

**Ontario Species at Risk Evaluation Report for
Unisexual *Ambystoma* (*Ambystoma laterale*)**

Committee on the Status of Species at Risk in Ontario
(COSSARO)

Small-mouthed Salamander dependent population (*Ambystoma
laterale - texanum*)
Assessed by COSSARO as Endangered

Jefferson Salamander dependent population (*Ambystoma laterale
- (2) jeffersonianum*)
Assessed by COSSARO as Endangered

Blue-spotted Salamander dependent population (*Ambystoma (2)
laterale - jeffersonianum*)
Assessed by COSSARO as Not at Risk

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Final

Ambystoma unisexué (Ambystoma laterale)

Les populations d'*Ambystoma unisexué* formées uniquement de femelles sont membres de la famille des salamandres fousseuses, soit les ambystomatidés, qui est endémique à l'Amérique du Nord. L'*Ambystoma unisexué* est la plus ancienne lignée connue de vertébrés unisexués. Le processus de fécondation et de formation des œufs nécessite un apport de sperme provenant d'une espèce sympatrique étroitement apparentée. Dès que leur formation est déclenchée, les œufs éliminent généralement le génome du sperme et se développent de manière asexuée. La plupart des descendants des individus asexués ont donc le même génotype que leur mère; ils sont le fruit d'un clonage. Dans de rares cas, l'ADN du sperme est incorporé, soit par la substitution d'une série de chromosomes par une autre, soit par l'augmentation de la ploïdie des embryons (par exemple, de triploïde à tétraploïde) avec l'ajout d'une série de chromosomes.

La morphologie de chaque groupe de salamandres dépend du génome nucléaire. Les individus unisexués qui possèdent au moins deux garnitures chromosomiques de la salamandre à points bleus (*A. laterale*) sont noirs et présentent divers degrés de mouchetures bleues; ils ont aussi des membres relativement courts et la tête étroite (forte ressemblance avec la salamandre à points bleus). Les individus unisexués qui possèdent au moins deux garnitures chromosomiques de la salamandre de Jefferson (*A. jeffersonianum*) sont quant à eux plus gros, leur peau est grise ou brune avec quelques mouchetures bleues, leurs membres sont assez longs et leur tête est relativement large (forte ressemblance avec la salamandre de Jefferson). Enfin, les individus unisexués qui possèdent au moins deux garnitures chromosomiques de la salamandre à petit bouche (*A. texanum*) sont gris, leur corps est plus mince et leur tête est étroite (forte ressemblance avec la salamandre à petit bouche).

Le CDSEPO a évalué trois unités désignables de salamandres asexuées, dont l'identification repose principalement sur l'espèce donneuse de sperme (voir ci-dessous).

Au Canada, l'espèce *Ambystoma unisexuée* dépendante de la salamandre à petit bouche (*Ambystoma laterale – texanum*) n'est présente que sur l'île Pelée, une île isolée du lac Érié, en Ontario, où elle dépend d'une espèce en voie de disparition, la salamandre à petit bouche (*Ambystoma texanum*) pour l'obtention de sperme. Cette unité désignable est menacée par la prédation et la modification de l'habitat par le dindon sauvage, une espèce introduite, les activités de drainage qui assèchent les étangs de reproduction, la mortalité attribuable à la circulation routière pendant les périodes de migration, le développement urbain et les activités récréatives. Étant donné que certaines menaces concernent tous les étangs de reproduction de l'île Pelée, ceux-ci devraient être considérés comme un seul endroit. Compte tenu de sa petite aire de répartition et du déclin de sa population, fondé sur les observations et les prévisions, l'*Ambystoma unisexuée* dépendante de la salamandre à nez court est considérée comme une espèce en voie de disparition.

L'*Ambystoma unisexuée* dépendante de la salamandre de Jefferson (*Ambystoma laterale – (2) jeffersonianum*) occupe des aires restreintes dans des zones perturbées

du Sud de l'Ontario, et dépend d'une espèce en voie de disparition, la salamandre de Jefferson (*Ambystoma jeffersonianum*), pour l'obtention de sperme. Cette unité désignable est menacée par les activités humaines qui mènent à la perte et à la fragmentation continues de son habitat, et par la mortalité attribuable à la circulation routière. Compte tenu du déclin de sa population et de sa petite aire de répartition, l'*Ambystoma* unisexuée dépendante de la salamandre de Jefferson est considérée comme une espèce en voie de disparition.

On ne connaît pas précisément la répartition de l'*Ambystoma* unisexuée dépendante de la salamandre à points bleus (*Ambystoma (2) laterale – jeffersonianum*) en Ontario, mais on sait que la salamandre à points bleus (*Ambystoma laterale*), l'espèce de laquelle elle dépend pour obtenir son sperme, est largement répartie en Ontario, des frontières manitobaines aux frontières québécoises, et des Grands Lacs à la baie James. L'*Ambystoma* unisexuée a été observée dans plusieurs lieux de cette zone, au nord de Wawa, et est probablement présente dans une grande partie de l'aire de répartition de la salamandre à points bleus. Étant donné que les menaces sont localisées et qu'il est peu probable que toute la population de l'Ontario soit concernée, l'*Ambystoma* unisexuée dépendante de la salamandre à points bleus est considérée comme une espèce non en péril.

Cette publication hautement spécialisée «COSSARO Candidate Species at Risk Evaluation for Unisexual Ambystoma» n'est disponible qu'en anglais conformément au Règlement 671/92, selon lequel il n'est pas obligatoire de la traduire en vertu de la Loi sur les services en français. Pour obtenir des renseignements en français, veuillez communiquer avec le ministère des Richesses naturelles et des Forêts au recovery.planning@ontario.ca.

Executive summary

The all-female populations of Unisexual *Ambystoma* are members of the Mole Salamander family Ambystomatidae, which is endemic to North America. The Unisexual *Ambystoma* represent the oldest known lineage of unisexual vertebrates. The fertilization of eggs and initiation of development requires sperm from a sympatric, closely related species. Once development is initiated, the eggs normally discard the sperm genome and develop asexually. As a result, most of the offspring of unisexuals have the same genotype as their mother and represent clonal reproduction. In rare cases, DNA from sperm is incorporated, thereby substituting one set of chromosomes for another or increasing the ploidy of the embryos (e.g., from triploid to tetraploid) by adding a set of chromosomes.

The morphology of each group of salamanders is determined by the nuclear genome. Unisexuals with two or more Blue-spotted Salamander (*A. laterale*) chromosome complements are black with various amounts of blue flecking, and have relatively short limbs and narrow heads (closely resembling Blue-spotted Salamander). Unisexuals with two or more Jefferson Salamander (*A. jeffersonianum*) chromosome complements are larger animals that are grey to brown in colour with a small amount of blue flecking, relatively long limbs, and a relatively broad head (closely resembling Jefferson Salamander). Unisexuals with two or more Small-mouthed Salamander (*A. texanum*) chromosome complements are grey with slender bodies and narrow heads (closely resembling Small-mouthed Salamander).

COSSARO assessed three Designatable Units (DUs) of unisexual salamanders, identified primarily on the basis of their sperm-donor species and resultant genomic DNA (see below).

The Unisexual *Ambystoma* (Small-mouthed Salamander dependent population) (*Ambystoma laterale* - *texanum*) exists only on Pelee Island in Canada, an isolated island in Lake Erie, Ontario, where they depend on the Endangered Small-mouthed Salamander (*Ambystoma texanum*) for sperm donations. This DU is threatened by predation and habitat modification by introduced wild turkeys, drainage activities that dry breeding ponds, road mortality during migration periods, urban development, and recreational activities. Some threats are common to all breeding ponds on Pelee Island, and therefore should be considered a single location. The Unisexual *Ambystoma* (Small-mouthed Salamander dependent population) is assessed as Endangered based on small distribution range and both observed and projected declines.

The Unisexual *Ambystoma* (Jefferson Salamander dependent population) (*Ambystoma laterale*- (2) *jeffersonianum*) occupies restricted areas within disturbed areas of southern Ontario and depend on the Endangered Jefferson Salamander (*Ambystoma jeffersonianum*) for sperm donation. This DU is threatened by human activities that lead to ongoing habitat loss and fragmentation, and road mortality. The Unisexual *Ambystoma* (Jefferson Salamander dependent population) is assessed as Endangered based on declining populations and small distribution range.

The Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

(*Ambystoma (2) laterale-jeffersonianum*) distribution in Ontario is not precisely known, but Blue-spotted Salamander (*Ambystoma laterale*), on which they rely for sperm donation, has an extensive distribution in Ontario that ranges from the Manitoba to Quebec borders, and from the Great Lakes to James Bay. Unisexuales have been identified in sites across much of this range north to Wawa, and likely occur throughout much of the range of the Blue-spotted Salamander. The Unisexual *Ambystoma* (Blue-spotted Salamander dependent population) is assessed as Not at Risk as threats are localized and are unlikely to impact the entire Ontario population.

1. Background information

1.1. Current designations

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

- GRANK: Not assessed (NatureServe accessed November, 2016)
- NRANK Not assessed (NatureServe accessed November, 2016)
- COSEWIC: Endangered (2016)
- SARA: No Status (No Schedule)
- ESA 2007: Not assessed
- Not assessed

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

- GRANK: Not assessed (NatureServe accessed November, 2016)
- NRANK Not assessed (NatureServe accessed November, 2016)
- COSEWIC: Endangered (2016)
- SARA: No Status (No Schedule)
- ESA 2007: Endangered (given the same as Jefferson Salamander)
- SRANK: Not assessed

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

- GRANK: Not assessed (NatureServe accessed November, 2016)
- NRANK Not assessed (NatureServe accessed November, 2016)
- COSEWIC: Not at risk (2016)
- SARA: No Status (No Schedule)
- ESA 2007: Not assessed
- SRANK: Not assessed

1.2. Distribution in Ontario

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

In Ontario, this population is found only on Pelee Island, Lake Erie (COSEWIC, 2016). Four subpopulations were identified in COSEWIC (2016); Fishpoint, Pond, Sheridan Road and Stone Road. T. Hossie (pers. comm.) and J. Hathaway surveyed six potential breeding sites in 2015/2016 and identified two breeding sites that were previously unidentified. One of these is in the same forest block as Stone Road and should be included as part of that location, leaving several 'Stone Road' sites that would be similarly affected by a single event e.g. adjustment to water table. The second site was not previously identified. In addition, although the location at Lighthouse Point was considered extirpated in the COSEWIC report, adults have since been found at that site, although T. Hossie and collaborators did not find a breeding pond (T. Hossie, pers. comm.). One site that is on private land has not been surveyed in >10 years, and may have few Small-mouthed Salamanders or Small-mouth Salamander dependents (i.e.

the majority of salamanders at this site are thought to be Blue-spotted dependents). The former location known as Girl Guide Pond is known to be extirpated. Therefore, there are up to six locations for this species.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

This population is found in a number of sites in built-up areas west of Toronto: Kitchener, Erindale, Waterdown, and Hilton Falls Conservation Area; COSEWIC, 2016).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Blue-spotted Salamander has an extensive range in eastern Canada, and relatively few ponds have been surveyed for Blue-spotted Salamander–dependent unisexuales. Figure 7 in COSEWIC (2016) shows that these unisexuales have been confirmed across much of Ontario north to approximately Wawa and Temiskaming. The current data likely represents only a subset of their distribution in the province.

1.3. Distribution and status outside Ontario

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

This form has been recorded from southern Michigan and northwestern Ohio to central Indiana (COSEWIC 2016).

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

This taxa has been recorded from southern Michigan and Indiana, through south-central Ontario east to northern New Jersey, western Massachusetts and Vermont (COSEWIC 2016).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

While not as extensively sampled, this taxon has been widely recorded from Minnesota southern Michigan and much of Ontario, east to northern New Jersey, Nova Scotia and New Brunswick (COSEWIC 2016).

1.4. Ontario conservation responsibility

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Since only found on Pelee Island, Ontario represents much less than 10% of the known range of this taxa.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Based on the range described above, Ontario represents roughly 20% of the known range of this taxa.

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Based on the range described above, Ontario represents roughly 25% of the known range of this taxa.

1.5. Direct threats

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Loss of the sperm donor species, the endangered Small-mouthed Salamander (*Ambystoma texanum*), would prevent the unisexual population from being able to reproduce. Other threats include drainage activities and climatic fluctuations that can lead to premature drying of ponds; habitat loss through development and agriculture; road mortality during dispersal events; and predation and habitat modification by wild Turkeys (COSEWIC, 2016; T. Hossie, pers. comm.). Overall threat impact calculated from threats calculator = high (COSEWIC, 2016).

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Loss of the sperm donor species, the endangered Jefferson Salamander (*Ambystoma jeffersonianum*), would prevent the unisexual population from being able to reproduce. Habitat loss may be caused by building, quarrying, and agriculture. Road mortality during seasonal dispersal and introduced predatory fish at breeding sites are also significant threats. Overall threat impact calculated from threats calculator = very high (COSEWIC, 2016).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

The Blue-spotted Salamander is relatively abundant through much of its Canadian range and threats to most populations are minimal. Conversion of natural habitat to agricultural and urban land uses has destroyed and fragmented much of this species' habitat throughout the southern portion of its range in Canada, particularly in Ontario. Furthermore, ongoing habitat destruction following urbanization, cottage development and road construction, continues to cause localized population declines. Road mortality can be a significant threat to this species when roads bisect spring migration routes. Pollution, such as herbicides, agricultural effluent and road salt can be detrimental to salamanders since toxins are easily absorbed through their skin. Climate change and introduced pathogens pose potentially serious future threats to Canadian salamanders.

Overall threat impact calculated from threats calculator = low (COSEWIC, 2016).

1.6. Specialized life history or habitat use characteristics

Unisexual *Ambystoma* requires sperm donations from sympatric bisexual *Ambystoma*. They are essentially sex parasites. In breeding ponds, unisexuals typically outnumber their bisexual hosts. For example, a survey conducted at breeding ponds on Pelee Island in the spring of 2015 and 2016 identified only 6 *Ambystoma* as bisexual *A. texanum* out of a sample of 430 individuals. The remainder were unisexuals, and no *A. laterale* were trapped (T. Hossie, pers. comm.). Sperm-donor species for two of the three unisexual forms are endangered, and this may be a severe limiting factor.

2. Eligibility for Ontario status assessment

2.1. Eligibility conditions

2.1.1. Taxonomic distinctness

The taxonomic status of unisexual lineages of *Ambystoma* has been debated for many years. The dependent populations of *Ambystoma* unisexuals comprise a monophyletic mitochondrial lineage that diverged from *Ambystoma* bisexuals 3-5 million years ago (Bi and Bogart, 2010). Within the unisexual lineage, informal names have been based on chromosome complements, e.g. a triploid unisexual possessing one Blue-spotted Salamander genome and two Jefferson Salamander genomes is *Ambystoma laterale* - (2) *jeffersonianum*, also described as LJJ. While all unisexual salamanders possess at least one set of Blue-spotted Salamander chromosomes, the other chromosome sets are derived from the sperm-donor species and hence the DUs can be differentiating both geographically and genetically. Furthermore, each DU has adaptations and an ecology that is similar to those of the sperm-donor species.

2.1.2. Designatable units

There are no official scientific and common names for unisexual salamanders in the genus *Ambystoma* (Crother, 2012). In the absence of formally described common names, COSSARO will follow the convention established by COSEWIC (2015) which is to use protocols specific to the taxonomic group under consideration. Although many publications include Unisexual *Ambystoma* in the Jefferson Salamander complex, not all populations actually possess Jefferson Salamander chromosomes (Bogart et al. 2009), and therefore it is more appropriate to identify three designatable units (DUs) within the Unisexual *Ambystoma* complex based on their sperm-donor species: Small-mouthed Salamander dependent, Jefferson Salamander dependent, and Blue-spotted Salamander dependent populations. Each DU can include diploid, triploid, or tetraploid unisexuals. In Canada, unisexual populations of salamanders occur in all known Jefferson Salamander and Small-mouthed Salamander populations, as well as in the majority of Blue-spotted Salamander populations that have been investigated (COSEWIC, 2016). Each DU has an ecology similar to that of their co-occurring sperm-donating species. Furthermore, each is significant because of quantitative genetic

differences in the alleles obtained from the sperm donor species, meaning that each would likely be extirpated following the extirpation of its sperm donor species (COSEWIC, 2016).

2.1.3 Native status

Distinct forms within the *Ambystoma* complex that may have been unisexuals were first identified by Clanton (1934), and all-female triploids were described in the 1960s (Uzzell, 1964; Uzzell and Golblatt, 1967). However, the recency with which this group was discovered is most likely a reflection of the relative recency with which researchers were able to assess ploidy levels of non-model species; the unisexual lineage, inferred from mitochondrial DNA (mtDNA) sequences is at least three million years old (Bi and Bogart, 2010), and they likely co-existed with three bisexual, sperm-donating species of *Ambystoma* in Ontario for a long period of time. Unisexual populations of *Ambystoma* comprise more than 20 diploid, triploid, tetraploid, and even pentaploid combinations of chromosomes from two or three of the Jefferson salamander the Blue-spotted Salamander, or the Small-mouthed Salamander (Bogart 2003; Bogart et al. 2009). All unisexual *Ambystoma* have at least one set of chromosomes from Blue-spotted Salamander plus mtDNA from a lineage that has diverged substantially from the lineages of bisexual *Ambystoma* (Hedges et al. 1992; Bogart 2003). Unisexual *Ambystoma* have been present in Ontario for more than fifty years (COSEWIC, 2016), and likely for a lot longer than that.

2.1.3. Occurrence

All three DUs occur in Ontario (see Ontario distribution).

2.2. Eligibility results

The three DUs of Unisexual *Ambystoma*, the Small-mouthed Salamander dependent population, the Jefferson Salamander dependent population, and the Blue-spotted Salamander dependent population are eligible for status assessment in Ontario.

3. Ontario status assessment

3.1. Application of Endangered/Threatened status in Ontario

3.1.1. Criterion A – Decline in total number of mature individuals

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Does not apply. Inferred decline in IAO based on loss of breeding ponds from 1991 to 2000 (COSEWIC 2004) may not be realized. Four subpopulations were identified in COSEWIC (2016), but as noted above, there are now potentially six locations. One location has been lost (Girl Guide Pond), and other recently discovered breeding ponds are likely not 'new'. Therefore, there has likely been a loss of at least one location since 2000, representing a decline in IAO. Further declines may be linked to endangered status of sperm-donor species (COSEWIC, 20014). However, the magnitude of decline

is unknown, and therefore does not meet criteria.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Meets Endangered A2bc+3c+4bc. There is an observed and projected decline of ~74% of suitable breeding ponds in the most recent generation for the Jefferson Salamander (COSEWIC, 2016), on which the unisexualls depend. Furthermore, habitat for both Jefferson Salamander and their dependent unisexualls is being lost and degraded through much of their Ontario range. Meets Endangered because there is an observed, inferred and suspected decline of >50% in number of mature individuals over the past 3 generations (since 1982) based on a decline in index of abundance (subcriterion b) and in IAO and quality of habitat (sub-criterion c); also meets A3 based on similar suspected future declines and A4 based on similar suspected declines that incorporate both the future and the past.

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Does not apply. Blue-spotted Salamander has an extensive range in the province and the associated unisexual is also widespread. It is likely that both are experiencing localized declines but there are likely few threats through much of their range. There is no evidence of widespread declines.

3.1.2. Criterion B – Small distribution range and decline or fluctuation

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Meets Endangered B1ab(ii,iii, iv)+2ab(ii, iii, iv). Taxa occurs only on Pelee Island in Canada, an island in Lake Erie that is approximately 4,262 ha. Two of the six known historical breeding populations were thought to have been lost after 2000 (COSEWIC (2016)). As described above, up to six breeding sites may remain; however, because some threats are relevant to all sites in the island (turkeys, endangered sperm donor species), Pelee Island is more appropriately identified as a single location. EEO = 20 km², IAO = 20 km². Population is not severely fragmented. Although the minimum distance between sites is ~630 m, most are separated by distances >1 km (T. Hossie, pers. comm.) which exceeds dispersal distances of most individuals (Bériault 2005); however, more than half of the occupied sites appear to support viable populations. EEO and IAO are within thresholds for Endangered, and meets criterion of <6 locations (a). Habitat quantity and quality are declining because of wild turkeys and other threats (b iii); there is a projected continued decline in subpopulations because <2% of 430 trapped *Ambystoma* on the Island were bisexual *A. texanuman* in 2015/16 (b iv) which will result in a decline in IAO (b ii). Furthermore, the loss of Girl Guide Pond since 2003 represents a decline in IAO. No evidence of extreme fluctuations (c).

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Meets Endangered B2ab(i,ii,iii,iv,v). Estimated 30 distinct locations. EEO = 9,457 km², IAO=188 km² (COSEWIC, 2016). Virtually all subpopulations (breeding ponds or locations) are below estimated MVPs for long-term persistence of vertebrates in general and for other species of *Ambystoma* (Reed *et al.* 2003). Connecting habitat between breeding ponds has in many cases been lost, and most of these ponds are separated by >1km which exceeds the expected dispersal distances for most salamanders (Bériault, 2005). Habitat is being lost and degraded over the range of Jefferson

Salamanders in Ontario. Observed and projected declines in subpopulations (corresponding to discrete breeding ponds), IAO, and EOO because an observed and projected decline for the Jefferson Salamander will impact the unisexuales that depend on this species for reproduction, i.e. 62% decline in EOO and 74% decline in suitable breeding ponds for Jefferson Salamanders (COSEWIC, 2016). Meets Endangered B2ab because IAO is within threshold; meets sub-criterion (a) because the population is severely fragmented; and meets sub-criterion (b) because there is a continuing inferred and projected decline in EOO (i), index of area of occupancy (ii), extent and /or quality of habitat (iii), number of locations and subpopulations (iv), and number of mature individuals (v).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Does not apply. Blue-spotted Salamander has a wide range with an estimated EOO of 671,668 km² in Canada while the unisexual occurs through much of this area (awaiting Ontario-specific info). The unisexual has an estimated IAO of 1932 km² in Canada which is likely to be a gross underestimate (Ontario-specific information not available). Based on range map for Blue-Spotted Salamander, exceeds threshold.

3.1.3. Criterion C – Small and declining number of mature individuals

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Not applicable as criteria for Endangered do not apply. Total number of estimated mature may be <1,000, but data are incomplete. It is highly unlikely that there are >2,500 individuals, but there are no data to suggest that additional criteria are met with respect to the rate of continued decline, extreme fluctuations in numbers of mature individuals, or the presence of >250 mature individuals in any population. Therefore, does not meet thresholds for Endangered. However, meets Threatened C1 a(i) since there are fewer than 10,000 individuals and no sub-population is estimated to contain more than 1000 individuals.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Not applicable as criteria for Endangered do not apply. The population is unknown but likely <10,000. There may be <2500 adult Jefferson Salamanders (COSEWIC 2010). Jefferson Salamander-dependent unisexuales are more numerous than their sperm donors and, over the range of Jefferson Salamanders, constitute 60% to 95% of subpopulations with an approximate average of 80%, resulting in probably <10,000 adult unisexuales. Meets Threatened under C2 a(i) because the population is <10,000 adults, there is continuing inferred and projected decline in numbers of individuals, and no subpopulation contains more than 1000 mature individuals.

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Does not apply. The population size is unknown but it is almost certainly far in excess of 10,000 given its broad distribution and therefore does not qualify under this criterion.

3.1.4. Criterion D – Very small or restricted total population

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Not applicable as criteria for Endangered do not apply. There could be more than 1000

individuals, but its single population meets the criterion of threatened.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Does not apply. There are an estimated 30 locations, and an IAO of 188 km². Population is not very small or restricted.

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Does not apply. The IAO and number of individuals greatly exceeds the threshold and many populations are unlikely to be restricted.

3.1.5. Criterion E – Quantitative analysis

Does not apply. No analysis available. Application of Special Concern in Ontario
Does not apply.

3.2. Status category modifiers

3.2.1. Ontario's conservation responsibility

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

Does not apply (~10% of known range is in Ontario).

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Does not apply (~20% of known range is in Ontario).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Does not apply (~25% of known range is in Ontario, but no evidence of small population sizes or decline).

3.2.2. Rescue effect

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population)

The population lives on Pelee Island which is an isolated island in Lake Erie. Rescue effect is exceedingly unlikely.

Unisexual *Ambystoma* (Jefferson Salamander dependent population)

Unisexual *Ambystoma* do not have a status in adjoining US states (Vermont, New York, Michigan and Ohio). The sperm donor, Jefferson Salamander, is a threatened species in Vermont and does not occur in Quebec or Michigan. Rescue effect therefore is very unlikely given their limited dispersal ability.

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population)

Unknown, but may be possible.

3.3. Other status categories

3.3.1. Data deficient

Does not apply.

3.3.2. Extinct or extirpated

Does not apply.

3.3.3. Not at risk

Does not apply to Small-mouthed Salamander dependent and Jefferson Salamander-dependent populations.

The Blue-spotted Salamander dependent population does not qualify under any of the above classifications, and therefore qualifies for Not at Risk.

4. Status

Unisexual *Ambystoma* (Small-mouthed Salamander dependent population) (*Ambystoma laterale*) is classified as Endangered based on meeting criteria B1ab(ii,iii,iv)+2ab(ii, iii, iv).

Unisexual *Ambystoma* (Jefferson Salamander dependent population) (*Ambystoma laterale*) is classified as Endangered in Ontario based on meeting criteria A2bc+3c+4bc; B2ab(i,ii,iii,iv,v).

Unisexual *Ambystoma* (Blue-spotted Salamander dependent population) (*Ambystoma laterale*) is classified as Not at Risk in Ontario.

5. Information sources

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Appendix 1: Technical summary for Ontario

Species: Unisexual *Ambystoma* - Small-mouthed Salamander dependent population

Demographic information

Demographic attribute	Value
Generation time. Based on average age of breeding adult: age at first breeding = X year; average life span = Y years.	8 years
Is there an observed, inferred, or projected continuing decline in number of mature individuals?	Yes, inferred and projected continuing decline based on the projected decline for the Small-mouthed Salamander (see COSEWIC 2004, 2014) and other identified threats.
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.	Unknown
Projected or suspected percent reduction or increase in total number of mature individuals over the next 10 years or 3 generations.	Projected and suspected reduction based on the Threats Calculation that summarized the overall threat impact as "high" (10 – 70% projected decline).
Observed, estimated, inferred, or suspected percent reduction or increase in total number of mature individuals over any 10 years, or 3 generations, over a time period including both the past and the future.	Unknown
Are the causes of the decline (a) clearly reversible, and (b) understood, and (c) ceased?	a. No b. Yes c. No
Are there extreme fluctuations in number of mature individuals?	There are fluctuations because recruitment varies greatly from year to year, but such fluctuations are probably not extreme.

Extent and occupancy information in Ontario

Extent and occupancy attributes	Value
Estimated extent of occurrence (EOO).	~20 km ² (approximate because of two newly discovered ponds – one viable – and one possibly extirpated).
Index of area of occupancy (IAO).	~20 km ² , based on five 2 x 2 km grids superimposed on four recently occupied breeding ponds. Approximate because of two newly discovered ponds – one viable – and one possibly extirpated).
Is the total population severely fragmented? i.e., is >50% of its total area of occupancy is in habitat patches that are: (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse?	(a) No; more than half of the occupied sites appear to support viable populations; (b) yes; the breeding ponds are separated by > 600 m, and salamanders are unlikely to disperse among them.
Number of locations.	4-6. Four recorded by COSEWIC, one of which wasn't found in 2015/6 survey. Two new ones discovered in that survey but one doesn't show signs of reproduction.
Number of NHIC Element Occurrences	No NHIC data for unisexuals but 5 for Small-mouthed Salamander
Is there an observed, inferred, or projected continuing decline in extent of occurrence?	Likely (projected) because of paucity of sperm-donor species and other threats
Is there an observed, inferred, or projected continuing decline in index of area of occupancy?	Likely (projected) because of paucity of sperm-donor species and other threats
Is there an observed, inferred, or projected continuing decline in number of populations?	Likely (projected) because of paucity of sperm-donor species and other threats
Is there an observed, inferred, or projected continuing decline in number of locations?	Likely (projected) because of paucity of sperm-donor species and other threats
Is there an observed, inferred, or projected continuing	Likely (projected) because

Extent and occupancy attributes	Value
decline in [area, extent and/or quality] of habitat?	of paucity of sperm-donor species and other threats
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 sub-population or total population (if known)

Sub-population (or total population)	Number of mature individuals
Fish Point (southern tip of Pelee Island)	Unknown
Pond (middle of Pelee Island)	Unknown
Sheridan Point (northern Tip of Pelee Island)	Unknown
Stone Road	Unknown
Additional (unnamed) pond identified by T. Hossie (pers. comm.)	Unknown
Additional (unnamed) pond identified by T. Hossie (pers. comm.)	Unknown

Quantitative analysis (population viability analysis conducted)

Probability of extinction in the wild is unknown (not done due to lack of data)

Threats

- i. Dams and water management/use (Threat 7.2). Drainage activities on Pelee Island can affect the breeding habitat by reducing breeding areas and cause premature drying of breeding ponds.
- ii. Housing and urban areas (Threat 1.1). Decline or destruction of habitat from clearing of wooded areas.
- iii. Annual and perennial non-timber crops (Threat 2.1)
- iv. Roads and railroads (Threat 4.1). Barriers to breeding migrations from new roads, as well as increased road mortality.
- v. Recreational activities (Threat 6.1)
- vi. Other ecosystem modifications (Threat 7.3). The introduction of Wild Turkeys on Pelee Island may pose a serious threat because Turkeys can destroy terrestrial hiding places for salamanders and may prey upon salamanders (Invasive non-native/alien species) (Threat 8.1).

Was a threats calculator completed for this species and if so, by whom?

Yes. Leslie Anthony, Jim Bogart, Joe Crowley, Yohann Dubois, Isabelle Gauthier, Bev McBride (COSEWIC secretariat)

Rescue effect

Rescue effect attribute	Value
Status of outside population(s) most likely to provide immigrants to Ontario	Unisexual <i>Ambystoma</i> have no status in Ohio or Michigan. The Small-mouthed Salamander is a threatened species in Michigan owing, in large part, to loss of suitable habitat.
Is immigration of individuals and/or propagules between Ontario and outside populations known or possible?	No
Would immigrants be adapted to survive in Ontario?	Yes
Is there sufficient suitable habitat for immigrants in Ontario?	No, these unisexuales depend on Small-mouthed Salamander males that, in Canada, only exist on Pelee Island, which is isolated from U.S. populations.
Are conditions deteriorating in Ontario?	Yes
Is the species of conservation concern in bordering jurisdictions?	Unisexual <i>Ambystoma</i> have no status in Ohio or Michigan. The Small-mouthed Salamander is a threatened species in Michigan owing, in large part, to loss of suitable habitat.
Is the Ontario population considered to be a sink?	No
Is rescue from outside populations likely?	No

Sensitive species

Yes, because these unisexuales depend on, and live with, the Endangered Small-mouthed Salamander, which is a data sensitive species.

Species: Unisexual *Ambystoma* - Jefferson Salamander
dependent population (*Ambystoma laterale*)

Demographic information

Demographic attribute	Value
Generation time. Based on average age of breeding adult: age at first breeding = X year; average life span = Y years.	11 years
Is there an observed, inferred, or projected continuing decline in number of mature individuals?	Yes, observed, inferred and projected continuing decline based on the decline for the Jefferson Salamander and threats to habitat
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.	Suspected decline of > 90% over the last 3 generations (33 years), based on long-term data sets for Jefferson Salamanders. Egg mass counts (index of abundance) from 1976 – 2006 show a >90% decline.
Projected or suspected percent reduction or increase in total number of mature individuals over the next 10 years or 3 generations.	Projected reduction based on the Threats Calculation that summarized the overall threat impact as “very high” (50 – 100% reduction).
Observed, estimated, inferred, or suspected percent reduction or increase in total number of mature individuals over any 10 years, or 3 generations, over a time period including both the past and the future.	Suspected decline of > 90% based on past and projected future declines.
Are the causes of the decline (a) clearly reversible, and (b) understood, and (c) ceased?	a. No, b. Yes c. No
Are there extreme fluctuations in number of mature individuals?	There are fluctuations because recruitment varies greatly from year to year, but such fluctuations are probably not extreme.

Extent and occupancy information in Ontario

Extent and occupancy attributes	Value
Estimated extent of occurrence (EOO).	9,457 km ² . EOO was calculated as 24,624 km ² when both historical and recent localities are included. However, these salamanders depend on the Jefferson Salamander, and the most recent EOO calculated for that species is 9,457 km ²
Index of area of occupancy (IAO).	188 km ² . IAO was calculated as 728 km ² , when both historical and recent localities were included using 2x2 km grids. The most recent calculation for the Jefferson Salamander is 188 km ² .
<p>Is the total population severely fragmented? i.e., is >50% of its total area of occupancy is in habitat patches that are:</p> <p>(a) smaller than would be required to support a viable population, and</p> <p>(b) separated from other habitat patches by a distance larger than the species can be expected to disperse?</p>	<p>a. Yes, most known sub-populations have small (<200) numbers of adult Jefferson Salamanders and are isolated from one another. Virtually all subpopulations (breeding ponds or locations) are below estimated MVPs for long-term persistence of vertebrates in general and for other species of <i>Ambystoma</i> (Reed <i>et al.</i> 2003).</p> <p>b. Yes, there is a loss of connecting habitat between breeding ponds, most of which are separated by >1 km. This distance would be greater than expected dispersal distances for the salamanders. Salamanders have limited dispersal capability and</p>

Extent and occupancy attributes	Value
	have breeding site fidelity.
Number of locations.	There are estimated to be ~30 geographically or ecologically distinct locations for the Jefferson Salamander. At each location, a single threatening event, such as a change in hydrology or hydroperiod from a variety of human activities, can rapidly affect all individuals.
Number of NHIC Element Occurrences	No NHIC data for unisexuals but 45 for Jefferson Salamander.
Is there an observed, inferred, or projected continuing decline in extent of occurrence?	Yes, present subpopulations are more closely linked with the Niagara Escarpment. Several historical subpopulations to the east and west of the Escarpment have been lost, resulting in a 62% decline in EOO.
Is there an observed, inferred, or projected continuing decline in index of area of occupancy?	Yes, there is an observed and projected decline for the Jefferson Salamander that will have an impact on the unisexuals that depend on this species. Comparing 1979-2003 and 2004-2015 data for Jefferson salamanders, the decline of suitable breeding ponds in the most recent generation (2004-2015) is 74%.
Is there an observed, inferred, or projected continuing decline in number of populations?	Yes, observed and projected declines in subpopulations (corresponding to discrete breeding ponds).
Is there an observed, inferred, or projected continuing decline in number of locations?	Yes, observed and projected decline in

Extent and occupancy attributes	Value
	locations (corresponding to discreet breeding ponds).
Is there an observed, inferred, or projected continuing decline in [area, extent and/or quality] of habitat?	Yes, observed and projected decline in area, extent, and quality of habitat.
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 sub-population or total population (if known)

Sub-population (or total population)	Number of mature individuals
There are estimated to be about 30 discrete breeding ponds, each of which represents a subpopulation with little or no genetic exchange expected between subpopulations.	Unknown but probably < 10,000. There may be <2500 adult Jefferson Salamanders (COSEWIC 2010). Jefferson Salamander-dependent unisexuales are more numerous than their sperm donors and, over the range of Jefferson Salamanders, constitute 60% to 95% of subpopulations with an approximate average of 80%, resulting in < 10,000 adult unisexuales.

Quantitative analysis (population viability analysis conducted)

Probability of extinction in the wild is unknown (not assessed due to lack of data).

Threats

- i. Residential & commercial development (Threat 1), particularly from housing and urban areas (Threat
- ii. Mining & quarrying (Threat 3.2)
- iii. Roads & railroads (Threat 4.1). Road mortality during breeding migrations.
- iv. Agriculture (Threat 2.1) causing loss and degradation of habitats
- v. Invasive non-native/alien species.

Was a threats calculator completed for this species and if so, by whom?

Yes. Leslie Anthony, Jim Bogart, Joe Crowley, Yohann Dubois, Isabelle Gauthier, Bev McBride (COSEWIC secretariat).

Rescue effect

Rescue effect attribute	Value
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Rescue effect attribute	Value
Status of outside population(s) most likely to provide immigrants to Ontario	Unisexuals have no status in adjacent states (Vermont, New York, Michigan and Ohio). The sperm donor, Jefferson Salamander, is a threatened species in Vermont and does not occur in Quebec or Michigan.
Is immigration of individuals and/or propagules between Ontario and outside populations known or possible?	Unlikely, but possible from Vermont.
Would immigrants be adapted to survive in Ontario?	Yes
Is there sufficient suitable habitat for immigrants in Ontario?	No, habitat is restricted to areas in southwestern Ontario that are diminishing.
Are conditions deteriorating in Ontario?	Yes, habitat is being lost and degraded over the range of Jefferson Salamanders in Canada.
Is the species of conservation concern in bordering jurisdictions?	Unknown
Is the Ontario population considered to be a sink?	No
Is rescue from outside populations likely?	No

Sensitive species

Yes, this is a data sensitive species because these unisexuals co-exist with the Endangered Jefferson Salamander, which is a data sensitive species.

Species: Unisexual *Ambystoma* - Blue-spotted Salamander dependent population (*Ambystoma laterale*)

Demographic information

Demographic attribute	Value
Generation time. Based on average age of breeding adult: age at first breeding = X year; average life span = Y years.	8 years
Is there an observed, inferred, or projected continuing decline in number of mature individuals?	Yes, inferred and projected decline. Although Blue-spotted Salamander-dependent unisexuals are wide-ranging and abundant in Canada, habitat and wetland loss in parts of the range of their sperm donor has resulted in a decline.
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.	Unknown
Projected or suspected percent reduction or increase in total number of mature individuals over the next 10 years or 3 generations.	Unknown
Observed, estimated, inferred, or suspected percent reduction or increase in total number of mature individuals over any 10 years, or 3 generations, over a time period including both the past and the future.	Unknown
Are the causes of the decline (a) clearly reversible, and (b) understood, and (c) ceased?	a. Partially; known in some geographical areas but not in others. b. Yes c. No
Are there extreme fluctuations in number of mature individuals?	No

Extent and occupancy information in Ontario

Extent and occupancy attributes	Value
Estimated extent of occurrence (EOO). <i>If value in COSEWIC status report is not applicable, then use geocat.kew.org. State source of estimate.</i>	___ km ²

Extent and occupancy attributes	Value
Index of area of occupancy (IAO). <i>If value in COSEWIC status report is not applicable, then use geocat.kew.org. State source of estimate.</i>	___ km ²
Is the total population severely fragmented? i.e., is >50% of its total area of occupancy is in habitat patches that are: (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse?	a. No b. No
Number of locations.	Unknown
Number of NHIC Element Occurrences	No NHIC data for unisexuals and Blue-spotted Salamander is not tracked.
Is there an observed, inferred, or projected continuing decline in extent of occurrence?	No
Is there an observed, inferred, or projected continuing decline in index of area of occupancy?	No
Is there an observed, inferred, or projected continuing decline in number of populations?	No
Is there an observed, inferred, or projected continuing decline in number of locations?	No
Is there an observed, inferred, or projected continuing decline in [area, extent and/or quality] of habitat?	No
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 sub-population or total population (if known)

Sub-population (or total population)	Number of mature individuals
Total population	Unknown

Quantitative analysis (population viability analysis conducted)

Probability of extinction in the wild is unknown (quantitative analysis not done).

Threats

- i. Roads & railroads (Threat 4.1) - Road mortality during breeding migrations

- ii. Logging & wood harvesting (Threat 5.3) - Wetland and terrestrial habitat alteration from forestry practices, include microhabitat degradation.

Was a threats calculator completed for this species and if so, by whom?

Yes. Leslie Anthony, Jim Bogart, Joe Crowley, Yohann Dubois, Isabelle Gauthier, Bev McBride (COSEWIC secretariat).

Rescue effect

Rescue effect attribute	Value
Status of outside population(s) most likely to provide immigrants to Ontario	Unisexual <i>Ambystoma</i> do not have a status in adjoining US states
Is immigration of individuals and/or propagules between Ontario and outside populations known or possible?	Not known but possible
Would immigrants be adapted to survive in Ontario?	Yes
Is there sufficient suitable habitat for immigrants in Ontario?	Yes
Are conditions deteriorating in Ontario?	Possibly
Is the species of conservation concern in bordering jurisdictions?	Unknown
Is the Ontario population considered to be a sink?	No
Is rescue from outside populations likely?	Possibly

Sensitive species

Not a data sensitive species.

Appendix 2: Adjoining jurisdiction status rank and decline

Information regarding rank and decline for Unisexual *Ambystoma* in adjoining jurisdictions is not available.