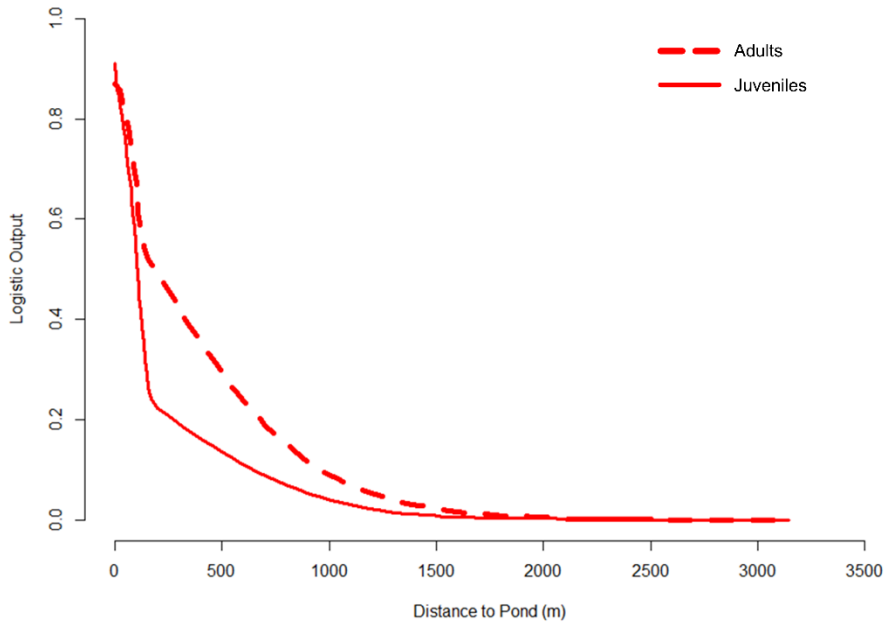


Figure S5: Effect of distance to breeding site (m) on predicted habitat suitability for adult and juvenile life stages of *Ambystoma* salamanders on Pelee Island, ON, Canada collected from 2015-2019. Dashed line depicts the relationship for adult *Ambystoma* salamanders. Solid line depicts the relationship for juvenile *Ambystoma* salamanders. Higher values on y-axis represent higher habitat suitability.

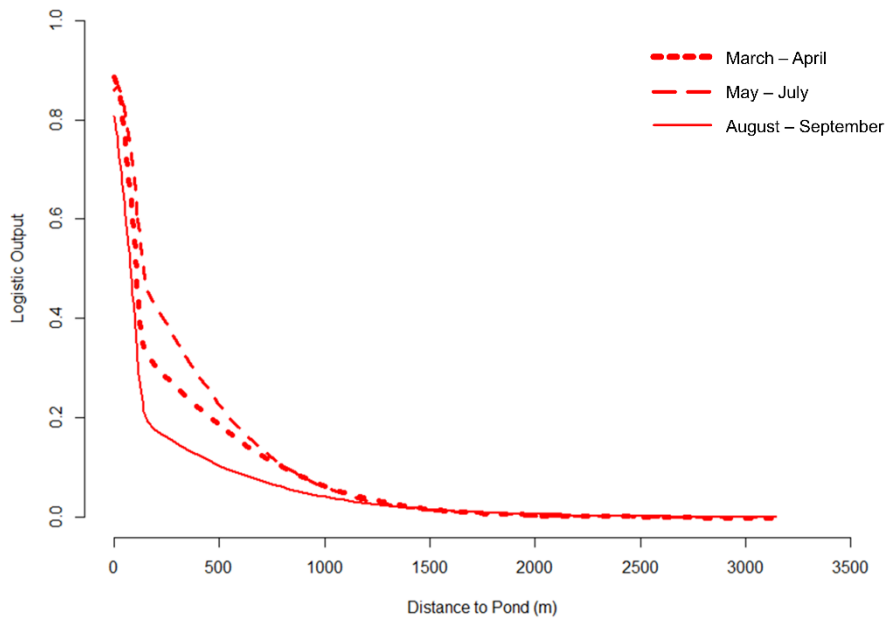


Figure S6: Effect of distance to breeding site (m) on predicted habitat suitability *Ambystoma* salamanders on Pelee Island, ON, Canada across seasons using data collected from 2015-2019. Dotted line indicates model response based on *Ambystoma* salamander locations from March-April ($n = 190$). Dashed line indicates model response based on *Ambystoma* salamander locations from May-July ($n = 233$). Solid line indicates model response based on *Ambystoma* salamander locations from August-September ($n = 136$). Higher values on y-axis represent higher habitat suitability.

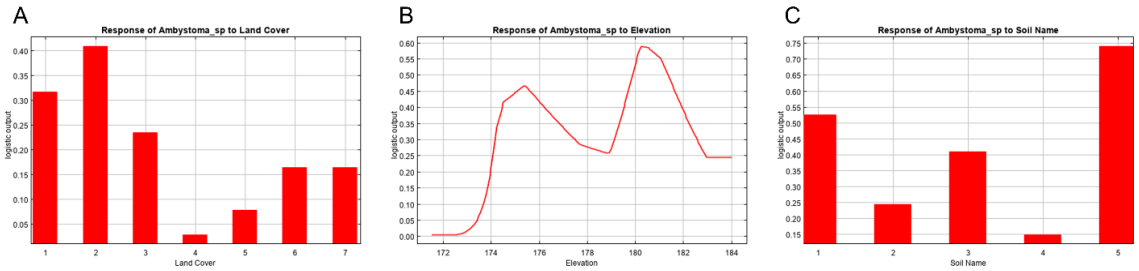


Figure S7: Effect of tested environmental variables on predicted habitat suitability for *Ambystoma* salamanders ($n = 476$) on Pelee Island, ON, Canada collected from 2015-2019. A) Effect of land cover classifications on Maxent model. Land cover classes were 1: Wetland, 2: Forest, 3: Tallgrass Community, 4: Agricultural, 5: Transportation, 6: Human Disturbance, 7: Open Water. B) Effect of elevation on Maxent model. C) Effect of soil type on Maxent model. Soil classes were 1: Eastport Sand, 2: Farmington Loam, 3: Brookston Clay, 4: Toledo Clay, 5: Marsh. Higher values on y-axis represent higher habitat suitability.

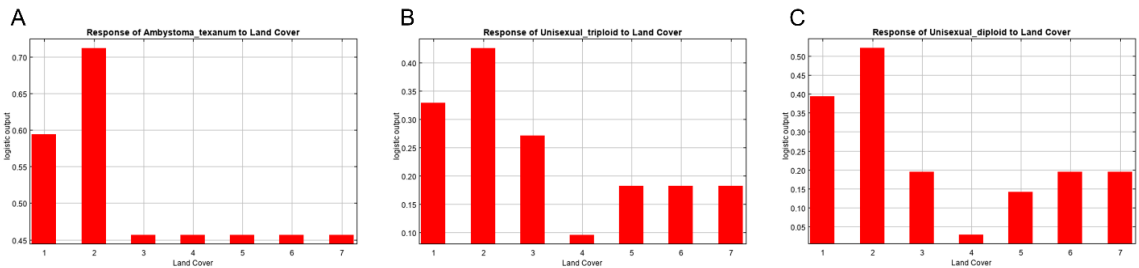


Figure S8: Effect of land cover type on predicted habitat suitability of *Ambystoma* salamanders on Pelee Island, ON, Canada collected from 2015-2018, based on tested genotypes. A) locations of *Ambystoma texanum* (TT) individuals ($n = 37$). B) locations of unisexual diploid (LT) individuals ($n = 286$). C) locations of unisexual triploid (LTT) individuals ($n = 156$). Land cover classes were 1 – Wetland, 2 – Forest, 3 – Tallgrass Community, 4 – Agricultural, 5 – Transportation, 6 – Human Disturbance, 7 – Open Water. Higher values on y-axis represent higher habitat suitability. Note the different scales on the y-axis.

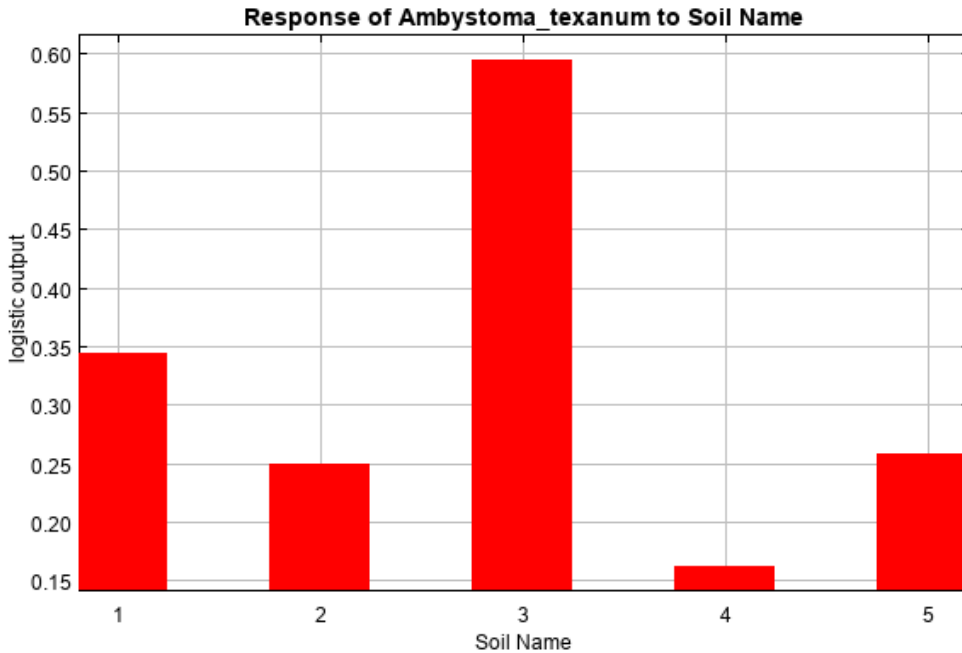


Figure S9: Effect of soil type on predicted habitat suitability of *Ambystoma texanum* (TT) salamanders on Pelee Island, ON, Canada collected from 2015-2018. Soil type classes were 1- Eastport Sand, 2- Farmington Loam, 3- Brookston Clay, 4- Toledo Clay, 5- Marsh. Higher values on y-axis represent higher habitat suitability.

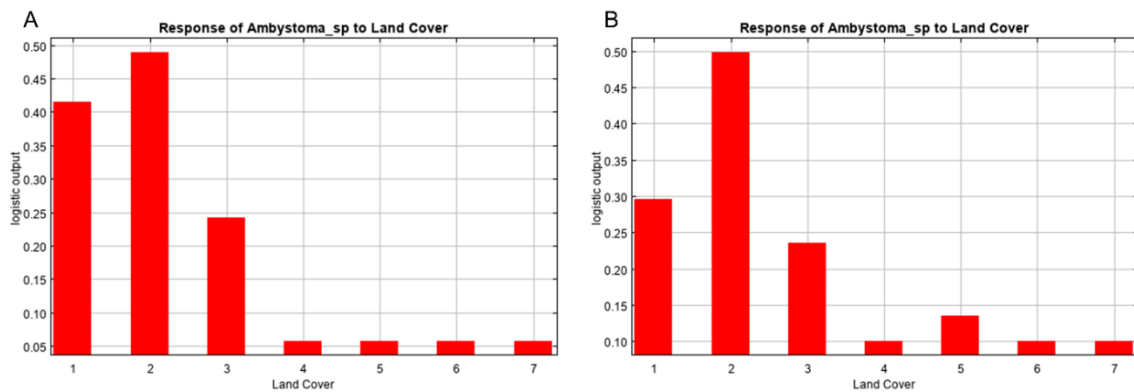


Figure S10: Effect of land cover type on predicted habitat suitability of *Ambystoma* salamanders on Pelee Island, ON, Canada collected from 2015-2019, based on tested life stages. A) locations of adult salamanders ($n = 369$). B) locations of juvenile salamanders ($n = 179$). Land cover classes were 1: Wetland, 2: Forest, 3: Tallgrass Community, 4: Agricultural, 5: Transportation, 6: Human Disturbance, 7: Open Water. Higher values on y-axis represent higher habitat suitability.

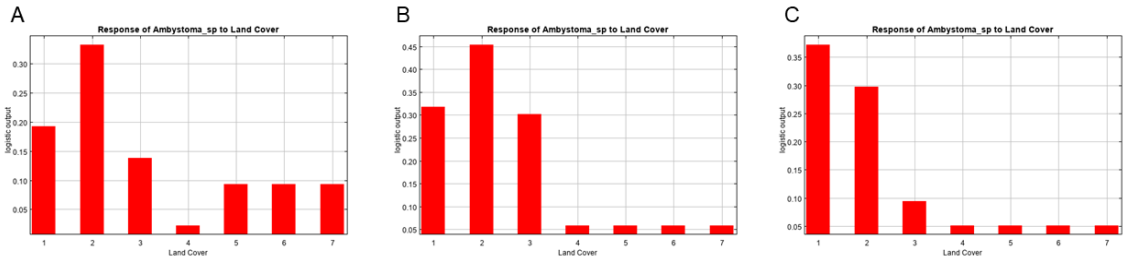


Figure S11: Effect of land cover type on predicted habitat suitability of *Ambystoma* salamanders on Pelee Island, ON, Canada collected from 2015-2019, based on tested seasons. A) locations collected in March and April ($n = 190$). B) locations collected from May-July ($n = 233$). C) locations collected in August and September ($n = 136$). Land cover classes were 1 – Wetland, 2 – Forest, 3 – Tallgrass Community, 4 – Agricultural, 5 – Transportation, 6 – Human Disturbance, 7 – Open Water. Higher values on y-axis represent higher habitat suitability. Note the different scales on the y-axis.

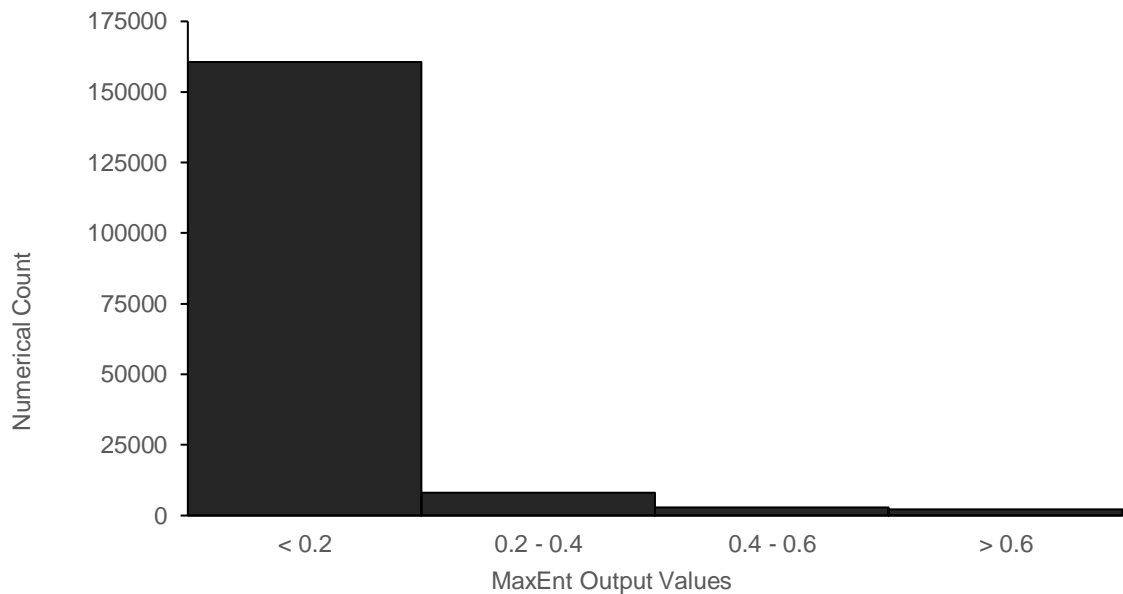


Figure S12: Distribution of habitat suitability of *Ambystoma* salamanders across Pelee Island, ON, Canada collected from 2015-2019, based on predicted suitability of individual 10x10m grid cells ($n = 173,764$) from model output using all salamander location data ($n = 476$). Suitability values ranged from 0 to 1: values <0.2 represented unsuitable habitat, values between 0.2 and 0.4 represented low suitability, values between 0.4 and 0.6 represented intermediate suitability and values >0.6 represented high suitability.

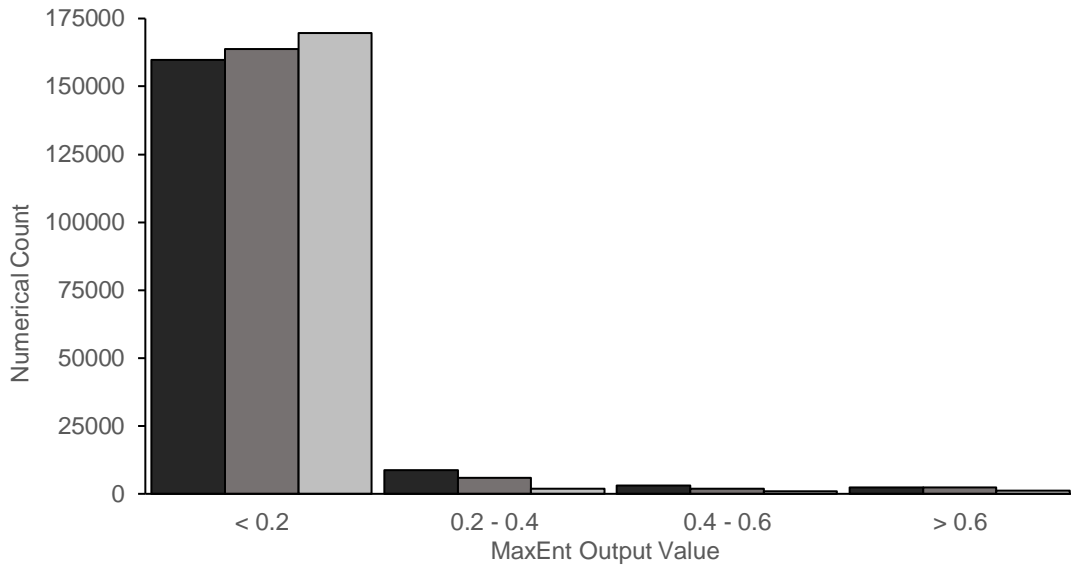


Figure S13: Distribution of habitat suitability of *Ambystoma* salamanders across Pelee Island, ON, Canada collected from 2015-2018, based on predicted suitability of individual 10x10m grid cells ($n = 173,764$) from model outputs of tested genotypes. Black represents all unisexual diploid (LT) locations ($n = 286$); Dark gray represents all unisexual triploid (LTT) locations ($n = 156$); Light gray represents all *Ambystoma texanum* (TT) locations ($n = 37$). Suitability values ranged from 0 to 1: values <0.2 represented unsuitable habitat, values between 0.2 and 0.4 represented low suitability, values between 0.4 and 0.6 represented intermediate suitability and values >0.6 represented high suitability.

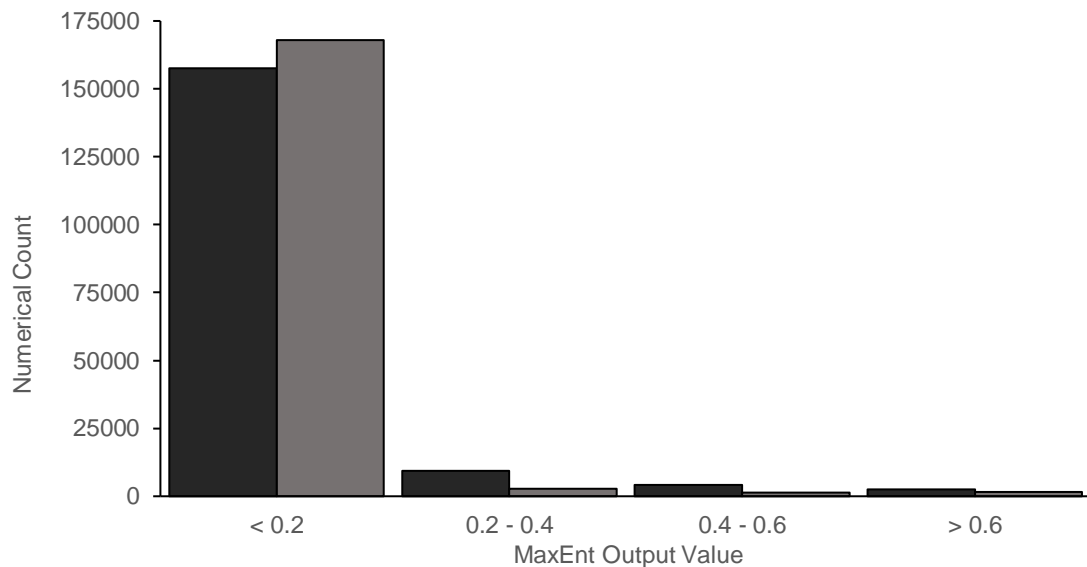


Figure S14: Distribution of habitat suitability of *Ambystoma* salamanders across Pelee Island, ON, Canada collected from 2015-2019, based on predicted suitability of individual 10x10m grid cells ($n = 173,764$) from model outputs of tested life stages. Black represents all adult salamander location data ($n = 369$); Gray represents all juvenile salamander location data ($n = 179$). Suitability values ranged from 0 to 1: values <0.2 represented unsuitable habitat, values between 0.2 and 0.4 represented low suitability, values between 0.4 and 0.6 represented intermediate suitability and values >0.6 represented high suitability.

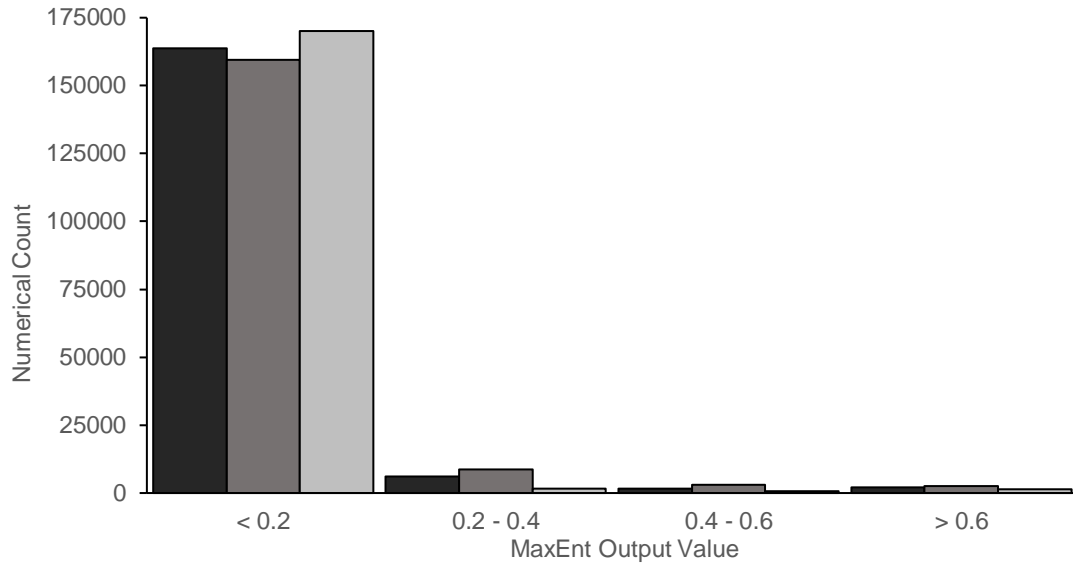


Figure S15: Distribution of habitat suitability of *Ambystoma* salamanders across Pelee Island, ON, Canada collected from 2015-2019, based on predicted suitability of individual 10x10m grid cells ($n = 173,764$) from model outputs of tested seasons. Black represents all locations collected in March and April ($n = 190$); Dark gray represents all locations collected from May-July ($n = 233$); Light gray represents all locations collected in August and September ($n = 136$). Suitability values ranged from 0 to 1: values < 0.2 represented unsuitable habitat, values between 0.2 and 0.4 represented low suitability, values between 0.4 and 0.6 represented intermediate suitability and values > 0.6 represented high suitability.