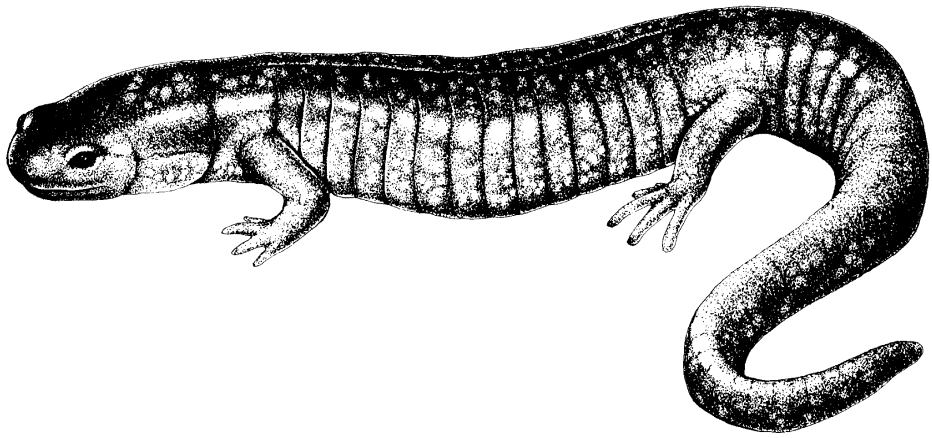
COSEWIC
Assessment and Update Status Report

on the

Small-mouthed Salamander
Ambystoma texanum

in Canada



ENDANGERED
2004

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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COSEWIC
Assessment Summary

Assessment Summary – May 2004

Common name
Small-mouthed salamander

Scientific name
Ambystoma texanum

Status
Endangered

Reason for designation
This salamander is restricted solely to Pelee Island in Canada. The extent of occurrence is only 40 km^2 (effectively the total area of Pelee Island). It occupies only three extant breeding sites and surrounding remnant forested habitat with total area of occupancy equalling not more than 5 km^2. It has exhibited declines in area, extent and quality of habitat, and in the number of locations on the island where it may be found. Threats to its continued existence include loss of wetland breeding sites and modified drainage patterns.

Occurrence
Ontario

Status history
Designated Special Concern in April 1991. Status re-examined and designated Endangered in May 2004. Last assessment based on an update status report.

COSEWIC
Executive Summary

Small-mouthed Salamander
Ambystoma texanum

In this update of the 1991 status report for the Small-mouthed Salamander, we bring together all available data that relate to the distribution of *Ambystoma texanum* on Pelee Island: the only Canadian locality for this species. We include locality information for *Ambystoma texanum*, *A. laterale* and diploid, triploid and tetraploid genomic hybrids that also exist on the island because: (1) the identification of *A. texanum*, and distinguishing that species from the genomic hybrids is confusing; and (2) both *A. texanum* and *A. laterale* males are used as sperm donors for the persistence of genomic hybrid combinations. Although this update only considers the status of "pure" diploid *A. texanum*, the presence of rare *A. texanum* males can be estimated through an examination of the more common genomic hybrids.

Preliminary DNA sequence information suggests that *Ambystoma texanum* on Pelee Island are the result of more than one historic invasion from the United States mainland. Known breeding sites from the 1980s were re-visited in the spring of 2000 to estimate trends and to establish a current list of important breeding sites. Two of the five breeding sites from 1991, on the north and east side of the Island, appear to have been eliminated. *Ambystoma texanum* appears now to be restricted to two areas (breeding sites) in the southern part of Pelee Island. Both areas are within protected zones. The presence of adults and larvae at both sites provides evidence that recruitment is occurring.

Diploid and polyploid hybrid combinations that incorporate *A. texanum* nuclear genomes are more common than the pure species and it is estimated that the breeding population would include fewer than 1000 pure, diploid *A. texanum* individuals on the Island. *Ambystoma texanum* is listed as threatened by the Ontario Ministry of Natural Resources (OMNR). Presently, *A. texanum* exists in an area of approximately 1000 ha across the southern quarter of Pelee Island. This is a considerable reduction in the range of this salamander on the island that coincides with the reduction in previously known breeding sites.

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. 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 and include 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 organizations (Canadian Wildlife Service, Parks Canada Agency, Department of Fisheries and Oceans, and the Federal Biosystematic Partnership, chaired by the Canadian Museum of Nature), three nonjurisdictional members and the co-chairs of the species specialist and the Aboriginal Traditional Knowledge subcommittees. The committee meets to consider status reports on candidate species.

DEFINITIONS (AFTER MAY 2004)

Species	Any indigenous species, subspecies, variety, or geographically or genetically distinct population of wild fauna and flora.
Extinct (X)	A species that no longer exists.
Extirpated (XT)	A species no longer existing in the wild in Canada, but occurring elsewhere.
Endangered (E)	A species facing imminent extirpation or extinction.
Threatened (T)	A species likely to become endangered if limiting factors are not reversed.
Special Concern (SC)*	A 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 species that has been evaluated and found to be not at risk.
Data Deficient (DD)***	A species for which there is insufficient scientific information to support status designation.

* 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.

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Update
COSEWIC Status Report

on the

Small-mouthed Salamander
Ambystoma texanum

in Canada

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2004

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INTRODUCTION

Pelee Island (Fig. 1) is the largest (4,262 ha) Island in Lake Erie and is inhabited by two species of mole salamanders (*Ambystoma laterale* and *A. texanum*) as well as diploid, triploid, and tetraploid genomic hybrids (Bogart *et al*. 1985; Bogart and Licht, 1986). *Ambystoma texanum* and hybrids also occur on other Islands in Lake Erie (Downs 1978; Bogart *et al*. 1987; King *et al*. 1997) and on the mainland in Ohio and Illinois (Downs 1978; Kraus 1985). Pelee Island is the only Canadian locality for *Ambystoma texanum*, the Small-mouthed Salamander (Bogart *et al*. 1985). In light of the previous Status Report on *Ambystoma texanum* (Bogart and Licht 1991), the species was assigned a status of Vulnerable because of its very restricted range in Canada. At the time the status report was written, the species was considered to be abundant on the Island.

The identification of the species of *Ambystoma* and the hybrids on the island was determined by allozyme electrophoresis and karyotypes by Bogart *et al*. (1985). At that time, only one of 34 collected specimens was *A. texanum* and only diploid and triploid hybrids were identified. Subsequent collecting yielded more *A. texanum* and documented the presence of tetraploid hybrids. It is now known that there exist diploid (*A. laterale* x *texanum* or LT), triploid (LLT and LTT) and tetraploid hybrids (LLLT, LLTT, and LTTT) on Pelee Island. The Island's salamanders later served to test various hypotheses related to the origin of the hybrids and consequences of polyploidy by Bogart and Licht (1986) and Licht and Bogart (1987; 1989). Although crosses of Pelee Island *A*. *texanum* and *A. laterale* have not been done in the laboratory nor observed in nature, it was generally assumed that the hybrids were produced through such a mechanism and that backcrosses were responsible for the various polyploid classes. But, in an examination of *mt*DNA from putative parental and hybrid *Ambystoma* over much of their known range, Hedges *et al*. (1992) showed that the hybrids possess a cytoplasmic genome that differed from any possible sperm-donating parent in eastern North America. Hybrids, irrespective of their nuclear genome or possible parental associations, have a similar "hybrid" cytochrome *b* sequence. They found that Pelee Island diploid, triploid, and tetraploid hybrids clustered with mainland hybrids that had nuclear contributions from *A. laterale* and *A. jeffersonianum* (not *A. texanum*). Additionally, Hedges *et al*. found that the two Pelee Island *A. texanum* used in their study were not homosequential and represented two distinctive haplotypes or *mt*DNA clones.

The new molecular data clearly show that the hybrids on Pelee Island were not produced through crosses involving the two parental species on the Island or, indeed, elsewhere in the range of the hybrids that have been examined. Apparently, the hybrids were isolated on the Island at the same time as the two diploid bisexual species and presently exchange nuclear genomes with those extant species (*A. laterale* and *A. texanum*). The significance of these findings is dealt with in detail by Hedges *et al*. (1992), Bogart and Klemens (1997), and Bogart (2003). The hybrids are virtually all females and the hybrids on Pelee Island must obtain a spermatophore from a male (*A*. *texanum* or *A*. *laterale*) for reproductive success (Bogart and Licht 1986; Bogart 2003). It is of interest that the two specimens of Pelee Island *A*. *texanum* that were

sequenced by Hedges *et al*. demonstrated greater sequence divergence than all *A*. *laterale* samples which included individuals from several Ontario populations as well as individuals from Prince Edward Island, Connecticut, Illinois, and Vermont.

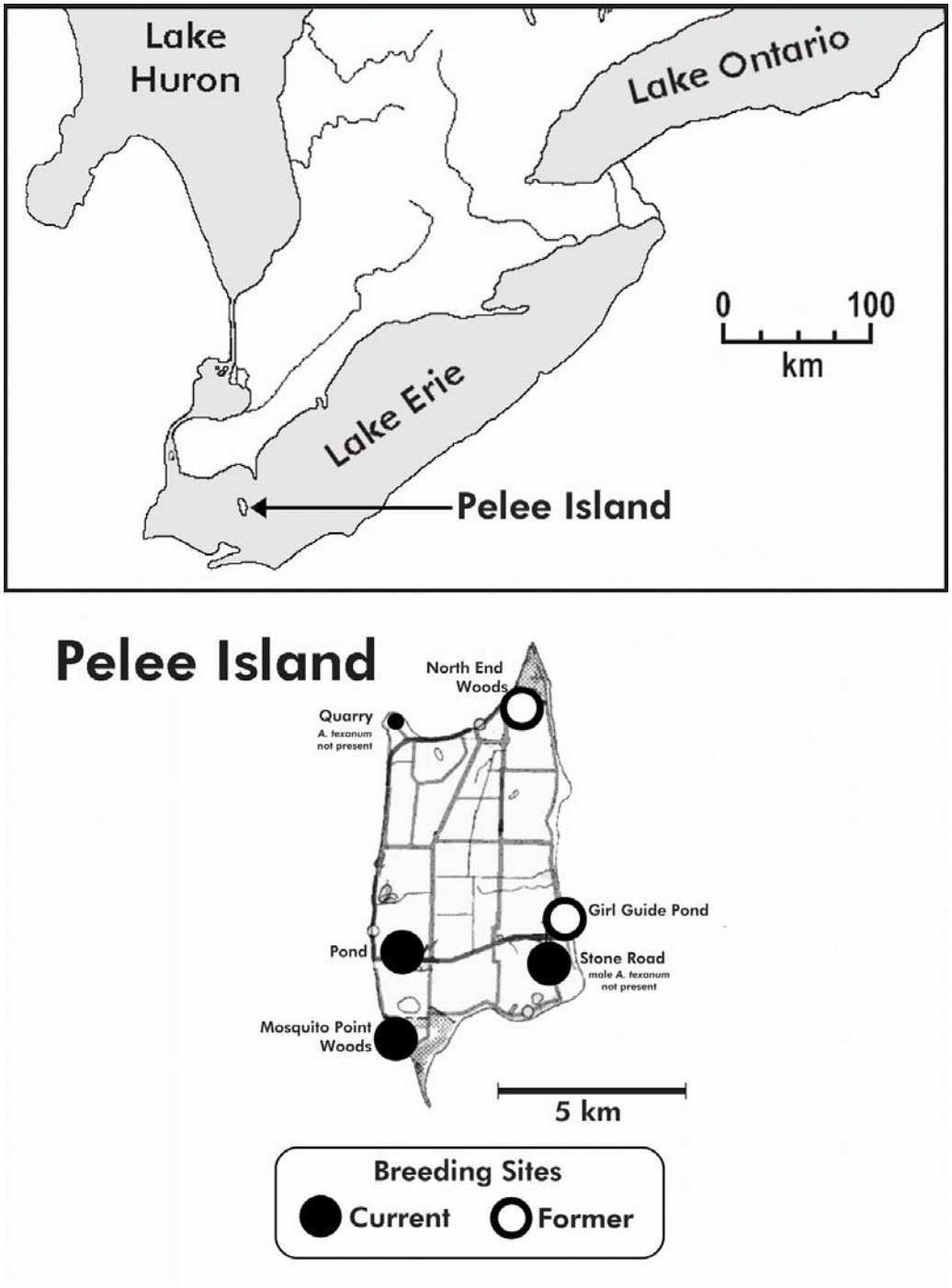

Figure 1. Pelee Island, the only locality for *Ambystoma texanum* in Canada, with localities on the Island as mentioned in the text.

Other than the molecular study by Hedges *et al*. (1992), no additional information on *A. texanum* from Pelee Island has been published subsequent to the 1991 Status Report and there are relatively few new studies that include *A*. *texanum* from elsewhere in its range. Petranka (1998) included a few new references and provided some additional detail concerning the ecology and ethology of *A*. *texanum* and *A*. *barbouri* (which was split taxonomically from *A*. *texanum* by Kraus and Petranka in 1989). The new references include southern range extensions, discovery of an albanistic individual, and the inclusion of *A. texanum* in some community ecological studies. *Ambystoma texanum* from Ohio and Texas were included in a mitochondrial sequence study by McKnight and Shaffer (1997) that compared 17 species of *Ambystoma*. A further resolution of the reproductive process used by nuclear hybrids and additional mitochondrial DNA sequences that show the unisexual lineage to be monophyletic were provided by Bogart (2003).

A trip was made to Pelee Island on the 1st and 2nd of April, 2000. Historic population sites were examined during the night and day to assess the conditions of these populations and to make observations with respect to successful breeding at those sites. Because individuals and/or egg masses were collected from these sites at the same time of year in the 1980s, the presence of individuals and/or egg masses would confirm that the habitat was suitable for current populations. Observations were also made with respect to any changes in the habitat that might have an effect on the current status of *Ambystoma texanum*.

DISTRIBUTION

Recent information on the distribution and the habitat association of *A. texanum* can be found in Petranka (1998). The species ranges from the coastal plain in eastern Texas, Louisiana, Mississippi, and western Alabama north to extreme southeastern Michigan, northern Ohio and Pelee Island in Ontario. Populations outside the coastal plain usually inhabit upland hardwood forests surrounding vernal ponds but some populations contain gilled, paedomorphic, adults that live in permanent or semipermanent ponds. Canadian *A. texanum* would represent a very small fraction of the range (less than 1% of the global range) and are only associated with vernal ponds and flooded wetlands.

Pelee Island - Specimens of *Ambystoma* were collected from various locations on Pelee Island from 1983 to 1995 (Fig. 1; Appendix 1). These specimens were used to test various hypotheses that related to genetics or to variation in fitness components that compared polyploids and diploids. Several papers have been published from these experiments but the actual distribution of *Ambystoma texanum* was not considered in these investigations. Because diploid and polyploid hybrids are much more common than either *A. texanum* or *A. laterale* on Pelee Island, a large sample size was necessary to estimate both the occurrence and the frequency of *A. texanum* in Island populations. Initially, specimens were collected as sexually mature adults but in the later collections, eggs and larvae were collected. Larvae that hatched from the eggs as well

as wild caught larvae were raised in the laboratory through metamorphosis so that the individuals' sex and genotype could be determined. Detailed methods for raising larvae, euthanizing, and processing transformed salamanders for the determination of genotype and ploidy are included in previous papers (Bogart 1982; Bogart *et al*. 1985; 1987; Bogart and Licht 1986).

Data were obtained (1984-1991) from more than 1200 larvae from the six breeding sites determined from our previous collections. Tissue samples from all individuals were analyzed for isozymes using starch-gel electrophoresis. Ploidy was determined by measuring the areas of erythrocytes and observations of allozyme band densities on the gels; if these other methods were inconclusive, karyotypes were examined. Only six breeding sites for *Ambystoma* have been found on Pelee Island and *Ambystoma texanum* was previously found in five of the six sites. The genotype frequencies were computed for comparisons (Table 1).

Table 1. Frequency and Occurrence of Genotypes of *Ambystoma texanum*, *A. laterale*, and Hybrids from Six Sites on Pelee Island (1984 to 1991).

Locality	Nuclear Genome								N
	2n			3n			4n		
	LL	TT	LT	LLT	LTT	LLLT	LLTT	LTTT	
Quarry	81	0	64	335	2	13	3	0	498
(Males)	(39)		(1)	(13)		(2)			
North End Woods	0	15	2	1	1	0	0	0	19
(Males*)		(1)							
Pond	1	5	98	109	108	9	14	7	351
(Males)		(3)	(7)		(4)				
Stone Road	1	56	20	4	15	0	0	0	96
(No males!)									
Girl Guide Pond	0	38	5	2	4	0	0	0	49
(Males)		(1)							
Mosquito Point	0	77**	83	23	79	0	0	12	274
(Males)		(24)		(2)	(2)				
Total	83	191	272	474	209	22	17	19	1287
Percentage	6.45	14.84	21.13	36.83	16.24	1.71	1.32	1.48	

*probably an underestimate of males from North End Woods as 13 of the 15 were larvae whose sex could not be determined.

**One female was a triploid (TTT)

Hybrids made up 78 percent of the salamanders on Pelee Island and, of the pure species, *Ambystoma texanum* was found to be more common and more numerous than *A. laterale*. These data (Table 1) also show that each of the populations was distinctive with respect to the frequency of pure species compared with the frequencies of the various genomic contributions found in hybrids. *Ambystoma laterale* was almost completely restricted to the Quarry area in the north of Pelee Island and the highest density of *A. texanum* occurred at the opposite end of the Island in the Mosquito Point woods (Fig. 1). In both these populations, hybrids outnumbered either species. But, in populations on the east side of the Island (Girl Guide Pond, Stone Road, and North End Woods), *A. texanum*

outnumbered the hybrids. The Pond locality was the only locality where both *A. laterale* and *A. texanum* as well as all combinations of hybrids were found to coexist.

Other islands in Lake Erie - *Ambystoma texanum* coexists with *A. tigrinum*, *A. opacum* and a suite of unisexual nuclear hybrids in Ohio on Kelleys Island, south of Pelee Island in Lake Erie (Bogart *et al*. 1987). Only unisexual nuclear hybrids have been found on the Bass Islands (also in Lake Erie) (Downs 1978). Downs assumed that the salamanders on those Islands reproduce by parthenogenesis (no males requied). However, a sperm cell was recovered from the cloaca of a Middle Bass Island, Ohio, female unisexual (Bogart 2003). The identity of the male is not known but, based on the fact that the female was a LTT unisexual, *A. texanum* was probably used as a sperm donor.

PROTECTION

Two of the five known breeding ponds or areas for *A. texanum* in Canada are found in nature reserves on Pelee Island. These would include the Stone Road site and the Mosquito Point Woods/Fish Point site. The secretive, nocturnal nature of breeding and the fact that the salamanders are subterranean during the tourist season alleviates collection of adults. In Canada, *A*. *texanum* was designated by COSEWIC as special concern (SC) in 1991 and, in Ontario, an OMNR designation of threatened. The major range of this species is in the central part of the United States. The species is considered to be secure over most of its known range and has a Global Heritage Status Rank of G5 (globally secure). *Ambystoma texanum* has the following ranks in the United States / Canada: Alabama (S3), Arkansas (S5), Illinois (S5), Indiana (S4), Iowa (S3), Kansas (S5), Kentucky (S5), Louisiana (S5), Michigan (S1), Mississippi (S5), Missouri (S?), Nebraska (S1), Ohio (S?), Oklahoma (S5), Tennessee (S5), Texas (S5), West Virginia (S1); Ontario (S1) [Subnational Heritage Status Rank Definitions: S1 = critically imperiled; S2 = imperiled; S3 = vulnerable; S4 = apparently secure; S5 = secure].

POPULATION SIZE AND TRENDS

It is clear (Table 1) that the genomic hybrids outnumber both *Ambystoma laterale* and *A. texanum* on Pelee Island. Most of the specimens that were used to derive the data in Table 1 came from random samples of eggs and larvae. The large number of individuals that were sampled provides a fairly accurate estimate of both the presence, absence, and the frequency of *Ambystoma texanum* in the six breeding sites for *Ambystoma*. Because it is very difficult to distinguish *A. texanum* from some genomic hybrids without genetic confirmation, finding large numbers of eggs, larvae, or even adults only confirms breeding success and does not provide density estimates for *A. texanum*. It is known, however, that reproductive success of the genomic female hybrids depends on their association with males of either *A. laterale* or *A. texanum* (Bogart and Licht 1986). Thus, finding larvae and juveniles in populations that have been found to contain only *A. texanum* attests to the continued existence of that species

at that site. Because of the problems identifying *A. texanum*, we do not know the population density of "pure" *A. texanum* and can not assess trends. All species of *Ambystoma* for which there are data on population sizes fluctuate extensively in abundance, as is typical of pond-breeding amphibians in general (Green, 2003). Two former breeding ponds have probably been extirpated (the North End Woods site and the Girl Guide Pond site). Both those sites are on private lands and the current vegetation is not typical of ephemeral pond habitat.

HABITAT

Known localities — General habitat requirements for *A. texanum* were provided in the 1991 status report (Bogart and Licht 1991) but limited information was provided with respect to the actual distribution of the Small-mouthed Salamander on Pelee Island. There are notable differences in the frequencies of *A. texanum*, *A. laterale* and the genomic hybrids from the five populations (Table 1). In this update, we include additional information on the sites that seems to be important for partitioning the salamanders on Pelee Island. The previous populations (Fig. 1) were re-visited and located using geographical coordinates (Appendix 1). Such data may eventually be combined with ongoing studies of the Blue Racer and the Eastern Fox Snake to document critical habitat for all these species.

At the Mosquito Point Woods locality, the habitat appeared virtually unchanged from the 1980s. There still exists a flooded woodlot with fallen logs and suitable habitat for the salamanders. The water level was low, compared with previous levels, which may pose a problem if the area dried before salamander larvae could transform. In one area where we observed breeding in 1984, the water depth was 50 cm and, at the same site in 2000, the water depth was only 20 cm. This area was normally dry by mid-August but transformation takes place through June and July. In April 2000, juvenile and adult salamanders were found under logs around the perimeter of the water. These salamanders were the only evidence that the population was still viable and that individuals were still being recruited into the population. Neither eggs nor larvae were observed. It was possible that breeding had not yet taken place or that breeding had been accomplished and the hatched larvae had dispersed through the thick layers of leaves that covered the bottom of the flooded woods.

The Quarry locality that has never yielded pure *A. texanum* individuals was also virtually unchanged from our previous investigations and many individuals of *A. laterale* and hybrids were found under rocks close to the water's edge. This locality is an abandoned quarry with emergent vegetation surrounded by rocky outcropping at Sheridan Point. Again, the water level in the Quarry was noticeably lower but the Quarry pond is deep and maintains water all year so a slight reduction in the water level would not appear to be detrimental**.** Based on the numbers of salamanders observed in 2000 and the different sizes present, this population is dense and probably has remained unchanged.

The Stone Road locality is a flooded woodlot with a limestone base. Most of the specimens that were previously examined from this site were raised from eggs that were found early in the spring. Adults have never been found under debris close to the water and the breeding activity of adults has not been observed. The water varies in depth from 50 cm to about a meter. The submerged grasses and reeds are dense which renders observations of salamanders in the water difficult. In 2000, a large number of newly hatched larvae were observed among the vegetation in the water. There was no noticeable difference in this locality from our earlier studies.

The Pond is the most interesting of all the localities because it is the only locality that has been found to have both *A. laterale* and *A. texanum* as well as all genomic combinations of hybrids (Table 1). The water level has not changed from our previous investigations and remains constant all summer (Licht, personal observations). The pond is discrete, has a diameter of about 10 meters and is surrounded by small trees and bushes that overhang and enter the water. In the spring, salamanders are very common under the rocks that surround the pond.

The Girl Guide Pond and North End Woods sites were flooded woodland areas on the East side of Pelee Island adjacent to the perimeter road (East Shore Rd). The Girl Guide pond was located on the south side of the road that led from East Shore Rd. into the Township Campground that was used by the Girl Guides. The North End Woods site was a flooded area at the northeast end of the Island just south of Garno Rd. between East Shore Rd. and Clutton Rd. It is adjacent to the border of the Lighthouse Point Nature Reserve. Egg masses were collected from the Girl Guide Pond and larvae were netted at the North End Woods site during the spring of 1987. In March of 1989 there was no water at the same sites but adults were collected under wet logs. The four adult salamanders collected in 1989 from the Girl Guide site consisted of three female genomic hybrids (1 LT and 2 LTT) and one male *Ambystoma texanum*. Three adults were collected at the North End Woods site (1 LTT, 1 male and 1 female *Ambystoma texanum*). In 2000 the sites were dry and no salamanders were found.

Habitat trends — No breeding activity or specimens were observed in two of the five historical sites that were visited in the spring of 2000. The Girl Guide Pond and the North End Woods localities no longer exist and are dry areas. Previously, these two sites had the highest frequency of *Ambystoma texanum* (Table 1). No male *A. texanum* have ever been found at the Stone Road site. All larvae that transformed in the laboratory were determined to be female. That site was virtually unchanged in 2000 from our earlier investigations and still seems to have a very viable population as evidenced by the observation of numerous larvae. It would appear that the number of breeding sites and perhaps the range of *A. texanum* has been reduced.

Habitat protection — The most important population for *A. texanum* is in Mosquito Point Woods that is within the Provincial Park System's Fish Point Nature Reserve. The Stone Road site is within a tract of land that is owned by the Federation of Ontario Naturalists and the Essex Region Conservation Authority. The Pond site is on privately

owned land but the isolation of that population and the distance from both the road and from houses may provide some protection.

GENERAL BIOLOGY

The general biology of *Ambystoma texanum* was outlined in the 1991 Status Report but some of that information requires amending based on more recent observations and genetic data. It is now known that genomic hybrids maintain a cytoplasm that is unlike that found in the bisexual species (Hedges *et al*. 1992; Bogart 2003). Therefore, the genomic hybrids have some unknown female ancestor that is unlike any of the known bisexual species that mate with the genomic hybrids. We also know that sperm from sympatric males of the bisexual species can be incorporated in the eggs of genomic hybrids (Bogart *et al*. 1989) and that the eggs can also develop without sperm incorporation (gynogenesis) (Elinson *et al*. 1992).

Unusual sex ratios in Ambystoma texanum — It is evident (Table 1) that fewer male *Ambystoma texanum* were found in the populations than would be expected. The sex ratio of the bisexual species is expected to be about 1 : 1 and the Quarry *A. laterale* fit this expectation. But there is a deficiency of male *A. texanum* in all of the sites, with the possible exception of the Pond site where three of the five *A. texanum* were males. In some sites (especially the North End Woods), many of the nuclear genotypes were electrophoretically determined from larvae and juveniles whose sex was not known. But at the Stone Road and Girl Guide Pond sites many larvae were raised through metamorphosis to a size that the gonads could be used to identify males and females. The only male *A. texanum* found at the Girl Guide Pond was an adult collected in 1989 and 13 individuals that transformed from two egg masses were all females. No males of 51 *A. texanum* were found at the Stone Road site and there are fewer males than expected at Mosquito Point Woods. It is also curious that the female *A. texanum* were raised from discrete egg masses that were collected in 1987 from the Stone Road and Girl Guide Pond sites. *Ambystoma texanum* normally lays single eggs or small groups of eggs that are attached to leaves and sticks on the substrate of the pond. This pattern is observed in Mosquito Point Woods. Egg masses, laid higher in the water column, are not usually found on Pelee Island and are more reminiscent of eggs laid by *A. jeffersonianum* or nuclear hybrids that include an *A. jeffersonianum* genome. One possible explanation would be that some *A. texanum* on Pelee Island are “hybrids” and may be derived from genomic hybrid females. For example, if an LT female that normally mated with *A. laterale* produced reduced T eggs, a *laterale* genome could be incorporated to maintain an LT genomic constitution. If, however, the same female were to mate with *A. texanum* and incorporated that genome, the offspring would be TT (*A. texanum*) but such an individual would possess a very recognizable “hybrid” *mt*DNA sequence. So far, no *A. texanum* individuals have been found that have a hybrid *mt*DNA sequence. Another possibility is that some *A. texanum* on Pelee Island are normally gynogenetic.

Genetics — The two individuals of Pelee Island *Ambystoma texanum* that were sequenced and included in the study by Hedges *et al*. (1992) came from different populations on the Island. One (catalogue number 17572; Genbank Accession # 12751) was a female that was raised in the laboratory after being collected as a larva in the spring of 1989 from Mosquito Point Woods. The other (catalogue number 15640; Genbank Accession #12757) was an adult male that was collected on March 27th, 1989 from the Girl Guide Pond area. Five Pelee Island genomic hybrids that were also sequenced in that study aligned with mainland hybrids. Additional samples were sequenced using frozen tissues from specimens that were previously identified using isozyme electrophoresis. For comparative purposes, we used the same primers that amplified 307 bases of the cytochrome *b* gene by Hedges *et al*. (1992). In addition, we also used another set of primers that amplified 660 bases in order to see if additional phylogenetically informative sites existed that might improve resolution of possible relationships of *A. texanum* both on Pelee Island and between mainland populations and those found on Pelee. The sequence data confirm that the Mosquito Point *A. texanum* and the specimens from the eastern populations (Girl Guide pond and Stone Road) have separate common ancestors that are more closely related to mainland populations in Ohio and Indiana. These data suggest that the two areas on Pelee are isolated from each other and individuals in those areas were derived from invasions of the two haplotypes from the United States.

Movement and dispersal — Other than the possible isolation of the *mt*DNA haplotypes (above), we have no new information on the movement and dispersal of *Ambystoma texanum*. All collections of adults, eggs, larvae and newly transformed juveniles were made at the breeding sites. Non-breeding adults are subterranean and have not been encountered after the breeding season away from the vicinity of their presumed breeding sites. Based on the co-occurrence of *A. texanum* with LTT triploid and LTTT tetraploid nuclear hybrids, *A. texanum* may rarely disperse to the Quarry. The Pond locality is the only site that was found to contain both *A. texanum* and *A. laterale* and that site had an equal number of LLT and LTT nuclear hybrids. We have no information on the history of that site that might provide a time scale for immigration of both species. *Ambystoma texanum* may have migrated to the Stone Road, Girl Guide Pond, and the North End Woods sites from a more southern population or provide evidence for remnants of a wider distribution for that species in the past. The mitochondrial data show that individuals from those eastern sites probably are derived from independant mainland ancestors. It is expected that additional genetic data will shed some more light on these alternate hypotheses.

LIMITING FACTORS

Ambystoma texanum depends on ponds that maintain water through the larval stage that normally lasts from March through July. The low water level at Mosquito Point Woods could pose a problem if that area dried too soon and rainfall was not adequate to replenish the flooded woods. Although there are no longevity records for adult *A. texanum* in nature, captive specimens survive for at least 15 years (Bogart,

unpublished) so unusually dry years with little or no recruitment would not be expected to be a major factor in the possible extirpation of *A. texanum*. Competition with nuclear hybrids may also be a factor that reduces the density of *A. texanum* but these hybrids require male *A. texanum* or male *A. laterale* for their continued survival. Perhaps, *A. laterale* could replace *A. texanum* but that species is mostly restricted to the Sheridan Point area where the habitat is quite different. It is also more likely that *A. texanum* would displace *A. laterale* because *A. texanum* are larger, can produce more eggs than *A. laterale*, and have been found in more diverse habitats over a larger area of the Island. We have no new information with respect to possible predators or unusual weather conditions that might have had a detrimental effect on *A. texanum*.

Climatic conditions are considered to be the most important factor limiting the northern spread of this species. *Ambystoma texanum* reaches the northern limit of their extensive range in Michigan where they are considered endangered (Harding 1997) with a heritage rank status of S1 (critically imperiled). *Ambystoma texanum* is fairly tolerant of human environmental disturbances throughout its range but does require shallow fish-free ponds that hold water into mid-summer. Because Pelee Island is also close to the northern limit of its range the species is probably limited by climatic conditions on the Island but we have no information on this aspect of its biology.

THREATS

In the 1991 Status Report, we suggested that, because of its very limited distribution in Canada, *Ambystoma texanum* was vulnerable to environmental degradation, modified drainage patterns, and other habitat destruction that could seriously affect existing populations in such a small area. Development has proceeded in the northern and eastern regions of the island where the historical sites appear to have been lost. Cutting of trees and removal of rotting trunks is detrimental to the salamanders because a canopy is important to retard evaporation of breeding ponds and flooded areas, and rotting trunks provide habitat for invertebrates that serve as food for transformed salamanders. The recognized breeding sites on Pelee Island are vulnerable to low water levels on Lake Erie as well as any draining activities that might be proposed in the area. The southern sites, in protected areas, still appear suitable but the water level reduction is a concern. Salamanders do cross the roads when migrating to and from the breeding areas so, although very few cars presently are on Pelee Island during the salamanders' breeding season, traffic through those areas at night in March and April has potential for serious negative consequences.

SPECIAL SIGNIFICANCE OF THE SPECIES

Ambystoma texanum has been present on Pelee Island for a very long time and the Island salamanders are most likely descended from migrants that came to the Island when it was connected to the Ohio mainland. Pelee Island is the only Canadian locality for *Ambystoma texanum* and the preservation of this species is deemed to be important

to maintain and preserve the natural diversity of Canadian fauna. In addition to this worthy endeavor, the existence and diversity of genomic hybrids on Pelee Island depend on the continued existence of *A. texanum*. The very interesting and complex interaction of *A. texanum*, *A. laterale*, and genomic hybrids appear to be unique to Pelee Island and it is important to maintain and to study this assemblage of genetic forms that might represent a unique biological phenomenon.

TECHNICAL SUMMARY

Ambystoma texanum
Small-mouthed Salamander — Salamandre à nez court
Range of Occurrence in Canada: Ontario

Extent and Area Information	
• *Extent of occurrence (EO)(km²)* **[Area of Pelee Island as given in the report: 4,262ha]**	43 km²
• *Specify trend in EO*	Stable
• *Are there extreme fluctuations in EO?*	No
• *Area of occupancy (AO) (km²)* **[estimate 1 km² for each breeding site in consideration of dispersal abilities of the salamanders and available terrain]**	5 km²
• *Specify trend in AO*	Decline
• *Are there extreme fluctuations in AO?*	No
• *Number of known or inferred current locations*	3
• *Specify trend in #*	Decline
• *Are there extreme fluctuations in number of locations?*	No
• *Specify trend in area, extent or quality of habitat*	Decline
Population Information	
• *Generation time (average age of parents in the population)*	3 yrs
• *Number of mature individuals*	Unknown
• *Total population trend:*	Unknown
• *% decline over the last/next 10 years or 3 generations.*	
• *Are there extreme fluctuations in number of mature individuals?* **[other species of *Ambystoma* have fluctuating population sizes]**	Highly probable
• *Is the total population severely fragmented?*	No
• *Specify trend in number of populations*	
• *Are there extreme fluctuations in number of populations?*	No
• List populations with number of mature individuals in each: unknown	
Threats (actual or imminent threats to populations or habitats)	
- very limited distribution in Canada - environmental degradation - modified drainage patterns - other habitat destruction	
Rescue Effect (immigration from an outside source)	
• *Status of outside population(s)?* **USA: N5** Alabama (S3), Arkansas (S5), Illinois (S5), Indiana (S4), Iowa (S3), Kansas (S5), Kentucky (S5), Louisiana (S5), Michigan (S1), Mississippi (S5), Missouri (S?), Nebraska (S1), Ohio (S?), Oklahoma (S5), Tennessee (S5), Texas (S5), West Virginia (S1)	
• *Is immigration known or possible?*	No
• *Would immigrants be adapted to survive in Canada?*	Yes
• *Is there sufficient habitat for immigrants in Canada?*	Yes
• *Is rescue from outside populations likely?*	No

Quantitative Analysis	Not Applicable
Other Status **COSEWIC: Special Concern (1991)** Ontario: S1	

Status and Reasons for Designation

Status: Endangered	**Alpha-numeric code:** [B1ab(ii,iii,iv)+2ab(ii,iii,iv)]

Reasons for Designation:
This salamander is restricted solely to Pelee Island in Canada. The extent of occurrence is only 40 km^2 (effectively the total area of Pelee Island). It occupies only three extant breeding sites and surrounding remnant forested habitat with total area of occupancy equalling not more than 5 km^2. It has exhibited declines in area, extent and quality of habitat, and in the number of locations on the island where it may be found. Threats to its continued existence include loss of wetland breeding sites and modified drainage patterns.

Applicability of Criteria

Criterion A:
Insufficient information to calculate precise numbers of individuals.

Criterion B:
Qualifies for Endangered B1,2 a, bii,iii,iv. Area of Occupancy is less than 500 km2 and fewer than 5 populations currently exist. There has been decline in Area of Occupancy, extent of habitat and numbers of populations due to the loss of breeding sites at Girl Guide Pond and North End Woods. Furthermore, extreme fluctuations in numbers of mature individuals are well documented in other species of Ambystoma and considered highly probable in A. texanum.

Criterion C:
May qualify for Endangered C2b as it is estimated that fewer than 1,000 adults exist on Pelee Island, there has been decline in abundance inferable from losses of breeding sites, and population sizes probably fluctuate to a great extent. However, these cannot be determined with as much certainty as can the qualifications under Criterion B.

Criterion D:
Meets the criterion for Threatened D2 because Area of Occupancy is less than 20 km2 and number of locations <5.

Criterion E:
Insufficient information to calculate.

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James (Jim) Bogart obtained his PhD from the University of Texas in Austin in 1969. He is presently a professor in the Department of Zoology at the University of Guelph. He has taught a senior undergraduate course in herpetology for 20 years and his research is mostly concerned with amphibian genetics. He has numerous publications on the *Ambystoma* salamander complex and several of his students have also worked on the complex.

Lawrence (Larry) Licht obtained his PhD from the University of British Columbia in 1970. He is presently a professor in the Department of Biology at York University. He teaches a senior level course in herpetology at York and his research is mostly concerned with ecology. Seventeen of his publications deal with Pelee Island salamanders.

Both Drs. Bogart and Licht have engaged in a collaborative effort to understand the interactions of pure species and genomic hybrids on Pelee Island for twenty years.

Appendix 1. Salamander breeding localities on Pelee Island and the species, genotypes and life history stage detected to be present at each site.

Girl Guide Pond

Ambystoma texanum: females, males, juveniles, larvae.
Hybrids: *Ambystoma laterale* X *texanum* (LT diploids) females, *Ambystoma laterale* X *laterale* X *texanum* (LLT triploids) females. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females

Mosquito Point Woods

Ambystoma texanum: females, males, juveniles, triploid female (TTT)
Hybrids: *Ambystoma laterale* X *texanum* (LT diploids) females, juveniles. *Ambystoma laterale* X *laterale* X *texanum* (LLT triploids) females, males. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females, males. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females.

North End

Ambystoma texanum: females, males, larvae
Hybrids: *Ambystoma laterale* X *texanum* (LT diploids) females. *Ambystoma laterale* X *laterale* X *texanum* (LLT triploid) females. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploid) females.

Pond

Ambystoma laterale: females
Ambystoma texanum: females, males
Hybrids: *Ambystoma laterale* X *texanum* (LT diploids) females, males. *Ambystoma laterale* X *laterale* X *texanum* (LLT triploids) females, juveniles. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females, males, juveniles. *Ambystoma laterale* X *laterale* X *laterale* X *texanum* (LLLT tetraploids) females. *Ambystoma laterale* X *laterale* X *texanum* X *texanum* (LLTT tetraploids) females, juveniles. *Ambystoma laterale* X *texanum* X *texanum* X *texanum* (LTTT tetraploids) females.

Quarry

Ambystoma laterale: females, males, juveniles
Hybrids: *Ambystoma laterale* X *texanum* (LT diploids) females, juveniles. *Ambystoma laterale* X *laterale* X *texanum* (LLT triploids) females, males, juveniles. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females. *Ambystoma laterale* X *laterale* X *laterale* X *texanum* (LLLT tetraploids) females, males, juveniles. *Ambystoma laterale* X *laterale* X *texanum* X *texanum* (LLTT tetraploids) females, juveniles.

Stone Road

Ambystoma laterale:	females
Ambystoma texanum:	females, larvae, juveniles
Hybrids:	*Ambystoma laterale* X *texanum* (LT diploids) females. *Ambystoma laterale* X *laterale* X *texanum* (LLT triploids) females. *Ambystoma laterale* X *texanum* X *texanum* (LTT triploids) females, juveniles.