COSEWIC
Assessment and Status Report

on the

Jefferson Salamander
Ambystoma jeffersonianum

in Canada

ENDANGERED
2010

COSEWIC
Committee on the Status
of Endangered Wildlife
in Canada

COSEPAC
Comité sur la situation
des espèces en péril
au Canada

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Jefferson Salamander — *Ambystoma jeffersonianum* female from Hamilton-Wentworth Region, Ontario. Photographs by J. P. Bogart.

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COSEWIC
Assessment Summary

Assessment Summary – November 2010

Common name
Jefferson Salamander

Scientific name
Ambystoma jeffersonianum

Status
Endangered

Reason for designation
This salamander has a restricted range within populated and highly modified areas. Over the past three generations, the species has disappeared from many historic locations and the remaining locations are threatened by development, loss of habitat and, potentially, the presence of sperm-stealing unisexual populations of salamanders.

Occurrence
Ontario

Status history
Designated Threatened in November 2000. Status re-examined and designated Endangered in November 2010.

COSEWIC
Executive Summary

Jefferson Salamander
Ambystoma jeffersonianum

Wildlife species information

Ambystoma jeffersonianum, Jefferson Salamander, is a long, slender, dark grey to brownish member of the mole salamander family with elongated limbs and toes. Light bluish-grey flecks may occur along the lower sides of the body and tail. Adults range in size from 60 to 104 mm snout-vent length with a tail that is nearly as long as the body and is laterally compressed. Males, in breeding condition, have a distinctly swollen cloacal region. Unisexual (all-female) *Ambystoma*, which co-exist with Jefferson Salamanders in all known Canadian populations, have a very similar morphology to female Jefferson Salamanders.

Distribution

The geographic range of Jefferson Salamander roughly coincides with upland deciduous forest in northeastern North America from New England to Indiana and south to Kentucky and Virginia. In Canada, the species is found only in isolated populations that are mostly associated with the Niagara Escarpment and Carolinian forest regions in Ontario.

Habitat

Adult Jefferson Salamanders, throughout their range, are found within deciduous or mixed upland forests containing, or adjacent to, suitable breeding ponds. Breeding ponds are normally ephemeral, or vernal, woodland pools that dry in late summer. Terrestrial habitat is in mature woodlands that have small mammal burrows or rock fissures that enable adults to over-winter underground below the frost line.

Biology

Adults migrate to and from breeding ponds at night very early in spring when temperatures are moderate. Most migration events to or from breeding ponds coincide with rain. Courtship and egg deposition may occur under the ice of vernal pools and individual males court several females. Within a day or two after mating, females deposit several egg masses on sticks or emergent vegetation. Duration of egg and larval development is variable and temperature-dependent. Carnivorous larvae normally transform in July or early August and leave the pond. Adults spend most of their time under rocks, logs, or in mammal burrows in the forest. Adults over-winter in the terrestrial environment below the frost line.

Unisexual *Ambystoma*, which are mostly polyploid, occur in all known Jefferson Salamander populations in Ontario. They are much more numerous than Jefferson Salamanders and, apparently, have the same behaviour as female Jefferson Salamanders. These females court male Jefferson Salamanders and use sperm from the males to initiate development of their eggs. The sperm may or may not be incorporated into the egg.

Population sizes and trends

Estimation of population sizes of the Jefferson Salamander is difficult because of the presence of unisexuals that are morphologically similar to female Jefferson Salamanders. Simply counting the number of salamanders migrating to or from a breeding pond would include unisexual individuals. Recent surveys show that very low numbers of pure Jefferson Salamanders actually exist in populations, even those that have a high density of salamanders. Most of the historical sites surveyed in 1990 and 1991 no longer supported populations of either the Jefferson Salamander or unisexuals in 2003 and 2004. Furthermore, at some sites where both Jefferson Salamanders and unisexuals still existed in 2003-04, there was a notable reduction in the number of egg masses compared to numbers found in the earlier surveys.

Limiting factors and threats

In Ontario, the Jefferson Salamander is limited by availability of suitable habitat that would include deciduous or mixed forested upland areas associated with fishless ponds that are most often temporary or vernal pools. Threats include the partial or absolute elimination of suitable habitat, construction of barriers (e.g., roads) across migratory routes to or from breeding ponds, stocking fish in breeding ponds, or reduction of the hydro period of breeding ponds so larvae do not have time to complete their development.

Special significance of the species

Jefferson Salamander is a large salamander and is considered to be a good biological indicator of a healthy environment in the United States. In Canada, it is only found in Ontario and is associated with upland, forested areas that are, historically, relatively unchanged. Unisexual (all-female) *Ambystoma*, which are more numerous than female Jefferson Salamanders, use male Jefferson Salamanders as sperm donors in all known Ontario populations. The co-evolution of Jefferson Salamander and the unisexuals has special significance because it appears to be a unique evolutionary system.

Existing protection

Over most of its range in the U.S., Jefferson Salamander is listed as secure but it is listed as imperiled in Vermont and Illinois. In Canada, the species was assessed as Threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2000, and listed as Threatened under Canada's *Species at Risk Act* (SARA) in 2002. It has also been assessed by the Committee on the Status of Species at Risk in Ontario (COSSARO), and listed as Threatened by the Ontario Ministry of Natural Resources (OMNR) in 2004. In 2008, the species was listed as Threatened in Regulation 230/08 (the Species at Risk in Ontario (SARO) List) under the new Ontario *Endangered Species Act (ESA), 2007*. The species received habitat protection under the *ESA, 2007*, in the form of a habitat regulation which came into force February 18, 2010 (Regulation 242/08). The Provincial Recovery Strategy for the Jefferson Salamander was published in February 2010.

TECHNICAL SUMMARY

Ambystoma jeffersonianum
Jefferson Salamander — Salamandre de Jefferson
Range of occurrence in Canada (province/territory/ocean): Ontario

Demographic Information

Generation Time = Age at maturity + 1/mortality. Where mortality = annual rate of mortality of adults. Mortality rate estimated from Weller (1980) and Downs (1989) as mean of 2, 12 and 27% = 14% (see **Life Cycle and Reproduction**)	Gen Time= 4 + 1/0.14 =11 years.
Is there an [observed, inferred, or projected] continuing decline in number of mature individuals?	Yes: Decline, observed and projected
Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations]	Unknown
[Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations]. See **Fluctuations and Trends section)** There were 87 historic sites of which 33 have been recently (since 2000) confirmed, representing a 59% decline over about three generations (1977-2010) (see Appendix 1 for complete list). This period goes back roughly to the first record of the species in Canada in 1976. There are now thought to be fewer than 30 extant locations as some of the 33 confirmed sites likely have lost Jefferson Salamanders since 'confirmed' a few years ago	Decline observed and suspected. Two long-term data sets (Location C, and Weller pond) indicate > 90% decline over < 3 generations (33 years)
[Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations].	Unknown, but likely a decline
[Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any [10 years, or 3 generations] period, over a time period including both the past and the future.	Unknown, but definitely a decline
Are the causes of the decline clearly reversible and understood and ceased?	Some causes are known but not ceased nor, usually, reversible
Are there extreme fluctuations in number of mature individuals?	No

Extent and Occupancy Information

Estimated extent of occurrence Populations exist in suitable habitat on the Niagara Escarpment from Grey County to Hamilton Region and on the Oak Ridge Moraine in York Region. A few isolated populations occur in Waterloo County, Brant County, and in Haldimand-Norfolk Region. The distribution is not continuous in any of these regions but there are zones of suitable habitat. The estimate covers all known locations at which both Jefferson Salamanders and LJJ unisexuals have been confirmed since 2000 (N=33)	6 913 km^2
Index of area of occupancy (IAO) (see Canadian Range section)	196 km^2 based on 300-m buffer for 33 locations with recently confirmed presence of Jefferson Salamander and unisexuals

Is the total population severely fragmented? Virtually all known populations have small (<200) numbers of adults and are isolated from one another by loss of connecting habitat and by the species' limited dispersal capability and breeding site fidelity. These populations (locations) are well below estimated MVPs for long-term persistence of vertebrates in general (Reed *et al*. 2003: Traill *et al*. 2007).	Yes
Number of "locations" It is assumed that each breeding pond qualifies as a "location" as per the COSEWIC definition as a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present. There are, at most, 33 extant locations (see **Canadian Range** section)	~33
Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? "New" populations were likely present historically and the number of populations lost exceeds these "new" populations, so there has been a large net decline.	Yes
Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy?	Decline observed and projected
Is there an [observed, inferred, or projected] continuing decline in number of populations? (see **Fluctuations and Trends** section). If the comparison between 1990-91 and 2003-4 is valid then decline in most recent generation = 15/18 = 80%. The data in the text in the **Fluctuations and Trends** section suggest that the decline over the whole range and three generations is similar or perhaps even higher.	Decline observed and projected.
Is there an [observed, inferred, or projected] continuing decline in number of locations?	Decline
Is there an [observed, inferred, or projected] continuing decline in [area, extent and/or quality] of habitat?	Decline observed, and projected
Are there extreme fluctuations in number of populations?	No
Are there extreme fluctuations in number of locations?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

Number of Mature Individuals (in each population)

Population	**N Mature Individuals**
Location A (> 200) Location B (< 100) Location C (?) Location D (?) Location E (< 100) Location F (?) Location G (?)	
Total	Unknown, but may be < 2500 adults

Quantitative Analysis

Probability of extinction in the wild is at least [20% within 20 years or 5 generations, or 10% within 100 years].	NA

Threats (actual or imminent, to populations or habitats)

- Habitat loss from development (housing, golf courses, roads, etc.), aggregates
- Microhabitat degradation and alteration
- Early drying of vernal pools from change in hydrology
- Road mortality during breeding migrations
- Agriculture causing loss of habitat and impacts from runoff of chemicals, and change in hydrology because of drainage systems
- Presence of large numbers of unisexual females that deprive Jefferson females of sperm could, hypothetically, limit reproductive success of Jefferson females. If this occurred as a result of anthropogenic changes, it would constitute a threat.

Rescue Effect (immigration from outside Canada)

Status of outside population(s)? **U.S.:** Secure. Variable among U.S. jurisdictions See Table 2. Unisexuals are usually not differentiated from Jefferson Salamander.	
Is immigration known or possible?	No
Would immigrants be adapted to survive in Canada?	Unknown
Is there sufficient habitat for immigrants in Canada?	Unknown
Is rescue from outside populations likely?	**No**

Current Status

COSEWIC: Endangered (November 2010)

Status and Reasons for Designation

Status:	**Alpha-numeric code:**
Endangered	A2bc+4bc; B2ab(i,ii,iii,iv,v)

Reasons for designation:
This salamander has a restricted range within populated and highly modified areas. Over the past three generations, the species has disappeared from many historic locations and the remaining locations are threatened by development, loss of habitat and, potentially, the presence of sperm-stealing unisexual populations of salamanders.

Applicability of Criteria

Criterion A (Decline in Total Number of Mature Individuals): Meets Endangered under A2bc+4bc as the total number of mature individuals has declined by more than 50% over the past 33 years. The decline has not ceased and may not be reversible. The decline is likely to continue at a similar rate or perhaps accelerate as potentially negative effects of the unisexuals increases. The decline is based on an appropriate index (number of locations) of abundance (subcriterion b) and a decline in IAO, EO and habitat quality (subcriterion c).

Criterion B (Small Distribution Range and Decline or Fluctuation): Meets Endangered under B2ab(i,ii,iii,iv,v) as the IAO (196 km^2) is lower than the Endangered threshold, the species' habitat is estimated to be severely fragmented, and there is an observed and inferred continuing decline in b(i,ii,iii,iv,v).

Criterion C (Small and Declining Number of Mature Individuals): Not applicable as the total number of mature individuals is unknown.

Criterion D (Very Small or Restricted Total Population): Not applicable.

Criterion E (Quantitative Analysis): Not performed.

PREFACE

Since the last report (COSEWIC 2000), several changes have occurred in the abundance and distribution of Jefferson Salamander (*Ambystoma jeffersonianum*) that increase the risk of extinction in this species. In addition, there has been significant new scientific information that allows more accurate and precise estimates of numbers and distribution. This new information clarifies further the relationship between Jefferson Salamander (JJ) and sympatric populations of all female unisexual *Ambystoma* (LJJ) salamanders.

Overall, 87 sites with Jefferson Salamanders and/or polyploid unisexuals with Jefferson genomes have been found since Jefferson Salamander was first discovered in Ontario in 1976. All populations in which *A. jeffersonianum* (JJ) have been found also contain unisexual *Ambystoma* (LJJ). *Ambystoma jeffersonianum* has not been found in all populations that contain LJJ unisexuals, but it is presumed that *A. jeffersonianum* is or was also present as a sperm donor in those populations. Since 2000, many populations of Jefferson Salamander have disappeared, so that from the original 87 sites it is now thought only about one third still have extant populations of Jefferson Salamanders. Furthermore, even those extant populations are now known to have many fewer individuals of Jefferson Salamanders than originally thought, because in most populations unisexual LJJ females outnumber Jefferson females, often by a wide margin. Until the past few years, these unisexuals could not be distinguished morphologically from Jefferson females. The only way that they could be distinguished required sacrifice of specimens. Now only a small piece of tissue is required to distinguish between JJ and LJJ and other polyploids, which allows much larger sample sizes to be evaluated. These larger recent samples show that most or all salamanders in all populations are LJJ. The absolute number of JJ and proportion of JJ to LJJ have declined in virtually all ponds where repeated tissue samples have been taken to conclusively identify JJ from unisexual LJJ using new molecular methods. Therefore, the many threats faced by these unusual salamanders in southern Ontario are continuing to increase the species' risk of extinction. These threats include loss of ponds and terrestrial habitat to development, fragmentation of locations by roads and uninhabitable terrain, changes in hydrology from a host of factors and introduction of predatory fish into ponds.

COSEWIC HISTORY

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) was created in 1977 as a result of a recommendation at the Federal-Provincial Wildlife Conference held in 1976. It arose from the need for a single, official, scientifically sound, national listing of wildlife species at risk. In 1978, COSEWIC designated its first species and produced its first list of Canadian species at risk. Species designated at meetings of the full committee are added to the list. On June 5, 2003, the *Species at Risk Act* (SARA) was proclaimed. SARA establishes COSEWIC as an advisory body ensuring that species will continue to be assessed under a rigorous and independent scientific process.

COSEWIC MANDATE

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses the national status of wild species, subspecies, varieties, or other designatable units that are considered to be at risk in Canada. Designations are made on native species for the following taxonomic groups: mammals, birds, reptiles, amphibians, fishes, arthropods, molluscs, vascular plants, mosses, and lichens.

COSEWIC MEMBERSHIP

COSEWIC comprises members from each provincial and territorial government wildlife agency, four federal entities (Canadian Wildlife Service, Parks Canada Agency, Department of Fisheries and Oceans, and the Federal Biodiversity Information Partnership, chaired by the Canadian Museum of Nature), three non-government science members and the co-chairs of the species specialist subcommittees and the Aboriginal Traditional Knowledge subcommittee. The Committee meets to consider status reports on candidate species.

DEFINITIONS
(2010)

Wildlife Species	A species, subspecies, variety, or geographically or genetically distinct population of animal, plant or other organism, other than a bacterium or virus, that is wild by nature and is either native to Canada or has extended its range into Canada without human intervention and has been present in Canada for at least 50 years.
Extinct (X)	A wildlife species that no longer exists.
Extirpated (XT)	A wildlife species no longer existing in the wild in Canada, but occurring elsewhere.
Endangered (E)	A wildlife species facing imminent extirpation or extinction.
Threatened (T)	A wildlife species likely to become endangered if limiting factors are not reversed.
Special Concern (SC)*	A wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats.
Not at Risk (NAR)**	A wildlife species that has been evaluated and found to be not at risk of extinction given the current circumstances.
Data Deficient (DD)***	A category that applies when the available information is insufficient (a) to resolve a species' eligibility for assessment or (b) to permit an assessment of the species' risk of extinction.

* Formerly described as "Vulnerable" from 1990 to 1999, or "Rare" prior to 1990.

** Formerly described as "Not In Any Category", or "No Designation Required."

*** Formerly described as "Indeterminate" from 1994 to 1999 or "ISIBD" (insufficient scientific information on which to base a designation) prior to 1994. Definition of the (DD) category revised in 2006.

Environment Canada / Environnement Canada
Canadian Wildlife Service / Service canadien de la faune

Canada

The Canadian Wildlife Service, Environment Canada, provides full administrative and financial support to the COSEWIC Secretariat.

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Table 2. NatureServe (2008) rank for *Ambystoma jeffersonianum* for all jurisdictions within its global range. S1 = critically imperiled, S2 = imperiled, S3 = vulnerable. S4 = apparently secure, S5 = secure, SNR = unranked. 27

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WILDLIFE SPECIES INFORMATION

Name and classification

Jefferson Salamander, *Ambystoma jeffersonianum* (Green), was first described as *Salamandra jeffersoniana*, by Green in 1827 (in Uzzell 1967) and the type locality given as, "near Chartier's creek in the vicinity of Jefferson college (formerly) at Cannonsburg, (Washington County, Pennsylvania)". The species was transferred to the genus *Ambystoma* by Baird in 1849 (Uzzell 1967). *Ambystoma jeffersonianum* was included, with other Mole Salamanders, in the family Ambystomatidae. The family is restricted to North America and, based on fossil ambystomatids found in lower Oligocene deposits in Saskatchewan (Holman 1968), has a fossil record dating to 30 million years ago. No fossils have been recorded for *A. jeffersonianum* per se. Frost *et al*. (2006) consider Dicamptodontidae with one genus (*Dicamptodon*) and four species in western North America to be the close sister family to Ambystomatidae, which has one genus and 31 species. Some phylogenies (e.g. Weins *et al*. 2005) include *Dicamptodon* in the family Ambystomatidae.

Most species of *Ambystoma* are included in the *Ambystoma tigrinum* complex that has members that range across North America and extend south to central Mexico (Shaffer and McKnight 1996). Using a combination of electrophoretic and morphological characters, Jones *et al*. (1993) included *A. jeffersonianum* in another cluster, or group of species, that also includes Blue-spotted Salamanders (*A. laterale*), Marbled Salamanders (*A. opacum*), and Long-toed Salamanders (*A. macrodactylium*), but Larsen *et al*. (2003) found the relationships among these species to be largely unresolved. Phylogenetically, *A. jeffersonianum* shares its most recent common ancestor with either *A. macrodactylum* (Shaffer *et al*. 1991) or *A. laterale* (Bogart 2003). Male *A. jeffersonianum* and *A. laterale* serve as sperm donors for unisexual (all-female) salamanders that co-exist with both species.

Identification of *A. jeffersonianum* became complicated following Clanton's (1934) observation that there were two distinct groups of individuals in populations of *A. jeffersonianum* in southern Michigan: a group of "dark" individuals and a group of "light" individuals. He noted that there seemed to be a 1:1 sex ratio among the "dark" individuals but that the "light" individuals were almost invariably female. These observations and the documented morphological variation, which seemingly ranged from *A. laterale* to *A. jeffersonianum*, prompted Bishop (1947) to consider *A. laterale* and *A. jeffersonianum* as a single, morphologically variable species (*A. jeffersonianum*). Thus, many museum specimens, including *A. laterale* and unisexual individuals, were catalogued as *A. jeffersonianum*. Logier and Toner (1961) published a checklist of the amphibians and reptiles in Canada in which they combined all known localities of *A. jeffersonianum* and *A. laterale* stating that "[t]hese two species have been confused for so long that it is impossible at present to separate the locality records pertaining to each". Minton (1954) clearly defined *A. laterale* and *A. jeffersonianum* in Indiana and posited that Clanton's "light" form was a hybrid of these two species.

Uzzell (1964) provided two species names for unisexual salamanders that were presumed to have arisen from the hybridization of *A. jeffersonianum* and *A. laterale*. Both were triploid, but one species, *A. platineum*, possessed a diploid chromosome set of *A. jeffersonianum* and a haploid set of *A. laterale* chromosomes (LJJ) while the other species, *A. tremblayi*, had a diploid chromosome set of *A. laterale* and a haploid *A*. *jeffersonianum* chromosome set (LLJ). *Ambystoma platineum* and *A*. *tremblayi* are not considered to be valid species (Lowcock *et al*. 1987). All offspring of unisexuals are female. It is now known (Bogart *et al*. 2009) that unisexual *Ambystoma* may incorporate nuclear genomes of at least five distinct species of *Ambystoma* and can be diploid, triploid, tetraploid and even pentaploid (Bogart 2003). It is also known that all of the unisexuals share (maternally inherited) mitochondrial DNA (*mt*DNA) that is distinctly different from that found in either *A. laterale* or *A. jeffersonianum* (Hedges *et al.* 1992). This eliminates the possibility that the unisexuals could have arisen from a hybridization event that involved females of either *A. laterale* or *A*. *jeffersonianum*. Unisexual *Ambystoma* are considered to have arisen at one time (about 5 million years ago) and share a maternal ancestor with a Kentucky population of Streamside Salamanders, *A. barbouri* (Bogart 2003, Bogart *et al*. 2007, Bi and Bogart 2010).

Morphological description

The morphology of *Ambystoma jeffersonianum* has been described by a number of authors (Bishop 1941, Minton 1954, Uzzell 1967, Cook 1984, Conant and Collins 1998, Petranka 1998). Jefferson Salamander is a robust, relatively large (65 to 96 mm snout to vent length (SVL)) salamander. Mature females are slightly longer than mature males. The costal grooves are distinct and the teeth are bicuspid with the premaxillary and maxillary teeth forming a single row (Uzzell 1967). It is a dark, brownish-grey salamander with small, pale blue flecks on the limbs and lower sides (Cook 1984; Conant and Collins 1998). It has relatively long toes such that when the front leg and hind leg are positioned along the flank (adpressed), the toes of males overlap by more than one and a half costal folds. The snout is relatively long and broad with an inter-narial distance of 0.062 or more of the SVL in males and 0.059 or more in females (Uzzell 1964). Males in breeding condition have conspicuously swollen cloacal glands and more compressed tails than do females. Male and female individuals are shown in Figure 1.

With the exception of populations in most of Pennsylvania and in some areas in south-central New York State, *A. jeffersonianum* lives with unisexual salamanders that have a nuclear genomic constitution consisting of one *A. laterale* genome and one (diploid unisexuals), two (triploid unisexuals), or three (tetraploid unisexuals) *A*. *jeffersonianum* genomes (Bogart 2003, Bogart and Klemens 1997, 2008). In these mixed populations, unisexuals are normally more abundant. In Maryland, Virginia, West Virginia, and Kentucky there are populations of *A*. *jeffersonianum* for which no unisexuals have been found. However, populations in these states have not been included in genetic screening that would be necessary to differentiate unisexual individuals from *A*. *jeffersonianum*. Unisexuals were recently identified in northern Kentucky (Bi and Bogart 2010) and it is possible that unisexuals also occur in other southern populations of *A. jeffersonianum*. All known Canadian populations of *A. jeffersonianum* also have unisexual salamanders. Morphological descriptions of genetically unknown adults, especially females, and larvae may include *A. jeffersonianum* and/or unisexual individuals. Larval keys (Petranka 1998) do not distinguish *A. jeffersonianum* larvae from those of *A. laterale* or unisexual *Ambystoma*.

Figure 1. *Ambystoma jeffersonianum* male (above) from Halton Region, Ontario and a female (below) from Hamilton-Wentworth Region, Ontario. These specimens show the range of colour and amount of blue flecking that may be found in this species. Note the swollen cloacal region in the male. (Photographs by J. P. Bogart.)

Genetic description

Genetic markers are used to identify *A. jeffersonianum* and to distinguish individuals of that species from unisexual individuals. *Ambystoma jeffersonianum* can easily be distinguished from *A. laterale* based on the presence of alternative electrophoretic alleles (allozymes) at several isozyme loci that are mostly homozygous (p > 0.90) in both species. Unisexuals that co-occur with *A. jeffersonianum* have allozymes of both species and the observed staining reaction provides information on the number of genomes of *A. laterale* or *A. jeffersonianum* that are present in a unisexual (Bogart 1982, Bogart and Klemens 1997, 2008). Also, because *A. jeffersonianum*, *A. laterale*, and unisexual individuals have distinctly different *mt*DNA genomes, they can easily be distinguished by sequencing mitochondrial genes (Hedges *et al*. 1992, Bogart 2003, Bogart *et al*. 2007, Noël *et al*. 2008, Bi and Bogart 2010) or by using restriction fragment length polymorphism (RFLP) of the mitochondrial genome (Spolski *et al*. 1992).

Julian *et al*. (2003) developed primers for several *A. jeffersonianum* microsatellite DNA markers or loci and provided allele sizes for those loci that could also be amplified in *A. laterale*. Using these primers, it is possible to identify *A. jeffersonianum* and unisexual individuals from DNA that can be extracted from a small tail tip or a toe and does not require sacrificing individuals for isozyme analyses (Ramsden *et al*., 2006, Bogart *et al*., 2007; 2009).

Designatable units

Phylogeographical studies over the range of *A. jeffersonianum* have not yet been done. A prerequisite to such studies would be to ensure that unisexuals are excluded. The primary focus of genetic studies of *A. jeffersonianum* has been to distinguish individuals of that species from sympatric unisexual individuals. A secondary focus has been to use genetics, and especially microsatellite DNA alleles, to test hypotheses that relate to the breeding system used by unisexuals (Bogart *et al*. 2007). Where data are available, isozyme analyses show that *A. jeffersonianum* has a low heterozygosity and few, if any, electrophoretic alleles that would indicate discrete populations or designatable genetic units (Bogart and Klemens 1997, 2008). These data are, however, somewhat misleading because the relatively few isozyme loci (eight to ten) that were chosen to identify *A. jeffersonianum* are conservative, or slowly evolving loci (Shaffer *et al*. 1991), that demonstrate very few, mostly homozygous, alternative allozymes in *A. jeffersonianum* and *A. laterale*. More rapidly evolving loci that have many allozymes or have allozymes with similar electrophoretic mobility in the two species are not useful for identification of the bisexual species and are difficult to score in unisexual individuals (Bogart and Klemens 1997).

Given that there is no evidence of major genetic differences in *A. jeffersonianum* in Canada and no significant natural disjunction and all populations occur in the same ecoregion, the species was considered to be a single designatable unit.

Unisexual salamanders represent a biological novelty unlike any other organism so far found in the animal kingdom and do not fit into any of the concepts that define a biological or an evolutionary species. They are often deemed to be hybrids and as such would not be considered for protection, but the unisexuals are not hybrids in the sense of first generation crossing of two species (like a mule). Attempts have been made in the U.S. to protect some of the rare, unisexual genomic combinations as genetically distinct units and even to consider the various biotypes as “species” for research and taxonomic purposes (as well as for conservation initiatives). Because the unisexuals require a male from one of five bisexual species for recruitment, the identity of the species donating the sperm is normally represented as a double or triple dose in polyploid unisexuals. In Ontario, there are populations of unisexuals that are in the process of switching sperm donors from *A*. *jeffersonianum* to *A*. *laterale*, and historical records reveal that this transition can happen very rapidly. Unisexual populations should probably be assessed as a distinct wildlife species separately from any species of *Ambystoma*, such as *A. jeffersonianum.*

DISTRIBUTION

Global range

The distribution of *A. jeffersonianum* is limited to parts of eastern North America (Figure 2). It ranges from New York and New England, south and southwestward to Virginia, West Virginia, Kentucky and Indiana (Petranka 1998, Bogart and Klemens 1997, 2008). Isolated populations have been recorded in east-central Illinois (Petranka, 1998, Mullen and Klueh 2009). For much of this range, genetic data are not available so the continental distribution of pure *A. jeffersonianum* and unisexuals that use *A*. *jeffersonianum* as a sperm donor is uncertain (Bogart and Klemens, 2008).

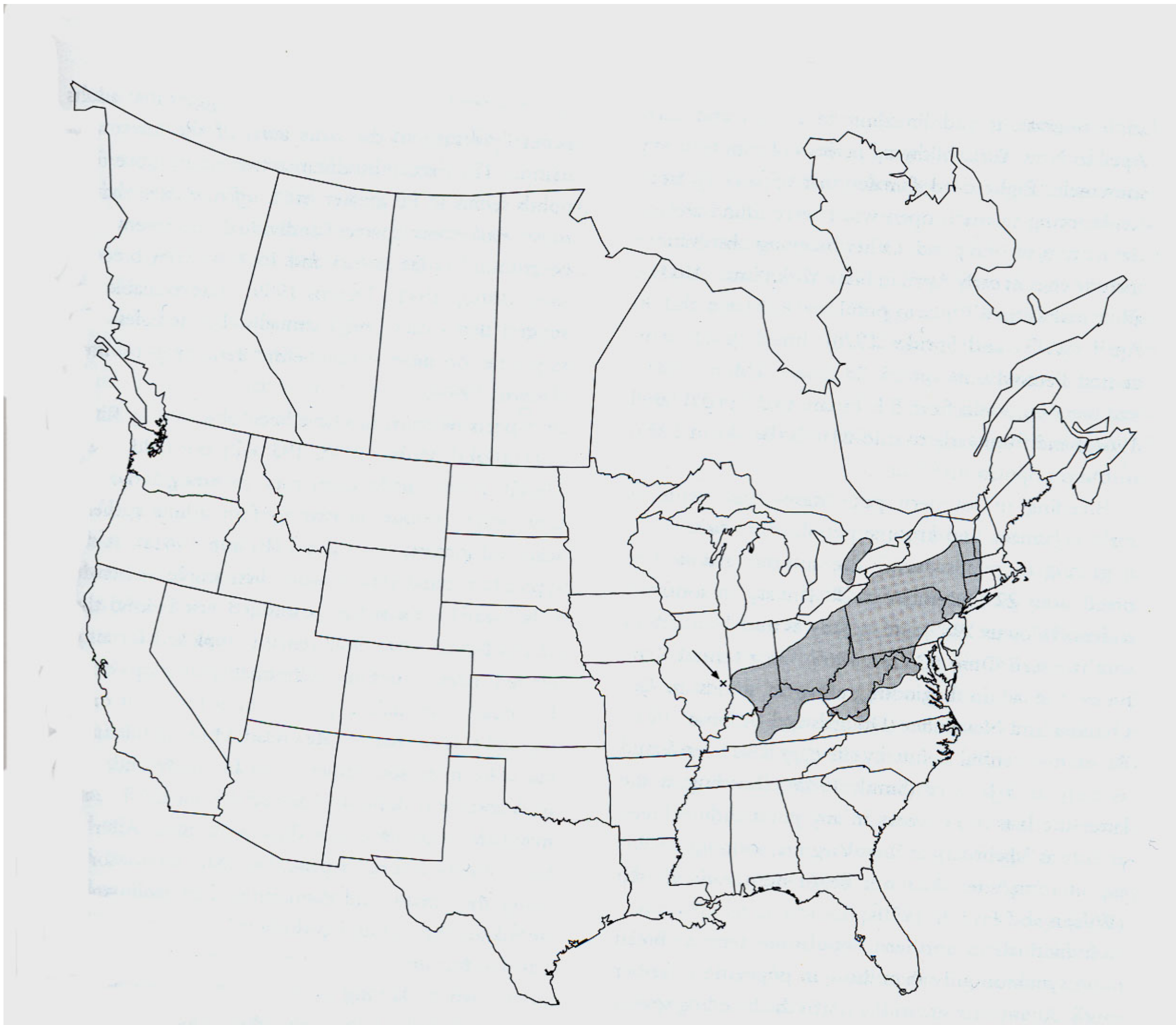

Figure 2. Global range of *Ambystoma jeffersonianum* (from Petranka1998). The arrow points to an isolated population in Illinois.

Canadian range

Ambystoma jeffersonianum has been known to exist in Canada only since 1976 when a population was discovered in southern Ontario (Weller and Sprules 1976). In Canada, the species is restricted to six regions of southern Ontario (Figure 3): 1) Haldimand and Norfolk Counties; 2) forested areas along the Niagara Escarpment from the Hamilton area to Orangeville; 3) isolated localities in Halton and Peel Regions; 4) Dufferin County east of the Escarpment; 5) Waterloo County; and 6) a few isolated ponds in York Region on the Oak Ridges Moraine. Sources for the Canadian distribution are provided in research catalogues of J.P. Bogart (University of Guelph) and include positively identified specimens reported to the Natural Heritage Information Centre (NHIC), and are included in the Ontario Ministry of Natural Resources (OMNR) database. Male *A. jeffersonianum* are important for the survival of unisexual populations that have a diploid (LJJ) or triploid (LJJJ) complement of *A. jeffersonianum* chromosomes. There are some localities outside the documented range of *A. jeffersonianum* from which LJJ or LJJJ unisexual *Ambystoma* have been positively identified. Presumably, *A. jeffersonianum* are or were also present in those populations.

Populations exist in suitable habitat on the Niagara Escarpment from Grey County to Hamilton Region and on the Oak Ridges Moraine in York Region. A few isolated populations occur in Waterloo County, Brant County, and in Haldimand-Norfolk Region. The distribution is not continuous in any of these regions, but there are zones of suitable habitat. A record from the former Camp Ipperwash on Lake Huron, a location disjunct from and significantly west of the current range of the species, reported salamanders of the "Jefferson salamander complex" (Sutherland *et al*. 1994). At the time that report was written, the salamanders (*A. laterale, A. jeffersonianum,* and the unisexuals) were all considered as part of the Jeffersonianum complex. In that report, the term 'Jefferson salamander complex' referred to specimens which had not been subjected to genetic analysis. Recent genetic analysis of specimens from around Sarnia and close to Ipperwash found them all to be *A. laterale* and LLJ unisexuals (J. P. Bogart pers. comm., August 27, 2010). There are many such references to "jefferson complex" and the taxonomy is confusing because *A. laterale* and *A. jeffersonianum* (including the unisexuals) were synonymized under *A. jeffersonianum* until the early 1960s. For the Ipperwash record, and likely other records as well, there is no evidence that *A. jeffersonianum* is present.

The COSEWIC Secretariat provided two calculations of extent of occurrence (EO) using a convex polygon. There is a total of 87 locations with historical or extant records of salamanders of the Jeffersonianum complex. Many of these have been lost or may no longer have *A. jeffersonianum* or only have unisexuals (see Appendix 1). The first EO calculation (6 913 km^2) was based on recently confirmed populations of Jefferson Salamanders and LJJ unisexuals (2000-2009) and included 33 sites (see Appendix 1). The second EO calculation (9 309 km^2) consisted of both recently and historically confirmed populations of Jefferson Salamanders and LJJ unisexuals and included 43 sites (see Appendix 1). It should be recognized that both of these calculated EO values included some ponds that may no longer support Jefferson Salamanders, ponds that no longer exist, as well as sites where only LJJ unisexuals have been found. Almost all of the records have come from breeding pond surveys. From radio tracking experiments, a 300-m distance from a breeding pond would be considered an adequate "home range" for this salamander. Assuming that all 33 recently confirmed populations of Jefferson Salamanders are still extant, and also assuming that all LJJ unisexuals must live with Jefferson Salamanders, the value of the EO used in this report is 6 913 km^2.

Two values of an index of area of occupancy (IAO) were also calculated using the same set of recently confirmed populations of Jefferson Salamander and LJJ unisexuals. The 33 recently confirmed sites gave an IAO of 196 km^2. The second IAO calculation used 43 recently and historically confirmed sites and gave an IAO of 256 km^2. The IAO value used in this report is 196 km^2.

HABITAT

Habitat requirements

Adult *A. jeffersonianum* throughout their range are found near or within deciduous or mixed upland forests (Klemens 1993) containing suitable breeding ponds. These sites include limestone sinkhole ponds, kettle ponds and other natural basins (Nyman *et al.* 1988). Breeding ponds are devoid of predatory fish, often ephemeral, and are filled by spring runoff, groundwater, or springs. *Ambystoma jeffersonianum* populations within Ontario seem to be more closely associated with Carolinian forests than with other types of deciduous forest.

<u>Breeding ponds</u>

Although there is evidence that *A. jeffersonianum* may not successfully reproduce in ponds with a pH at or below 4.5 (Horne and Dunson 1994a, 1994b, 1995), Bériault (2005) found that larvae were not particularly susceptible to relatively low pH in Ontario ponds. Success of larvae might be affected by reduced availability of prey items that are more susceptible to low pH (Sandinski and Dunson 1992) than are the salamander larvae themselves. No other parameters of water chemistry, water depth, temperature, or quality were found to be good predictors of the use or non-use of particular ponds for breeding (Bériault 2005).

Breeding ponds must contain attachment sites for egg masses and ephemeral ponds must exist for the duration of larval development. Egg masses are normally attached to submerged twigs or branches but submerged riparian vegetation or emergent grasses and sedges may also be utilized. Prey items in ponds include a variety of invertebrates as well as other amphibian larvae or tadpoles.

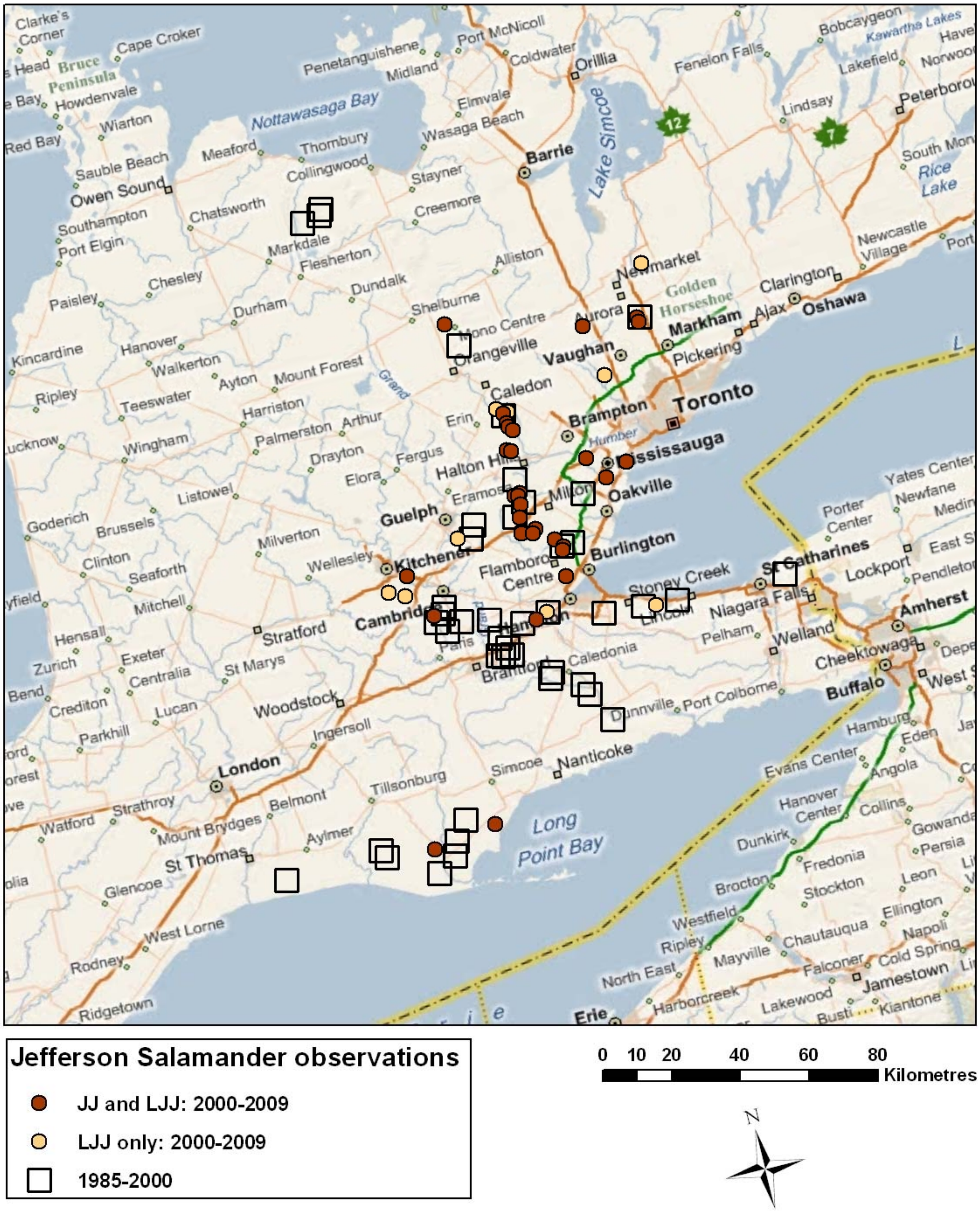

Figure 3. Documented locations of *Ambystoma jeffersonianum* in Ontario (Jefferson Salamander Recovery Team 2010).

Terrestrial habitat

Other than the few days spent in the breeding pond, adults live and forage in deciduous or mixed woodlands. Mole salamanders are seldom seen on the forest floor except when they are migrating to or from a breeding pond. Most of the information on the terrestrial ecology has been derived from experiments that have employed radio transmitters (Faccio 2003, Bériault 2005, OMNR, unpublished data). Microhabitats used by *A. jeffersonianum* include small mammal burrows, rock fissures, tree stumps, leaf litter, logs, and woody debris on the forest floor. *Ambystoma jeffersonianum* spend the winter underground below the frost line. They utilize deep rock fissures and small mammal burrows. Salamanders use horizontal burrows in the summer, but in winter use vertical fissures and burrows (Faccio 2003).

Habitat trends

Habitat for *A. jeffersonianum* in southern Ontario is restricted to fragmented woodlands on marginal agricultural land, including portions of the Niagara Escarpment. Development activities associated with urbanization, aggregate extraction, and resource development lead to an ongoing loss of suitable habitat and an increase in the fragmentation of habitat and populations. In addition to direct habitat loss, resource development can alter the water table or affect groundwater flow, which adversely affects moisture regimes in adjacent wetlands and soil substrates. These changes can shorten the hydro period of ephemeral ponds and thereby lead to a loss of suitable habitat for *A. jeffersonianum*.

In the previous COSEWIC report (COSEWIC 2000), it was assumed that breeding ponds were of primary importance and that salamanders could survive in a 30-m "buffer zone" around a breeding pond. These criteria were used to protect the habitat and for mitigation. New data from radio telemetry and drift fence surveys indicate that the salamanders' habitat includes up to 1 km or occasionally more from a breeding pond, and that a "core area" with a radius of at least 300 m would be required around breeding ponds to maintain a breeding population. Currently, a 1-km radius around breeding ponds is protected under habitat regulation to allow for population expansion, immigration and dispersal. Because planning authorities were using a 30-m criterion until recently, substantial foraging and overwintering habitat has been lost. These losses are reflected in reduced population sizes and disappearances of entire populations at some historical sites (see section on **Fluctuations and Trends**).

Habitat protection/ownership

Most of the known populations of *A. jeffersonianum* (Figure 3) have been found on land under private ownership. About one third of the known populations are found in suitable habitat (forests with small ponds) within provincial parks along the Niagara Escarpment and on lands held by conservation authorities. The inclusion of suitable habitat within a provincial park, conservation area or along the Niagara Escarpment does not necessarily guarantee protection of that habitat for species of *Ambystoma* because these areas serve multiple recreational uses. Some small permanent ponds on private lands and within conservation areas have been stocked with predatory fish to provide recreational fishing opportunities (J.P. Bogart, W.J. Cook, L. Rye pers. obs.), rendering the ponds unsuitable for *A. jeffersonianum*.

BIOLOGY

The general biology of *A. jeffersonianum* has been summarized by Downs (1989), Petranka (1998) and on web sites (e.g. NatureServe 2008, AmphibiaWeb 2010). Life-history observations have been made in some populations where unisexual *Ambystoma* are probably syntopic with *A. jeffersonianum* but, other than the expected complications with sex ratios and egg mass mortality, the biology of *A. jeffersonianum* and unisexuals (LJJ and LJJJ) is similar. In other words, these unisexuals mimic the normal, observable, behaviour of female *A. jeffersonianum*.

Kleptogenesis

All known Ontario populations of *A. jeffersonianum* also have unisexual individuals that may be diploid (LJ), triploid, (LJJ), tetraploid (LJJJ), or even pentaploid (LJJJJ). Unisexuals have a higher population density than do bisexual *A. jeffersonianum*. Successful recruitment of unisexuals requires sperm from a bisexual sperm donor that, over the range of unisexual *Ambystoma*, may be from a male from as many as five distinct sperm donating species (Bogart 2003, Bogart *et al*. 2009). Because unisexuals steal sperm from males, this unique reproductive mode is termed kleptogenesis (Bogart *et al*. 2007). Although sperm are required for egg development, the male’s genome is not always incorporated in the zygote and the zygote can develop through gynogenetic reproduction. If the male’s genome is incorporated, it can replace an existing unisexual genome through genome replacement (Bi *et al*. 2008) or add a genome to elevate the ploidy level of an offspring (e.g. a tetraploid offspring from a triploid mother).

Unlike most populations in the United States (Bogart and Klemens 1997, 2008), *A*. *jeffersonianum* and *A. laterale* in Ontario are often parapatric and the sympatric occurrence of these species has been documented in one population (Bogart *et al*. 2007). In that population, a unisexual LJJ female, normally expected to use sperm from *A*. *jeffersonianum*, probably used *A. laterale* as a sperm donor because some of her offspring were LJJ, assuming gynogenetic reproduction, and her ploidy-elevated offspring were LLJJ rather than the expected LJJJ. In a study that examined 1377 individuals from 118 sites, only one LLJJ individual was found by Bogart and Klemens (2008). This evidence suggests that unisexual *Ambystoma* will accept any suitable sperm donor and the identification of that sperm donor can be revealed in ploidy elevated individuals found among offspring of mixed ploidy egg masses (see also Bogart *et al*. 2009).

Life cycle and reproduction

Ambystoma jeffersonianum breed in the very early spring (generally late March in Ontario). Salamanders emerge from over-wintering sites and migrate during rain or on very humid nights to the breeding ponds with males usually preceding females to the ponds (Weller 1980, Nyman 1991). Males at a Kentucky site that presumably did not contain unisexual individuals outnumbered females by more than two to one (Douglas 1979) which is typical of the ratio found in other species of *Ambystoma* (Petranka 1998).

Courtship of *A. jeffersonianum* was described by Mohr (1931). Once in the ponds, males will court females, then deposit spermatophores on the bottom of the pond. The female will pick up a spermatophore with her cloaca and, within 1-2 days, will lay several clutches of approximately 30 eggs each on stems of fallen tree branches or submerged vegetation, such as willow (*Salix*) branches, near the periphery of the pond (Brodman 1995). Although male *A. jeffersonianum* will court both *A. jeffersonianum* and unisexual females, Dawley and Dawley (1986) showed that males can discriminate between *A. jeffersonianum* females and unisexual females and that the males used in their experiments preferred *A. jeffersonianum* females.

Little is known about either the age of first reproduction or the frequency of reproduction for either sex. Based on growth rates and sizes of first-time breeders, Weller (1980) estimated that male *A. jeffersonianum* return to the breeding pond 22 months after metamorphosis. Females, however, required 34 months or more before returning. There is some evidence that unisexual females generally take longer to reach sexual maturity than do bisexual females (Licht and Bogart 1989, Lowcock *et al*. 1992). Weller (1980) found that the breeding frequency varies among individual salamanders. Of 26 males that arrived at and left the breeding pond in the first year of his study, 12% returned in each of the next 4 years, 4% did not return until 4 years later, and the remainder returned to the pond and skipped years in various combinations. Females, including both *A. jeffersonianum* and unisexuals, followed a similar pattern with 10% of 206 females returning each year, 6% not returning until 4 years later, and the remainder of the females returning/skipping years in various combinations.

The embryonic period from egg deposition to hatching varies from 3 to 14 weeks and is dependent on the seasonal time of egg deposition and water temperature (Smith 1983) with an average of about 28 days (Brodman 1995) in northern Ohio. Brodman also found that embryonic survival rate was moderately high (87%). An embryonic survival rate of 60-88% was reported by Cook (1983) from five Massachusetts ponds and was independent of pond pH. In contrast, unisexual eggs typically have a very low embryonic survival rate (16%) (Bogart and Licht 1986) and embryo mortality, observed in many egg masses in a breeding pond, often signifies the presence of unisexual *Ambystoma*. Clanton (1934) mentions egg mortality in southern Michigan and Piersol (1910) observed high egg mass mortality in a Toronto, Ontario, pond that, at that time, could have been an *A. jeffersonianum* population with a high frequency of unisexual individuals.

When hatched, larvae feed on zooplankton until they are large enough to feed on larger invertebrates that include nematodes, water mites, cladocerans, copepods, collembolans, mosquito larvae, chironomid larvae, snails, and assorted insects (Smith and Petranka 1987). Larvae are often cannibalistic and will also feed on larvae of sympatric species of *Ambystoma* and unisexual larvae (Brandon 1961, Smith and Petranka 1987, J. Bogart pers. obs.). The larval period varies from 2 to 4 months and is likely related to water temperature, available food and hydro period (Downs 1989). In Ontario, transformation has been observed from mid-July to mid-September (Bogart pers. obs.). By early November, juveniles have an average total length of 62 mm (Downs 1989). Based on studies in Maryland (Thompson *et al*. 1980), Ohio (Downs 1989), and Illinois (Mullen and Klueh 2009) pre-metamorphic survival rates and recruitment rates are believed to be very low (0.0 to 0.7%) and little is known about the ecology of juveniles. Downs reported that juveniles could be found as far as 92 m from the breeding pond in a 10-day period.

There are no studies that examine age-specific survivorship in adult *A. jeffersonianum*. In a mark-recapture study, Downs (1989) estimated that 10-18% of adults survive a 3-year period. Weller (1980) estimated an extremely high, annual adult survivorship (0.981 for females (including unisexuals) and 0.883 for males). A demographic study of adult Spotted Salamanders (*A. maculatum*) using skeletochronology suggests that most of the salamanders in the population under study were between 2 and 18 years of age but some may live as long as 32 years (Flageole and Leclair 1992). It is not known if a similar age structure exists in populations of *A. jeffersonianum*.

Generation Time = Age at maturity + 1/mortality, where mortality = annual rate for adults. Mortality rate estimated from Weller (1980) and Downs (1989) as mean of 2, 12 and 27% = 14%. Age at maturity of females is estimated at 4 years (Weller 1980). Therefore, GT = 4 + 1/M = 4 + 1/.14= 11 years. Three generations is approximately 33 years.

Predation

Most predation occurs in the egg and larval stage. In both laboratory predation experiments and field observations (Rowe *et al*. 1994), *A. jeffersonianum* eggs in Pennsylvania were eaten by caddisfly larvae (*Ptilostomis postica*). There are also several reports of caddisfly larvae of this genus preying on eggs of congeneric species, *A. maculatum* and *A. tigrinum* (Eastern Tiger Salamander) (Murphy 1961, Dalrymple 1970). Caddisfly larvae burrow through the outer jelly coat of the egg mass and eat the eggs inside. Dalrymple (1970) suggests that this predation “may be a significant source of mortality in salamander populations”. Leclair and Bourassa (1981) report that egg masses of *A. maculatum* have also been eaten by chironomid larvae (genus *Parachironomus*). Both types of predators have been observed on or within egg masses of *A. maculatum* in Ontario (J.P. Bogart, W.J. Cook, L. Rye pers. obs.), but there have been no reports of predation on *A. jeffersonianum* egg masses. Aquatic insects (e.g. odonatan larvae, dytiscid adults and larvae) have been observed feeding on larvae of

Ambystoma in Ontario (J. Bogart pers. obs.). Larvae are also eaten by conspecifics and larvae of other ambystomatids. Eggs may also die if early spring temperatures drop and the egg masses freeze or if the water level falls and the eggs desiccate.

Little is known about predation on juvenile and adult *A. jeffersonianum*. Previously implanted radio transmitters have later been recovered from the forest substrate, which suggests that the salamanders were preyed upon by some vertebrate. One transmitter was tracked to an Eastern Gartersnake (*Thamnophis sirtalis*) and later retrieved from the snake's feces (Bériault 2005). Adults are susceptible to predation during their migration to and from breeding ponds, and it is presumed that skunks (*Mephitus mephitus*) and raccoons (*Procyon lotor*) prey on migrating adults. These mammals have been observed feeding on road-killed individuals (J Bogart pers. obs.) but it was not evident that they actually killed the salamanders. Observed road mortality can be severe when roads intersect migratory routes to and from breeding ponds.

Assuming high adult survivorship, the large number of eggs produced, and the high hatching rate of *A. jeffersonianum* eggs, it is probable that the life-history stages of aquatic larvae and terrestrial juveniles represent periods of highest rates of natural predation or mortality.

Physiology

Similar to most amphibians, *A. jeffersonianum* has a skin that is permeable to water and oxygen. To avoid desiccation and anoxia, juveniles and adults normally occur in cool, damp environments and surface movement is restricted to rainy, or humid, nights.

Over its range, *A. jeffersonianum* is one of the earliest seasonal breeders among salamanders (Petranka, 1998) and is active at < 1^{o}C (Feder *et al*., 1982). Migration and breeding often occur when ponds are ice-covered and before the ground has completely thawed (Bishop 1941, J Bogart pers. obs.). *Ambystoma jeffersonianum* is not known to posses any cryoprotectants. Frozen adults and eggs normally die (J. Pisapio, J. Bogart pers. obs.).

Although the poison that is present in the skin of *A. jeffersonianum* has not been analyzed, it is suspected of being important as an anti-predator defence. Poison glands are especially concentrated in the skin on the dorsal surface of the tail. When confronted with a possible predator or during manipulation by a human, a salamander will present and elevate its tail to the threat. Waving the tail and oozing poison are typical responses to a predator (Ducey and Brodie 1983, Brodie 1989).

Dispersal/migration

In southern Ontario, there are two periods of movement for *A. jeffersonianum*: dispersal of newly metamorphosed juveniles from ponds to surrounding forest, which normally takes place in July or August, and migration of adults from over-wintering sites to and from breeding ponds that takes place each spring (late March to mid-April).

Adult *A. jeffersonianum* likely move farther from their breeding pond than do other species of *Ambystoma* (Petranka 1998). Migratory distance from the breeding pond to surrounding terrestrial habitat can exceed 1 km (Downs 1989) but the distance travelled varies among individuals and populations. Ninety percent of radio-tracked adults reside in suitable habitat within 300 m of the breeding pond (Faccio 2003, Bériault 2005, OMNR unpublished data). During migratory movements, salamanders may traverse terrestrial habitat that would not be considered suitable habitat, such as agricultural fields, plantations, and roads. Because *A. jeffersonianum* adults and juveniles move only on rainy or humid nights, they may be found in such habitats when conditions are not suitable to complete their migration.

Individuals in a Kentucky population of *A. jeffersonianum* enter and exit a breeding pond from the same point as well as returning to the same area of the forest after breeding (Douglas and Monroe 1981). Pond fidelity, where individuals continually return to the same pond for breeding, has also been confirmed in Mole Salamanders (*A. talpoideum*) (Raymond and Hardy 1990).

Interspecific interactions

Over the range of *A. jeffersonianum*, there are other species of *Ambystoma* that have similar attributes and share similar habitats. Predation by larval Marbled Salamanders (*A. opacum*) on larval *A. jeffersonianum* can significantly reduce survivorship (Cortwright 1988). Marbled Salamanders do not. however, occur in Canada. Otherwise, in mixed populations of *Ambystoma*, *A. jeffersonianum* is considered to be the top predator (Petranka 1998). *Ambystoma maculatum* is syntopic in virtually all Ontario populations of *A. jeffersonianum,* but there is no indication that either species has a serious impact on the other. Eastern Newts (*Notopthalmus viridescens*) are commonly found breeding in the same ponds with *A. jeffersonianum* with no apparent conflict, but data that address possible competition are not available.

It has been proposed that unisexual *Ambystoma* might affect population densities of *A. jeffersonianum* (and other possible sperm donors). Clanton (1934) hypothesized that spermatophores could be limiting in unisexual dominated populations and that female *A. jeffersonianum* might lose out to unisexual females, which would continually reduce the population of *A. jeffersonianum* until there was no longer any recruitment. This so-called Clanton effect (Minton 1954) would result in a population crash of both *A. jeffersonianum* and unisexuals because there would no longer be any spermatophores. The Clanton effect has not been documented in any population.

Adaptability

All known populations of *A. jeffersonianum* in Ontario have specific habitat requirements (above) and can persist for an unknown period of time in a fragmented landscape if these requirements are met. Because of documented pond fidelity, it is unlikely that adults, which have previously bred in a pond, would subsequently use newly created ponds for breeding. However, *Ambystoma maculatum* are known to breed in newly created temporary and permanent ponds or lakes (J.P. Bogart, K. Bériault pers. obs.). In a few instances, *A. jeffersonianum* have been observed breeding in abandoned quarry ponds, but it is not known if these ponds represent historical pond sites. Colonization must occur, but data on immigration or emigration of *A. jeffersonianum* individuals, especially juveniles, are lacking.

POPULATION SIZES AND TRENDS

Search effort

Considerable effort has been made by Recovery Team members to survey historical breeding ponds and to find new occupied ponds. Because breeding might be limited to a few nights in March and early April, it is not possible to observe breeding activity over the complete range of *A. jeffersonianum* in Ontario in a single season. Egg masses are surveyed during the day from mid-April to mid-May and larval surveys from mid-May through July. Unisexual *Ambystoma* confound all search efforts because they are more commonly found than *A. jeffersonianum* (see Table 1) and genetic testing is necessary to distinguish between *A. jeffersonianum* and unisexuals (adults, eggs, or larvae). Prior to 2004, a pond was designated as a breeding pond for *A. jeffersonianum* if a few individuals were genetically confirmed, using isozymes (Bogart 1982), to be *A. jeffersonianum* or unisexuals on a presence/absence basis. Since 2004, microsatellite gene loci have provided a better method for genetic testing (Julian *et al*. 2003, Ramsden *et al*. 2006, Bogart *et al*. 2007). Individuals do not have to be sacrificed for microsatellite analyses, so many individuals can be safely genotyped in a relatively short period of time. Some recent intensive surveys have targeted suspected breeding ponds using drift fences combined with pit-fall traps to capture individuals migrating to and from breeding ponds (see Heyer *et al*. 1994 for methodology) as well as capturing breeding adults using minnow traps in the breeding ponds. Table 1 provides data derived from four such surveys.

Abundance

In those populations that contain unisexual females, females greatly outnumber males and it may appear that the percentage of *A. jeffersonianum* males is lower than *A. jeffersonianum* females. It was found, however, in breeding ponds at Location A, that 128 of 168 genetically identified *A. jeffersonianum* were males (76%) (Ramsden 2005). Males and females in this population are not significantly different in size, and breeding males ranged from 60 to 87 mm (SVL) (Figure 4). Unisexual *Ambystoma* outnumber

A. jeffersonianum in all known Ontario populations, and there are populations where only unisexual individuals have been found. The lack of *A jeffersonianum* in these populations is likely partly a reflection of sampling effort and the much higher density of unisexual *Ambystoma*. In three separate studies that involved sample sizes > 100 individuals, the percentage of *A*. *jeffersonianum* ranged from ~ 8 to 33% of sampled individuals (Table 1). Overall, most sampled populations likely have at most only a few hundred adult Jefferson Salamanders, but most have many fewer and the total number of adults in Ontario may be < 2500 (J. Bogart pers. obs.). These small populations are well below minimum population sizes estimated to be needed for long-term survival of vertebrate populations in general (~4000-7000: Reed *et al*. 2003; Traill *et al*. 2007 and references therein). As most of the extant *A. jeffersonianum* populations are much smaller than 200 adults, and are isolated from one another (see Figure 3), the species can be described as severely fragmented. Even when breeding ponds are fairly close to one another (1-2 km apart), adults show strong fidelity to their own breeding pond, and there is likely little or no mixing between populations from different ponds. The degree of mixing is being investigated currently (J.P. Bogart pers. comm. August 2010). Regardless, the map in Figure 3 demonstrates that most populations are isolated sites of one to three locations and these would have small total numbers (fewer than 500) of adults.

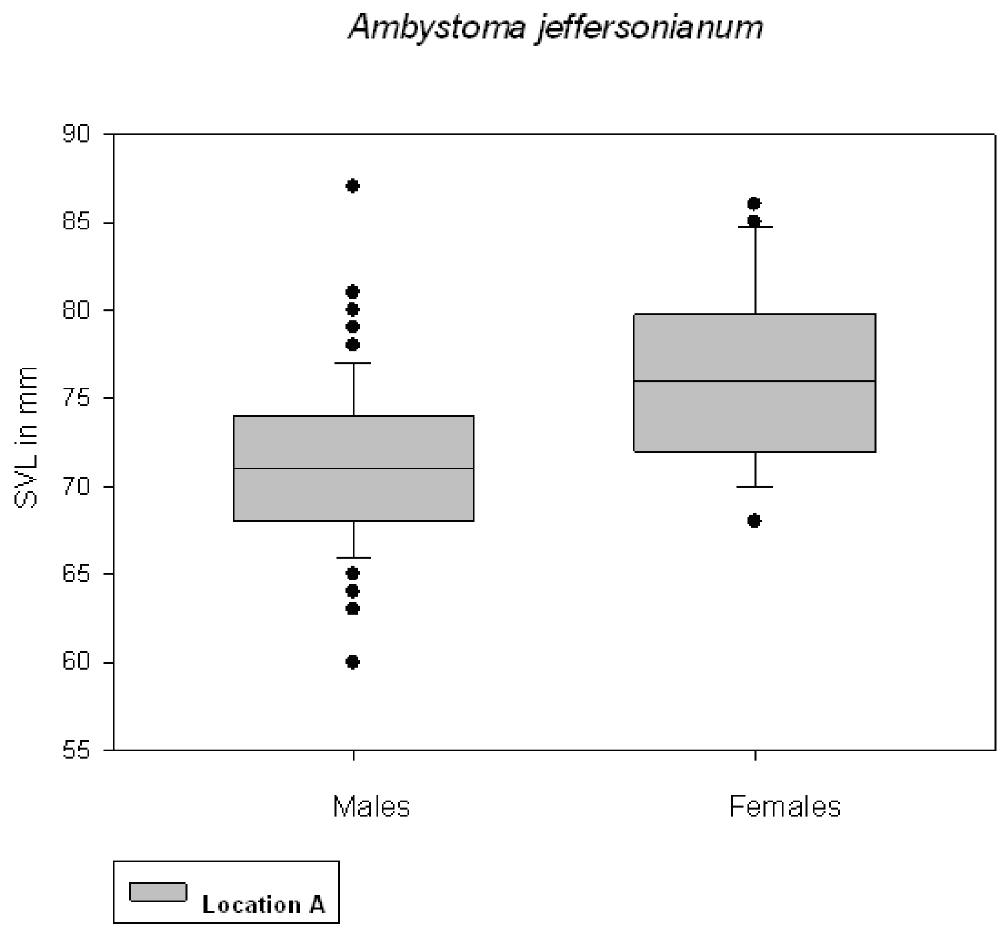

Figure 4. Box plot graphs to compare snout-vent lengths (SVL) of *Ambystoma jeffersonianum* males (n = 128) and females (n = 40) found during a survey at Location A in Ontario. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Dots are outlying individuals. These data are unpublished and are derived from studies conducted by the Bogart lab (Location A).

Table 1. Frequency of *Ambystoma jeffersonianum* and unisexuals found in three Ontario breeding ponds. Size data, measured from the snout to the vent (SVL), are only available for Location A and the first year of the Location B survey. Genomes are L (*A. laterale*) and J (*A. jeffersonianum*). LJJJ would be a tetraploid unisexual containing three J genomes and one L genome. JJ are *A. jeffersonianum*. These data are unpublished and are derived from studies conducted by the Bogart lab (Location A), the OMNR (Location E), and Ecologistics Limited (LGL) environmentalists (Location B). All identifications were by the Bogart lab.

Genotype (n)	SVL mean (SD)	SVL median	SVL range	Percent
Location A (2004) (n = 519) (Ramsden 2005)				
JJ (168)	72.4 (± 4.9)	72.0	60.0 – 87.0	32.37
LJJ (336)	81.2 (± 6.3)	82.0	56.0 – 95.0	64.74
LJJJ (15)	82.3 (± 4.4)	82.0	75.0 – 90.0	2.89
Location B (2007) (n = 141)				
JJ (12)	77.4 (± 6.0)	76.0	70.0 – 104.0	8.51
LJJ (110)	86.5 (± 6.6)	86.5	70.0 – 105.0	78.01
LJJJ (19)	85.9 (± 6.2)	85.0	75.0 – 100.0	13.48
Location B (2008) (n = 191)				
JJ (16)				8.38
LJJ (139)				72.77
LJJJ (35)				18.32
LJJJJ (1)				0.52
Location E (n = 118) (OMNR unpublished data)				
JJ (12)				10.17
LJJ (102)				86.44
LJJJ (4)				3.39

The data in Table 1 clearly show that unisexual *Ambystoma* that use *A. jeffersonianum* as a sperm donor are much more abundant than *A. jeffersonianum* and that tetraploid unisexuals are not larger than triploid unisexuals. Size data are available only for Location A and Location B (Table 1), The salamanders tend to be smaller at Location A, and perhaps this reflects more recent or more consistent recruitment over time and that larger individuals are, on average, older. Recruitment at Location B may not be as successful as it is at Location A. Future surveys at these and other sites will be necessary to assess recruitment and to monitor the percent differential between *A. jeffersonianum* and unisexuals. The percentage of *A. jeffersonianum* individuals at Location B did not change between the 2007 (8.51%) and 2008 (8.38%) surveys (Table 1).

A few new populations of *A. jeffersonianum* have been found on the Oak Ridges Moraine in York Region, in Waterloo Region, and in Hamilton-Wentworth Region (Figure 3). These are all isolated populations in typical *A. jeffersonianum* habitat (see **Habitat requirements**, above).

Fluctuations and trends

Ambystoma jeffersonianum is assumed to be a fairly long-lived species so adults have several yearly breeding opportunities that may compensate for extrinsic factors such as a cold snap that freezes eggs or a dry spring and summer that evaporates vernal pools and kills larvae. Fluctuations in the number of breeding adults in any year could be related to the cohorts of previous “good” and “bad” years for recruitment. Trends in population density can only be estimated through repeated yearly surveys of the same ponds combined with surveying several ponds in the same year.

A few of the documented populations in Ontario have been repeatedly surveyed, and some of these populations have been stable over a relatively long period of time (e.g., Location A and Location D) based on surveys performed in 1979, 1981, 1990 (NHIC 1998), and 2004-2006 (Bériault 2005, Ramsden 2005). The Location C population in Norfolk County also appears to be persistent. However, annual observations that document a severe reduction in number of egg masses from hundreds in 1979 to fewer than 10 in 2006 suggest that this population has declined considerably (> 90%) over this period (J.P. Bogart pers. obs.). Weller (1980) estimated that the population at his study site in Peel Region contained 80 breeding males and about 838 breeding females (including unisexuals). His study also recorded the actual number of individuals captured while migrating to the breeding pond. The numbers diminished over the three years of his study from 624 in 1975 to 513 in 1976 to 324 in 1977. Recent observations and egg collections at Weller’s study site (2006-2008) confirmed the continued presence of *A. jeffersonianum* but very few individuals or egg masses could be found (H. Lynn pers. obs.). In 2003 and 2004, researchers surveyed historically known breeding sites along the Niagara Escarpment that were documented in 1990 and 1991. Eighteen sites were searched for *A. jeffersonianum* egg masses by staff from Ontario’s Niagara Escarpment (ONE) Monitoring Program. Only three sites were confirmed to have *A. jeffersonianum* with the remaining 15 sites no longer supporting *A. jeffersonianum* or unisexuals (Jefferson Salamander Recovery Team 2009).

From repeated surveys within about a 15-year time frame (1990,91-2004,5), no population was estimated to be larger than the initial survey in subsequent surveys. Most populations were observed to be declining in numbers of individuals and some populations are probably extirpated. There are now fewer than 30 extant populations (defined here as equivalent to number of known, extant breeding ponds or locations) that still maintain *A. jeffersonianum*. A few new populations have been found since the last report, but the continued existence of about 25 historical populations cannot be confirmed. There are few or no egg masses in many ponds that formerly had many egg masses. Some historic ponds have been stocked with carnivorous fishes, some no longer hold water for the necessary time for larval development, and some have been lost to development.

The previous report (COSEWIC 2000) mostly dealt with populations along the Niagara Escarpment. In addition, that report provided estimates based on a relatively small number of individuals because specimens had to be killed to identify them. Using new methods, samples can be much larger and have shown that *A. jeffersonianum* makes up only a small percentage of any population. Most are unisexual individuals. Many unisexual females come to ponds and don’t find males. This is reflected by the unexpected low number (or absence of) egg masses in ponds where the population (of unisexuals) is reasonably large. These salamanders can live for ~ 25 yrs, so unisexuals could continue to come to breeding ponds for several years even if *A. jeffersonianum* males did not. However, without males, the unisexuals do not deposit eggs.

Rescue effect

Ambystoma jeffersonianum is apparently secure in several populations in Pennsylvania where it does not live with unisexual *Ambystoma* (Bogart and Klemens 1997), and has large population densities (M.W. Klemens pers. obs.). The closest U.S. populations of *A. jeffersonianum* to Ontario populations are in Cattaraugus and Wayne counties in New York where unisexual LJJ are also found (Bogart and Klemens 2008). Considering the limited movements in this species, current distribution, and barriers to dispersal, rescue from the U.S. is highly improbable.

LIMITING FACTORS AND THREATS

Habitat loss

The most probable cause of low numbers of *A. jeffersonianum* in Canada is the limited amount of suitable habitat, both terrestrial habitat and breeding ponds. The Carolinian forest with its associated fauna naturally reaches the northern limit of its distribution in southern Ontario, but the vast majority of this habitat in Ontario has been cleared, initially for agriculture and subsequently for urban development, aggregate extraction, and resource development.

Habitat alteration

Suitable or historical habitat for *A. jeffersonianum* may be impacted (e.g., a pond may be stocked with fish) (L. Rye, W.J. Cook, J.P. Bogart pers. obs.). Migratory paths between a breeding pond and summer habitat may be blocked by development, silt fencing, drainage ditches, some plantations, or other barriers. Hydrological alterations can reduce the hydro period of a breeding pond so that the pond consistently dries up before the larvae can transform.

Microhabitat alteration

Clearing fallen trees or debris from summer habitat and from the edges of breeding ponds limits the food, protective cover, and dispersal abilities for *A. jeffersonianum*. Clearing breeding ponds of sticks and other attachment sites for egg masses is also detrimental. Small mammal burrows are used by salamanders for hiding, feeding, and over-wintering, so reduction of the small mammal population could have a detrimental effect.

Road mortality

Individuals are frequently killed on roads by vehicles while migrating to or from a breeding pond. Curbs and catch basins can act as barriers or traps, respectively, and roads are often a source of chemical pollution (e.g., salt, metals, products of combustion) that degrade adjacent aquatic and terrestrial habitat. Toxic effects of salts applied for road de-icing can extend considerable distances into wetlands and have been demonstrated to be detrimental to *A. maculatum* (Turtle 2000, Karraker *et al.* 2008, Collins and Russell 2009). Roads also increase the vulnerability of migrating adults to predators.

Clanton effect

As noted in the section on **Interspecific Interactions,** the so-called Clanton effect, a term coined by Minton (1954), would result in a population crash of both *A. jeffersonianum* and unisexuals because there would no longer be any spermatophores. The reason for this loss of spermatophores is that as the unisexuals increase they would take more and more spermatophores leading to a decline in reproductive success of Jefferson females. Once the Jefferson females had disappeared there would be no new males being recruited and once all males were gone the unisexuals would become the “walking dead” as they could not reproduce. The Clanton effect has not been documented in any population; however, there is current research to test this hypothesis (J.P. Bogart pers. comm. August 2010). If male *A. jeffersonianum* are extirpated, the unisexual population crashes unless they can use another species as a sperm donor. It is also interesting to realize that unisexuals can only successfully immigrate to ponds that already have acceptable sperm donors.

SPECIAL SIGNIFICANCE OF THE SPECIES

Larvae of *A. jeffersonianum* are voracious aquatic predators that feed on moving prey items such as insect larvae, small crustaceans, and amphibian larvae. Adults are prey items for wetland predators such as snakes, birds, and mammals. *Ambystoma jeffersonianum* is considered to be an indicator species of high quality vernal pools.

The unisexuals represent a biological novelty that is unlike any other organism so far found in the animal kingdom. Unisexual *Ambystoma* are known to steal sperm from males of five different ambystomatid species (*A. barbouri, A. jeffersonianum, A. laterale, A. texanum, A. tigrinum*) in eastern North America (Bogart *et al*. 2009). The interaction of unisexual *Ambystoma* with their sperm donors may be an evolutionary novelty, but the unisexual lineage has probably persisted for millions of years (Bogart *et al*. 2007, Bi and Bogart 2010). Over the range of unisexuals, *A. jeffersonianum* is the most commonly used male sperm donor (Bogart and Klemens 2008). In Ontario, unisexuals also use males of *A. laterale* and *A*. *texanum*, a species found only on Pelee Island. Two species of sperm donors rarely occur in the same breeding ponds but two such ponds have been found in Ontario (Bogart *et al*. 2007; and unpublished). Unisexuals must be able to switch sperm donors and this switch has been documented in one Ontario population (Location G) (Bogart *et al*. 2007). Nevertheless, there are many questions left to be answered with respect to the interactions of unisexual salamanders and their sperm donors. Ontario populations are important to improve our understanding of this biological phenomenon. For example, they can expand our understanding of genomic interaction, genome organization, gene dosage compensation, cell regulation and other significant evolutionary components of biology.

EXISTING PROTECTION OR OTHER STATUS DESIGNATIONS

Ambystoma jeffersonianum received a COSEWIC status of Threatened in November 2000 (COSEWIC 2000). It was listed as Threatened in the Canadian *Species at Risk Act* (SARA) in 2002 as well as being designated by SARO (Species at Risk in Ontario) as Threatened, and listed by OMNR in 2004. In Canada, it has a NatureServe rank of N2 (imperiled), and in the U.S., and globally, it has a rank of N4 or G4, (apparently secure, i.e., uncommon but not rare) (NatureServe 2008).

Ambystoma jeffersonianum and *A. laterale* present problems for conservationists because they both coexist with unisexual individuals that normally do not have a conservation status (Kraus 1995). Connecticut lists *A. jeffersonianum* complex and *A. laterale* complex as state species of special concern because it is difficult to distinguish the unisexuals from the bisexual species, *A. jeffersonianum* and *A. laterale* (Klemens 2000). Connecticut also lists pure diploid populations of *A. laterale* in the eastern portion of the state as a threatened species, but there is no status given for *A. jeffersonianum* (only *A. jeffersonianum* “complex”) (Klemens 2000). There are other problems that relate to the use of museum specimens to establish temporal trends in ranges and populations. Museum specimens may be misidentified and the occurrence of unisexual individuals not recognized. This problem certainly justifies additional genetic confirmation of individuals throughout the range of *A. jeffersonianum*, other sperm donating species and unisexuals. A summary of the recent conservation status rankings for *A. jeffersonianum* is provided in Table 2.

Table 2. NatureServe (2008) rank for *Ambystoma jeffersonianum* for all jurisdictions within its global range. S1 = critically imperiled, S2 = imperiled, S3 = vulnerable. S4 = apparently secure, S5 = secure, SNR = unranked.

State/Province	S1	S2	S3	S4	S5	SNR
Connecticut*			X			
Illinois		X				
Indiana				X		
Kentucky				X		
Maryland			X			
Massachusetts		X	X			
New Hampshire		X	X			
New Jersey			X			
New York				X		
Ohio						X
Ontario		X				
Pennsylvania				X		
Vermont		X				
Virginia				X		
West Virginia			X			

*The ranking for Connecticut refers to *A. jeffersonianum* "complex" that would include unisexual individuals.

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BIOGRAPHICAL SUMMARY OF REPORT WRITER

James (Jim) Bogart is Professor Emeritus in the Department of Integrative Biology at the University of Guelph. His research examines evolution at the species level and involves molecular biology, cytogenetics, ecology and evolution with a focus on polyploid amphibians. His studies on unisexual *Ambystoma* and their sperm donors were initiated in 1975 and have continued to the present time. He has advised several graduate students who have studied various aspects of these salamanders in Canada and in the United States.

COLLECTIONS EXAMINED

Most *Ambystoma jeffersonianum* and unisexual salamanders examined from the United States are deposited in the American Museum of Natural History (AMNH). Canadian specimens are deposited at the Royal Ontario Museum (ROM) or the Canadian Museum of Nature (CMN). A frozen collection of tissues and DNA extractions is catalogued and stored at the University of Guelph.

Appendix 1. Locality information for *Ambystoma jeffersonianum* in Ontario.

All populations where *A. jeffersonianum* (JJ) have been found also contain unisexual *Ambystoma* (LJJ). *Ambystoma jeffersonianum* has not been found in populations that are listed as LJJ but it is presumed that *A. jeffersonianum* is also present as a sperm donor in those populations. The reference numbers are voucher specimens or DNA extractions from the catalogue of James P. Bogart (JPB). The temporal data relate to the first time that the population was discovered (First Year), and the subsequent years that the population was still found to contain *A. jeffersonianum* and/or unisexual individuals. The Jefferson Salamander Recovery Team members have visited most of the historic sites but only the **confirmed existence** of *A*. *jeffersonianum* or unisexual LJJ is recorded in Subsequent Years.

Location #	ID	County	JPB Ref. #	First Year	Subsequent Years	
1	LJJ	Grey	20236	1992		
2	LJJ	Grey	21464	1992		
3	LJJ	Grey	20136	1991		
4	JJ	Dufferin	19961	1990	2004	
5	JJ	Dufferin	21672	1992		
6	JJ	York	38657	2002	2009	
7	JJ	York	36303	2005		
8	JJ	York	35636	1978	2004	
9	JJ	York	33037	2002		
10	JJ	York	32751	2002	2009	
11	LJJ	York	33203	2002		
12	LJJ	York	36541	2005		
13	JJ	Peel	84807	1991	2004	
14	JJ	Peel	20013	1991	2002	
15	JJ	Peel	32717	1991	2009	
16	JJ	Peel	32800	2009		
17	JJ	Peel	39063	2009		
18	LJJ	Peel	35298	2004		
19	LJJ	Peel	34405	1990	2003	
20	LJJ	Peel	18335	1986	1990	2008
21	LJJ	Halton	19924	1991		
22	JJ	Halton	40002	2009		
23	JJ	Halton	35628	1991	2004	
24	JJ	Halton	35666	1991	2004	

Location #	ID	County	JPB Ref. #	First Year	Subsequent Years	
25	JJ	Halton	36093	2006		
26	JJ	Halton	33324	1980	2002	
27	JJ	Halton	34727	1990	2003	
28	JJ	Halton	32106	2003	2006	2008
29	JJ	Halton	32786	1990	2005	2009
30	JJ	Halton	19304	1990		
31	JJ	Halton	35510	2004	2009	
32	JJ	Halton	37574	1990	2006	
33	JJ	Halton	37501	2006		
34	JJ	Halton	18695	1990	2008	
35	JJ	Halton	35690	2004		
36	JJ	Halton	36458	2006		
37	JJ	Halton	17129	1989	2006	
38	JJ	Halton	38766	1990	2008	
39	JJ	Halton	37625	2006		
40	LJJ	Halton	19283	1990		
41	LJJ	Halton	18054	1990		
42	LJJ	Halton	17985	1990		
43	LJJ	Halton	16479	1989		
44	LJJ	Halton	17130	1989		
45	JJ	Wellington	18985	1990		
46	JJ	Wellington	35375	1986	1989	
47	JJ	Wellington	18985	1987	1993	
48	LJJ	Wellington	39949	2009		
49	LJJ	Wellington	18343	1990		
50	JJ	Waterloo	39124	2008	2009	
51	JJ	Waterloo	16873	1989		
52	JJ	Waterloo	36857	1979	2006	
53	LJJ	Waterloo	39886	2009		
54	LJJ	Waterloo	39096	2008	2009	
55	JJ	Hamilton-Wentworth	33548	1979	2004	2006
56	JJ	Hamilton-Wentworth	16977	1989		
57	JJ	Hamilton-Wentworth	18989	1990		
58	JJ	Hamilton-	17167	1989		

Location #	ID	County	JPB Ref. #	First Year	Subsequent Years	
		Wentworth				
59	LJJ	Hamilton-Wentworth	30858	1991		
60	LJJ	Hamilton-Wentworth	18288	1990		
61	LJJ	Hamilton-Wentworth	32605	2002		
62	LJJ	Hamilton-Wentworth	32597	2002		
63	JJ	Brant	16576	1989		
64	LJJ	Brant	16828	1989		
65	LJJ	Brant	16819	1989		
66	LJJ	Brant	16852	1989	1990	
67	LJJ	Brant	16588	1989		
68	LJJ	Brant	16634	1989		
69	LJJ	Brant	16920	1989		
70	LJJ	Brant	16659	1989		
71	LJJ	Brant	16751	1989		
72	LJJ	Niagara	17161	1989		
73	LJJ	Niagara	17024	1989		
74	LJJ	Niagara	20975	1991		
75	JJ	Haldimand	16584	1989		
76	JJ	Norfolk	11228	1985		
77	JJ	Norfolk	11986	1986		
78	JJ	Norfolk	18725	1989	1990	
79	JJ	Norfolk	11937	1986		
80	JJ	Norfolk	35744	1986	2004	
81	JJ	Norfolk	25779	1980	1991	2006
82	JJ	Norfolk	14191	1988		
83	LJJ	Norfolk	11065	1985		
84	LJJ	Norfolk	11221	1985		
85	LJJ	Norfolk	11769	1985		
86	LJJ	Elgin	21699	1992		
87	LJJ	Elgin	12194	1986		