Herpetological Conservation and Biology 21(2):308–319.
Submitted: 8 October 2025; Accepted: 17 April 2026; Published: 31 August 2026.

Field Surveys Indicate Contiguous Distributions of Tiger Salamanders (*Ambystoma mavortium and A. tigrinum*) in Manitoba, Canada

***Neil R. Balchan*[1,3] *and Owen M. Edwards*[2]**

[1]*Department of Biology, 123 North Monroe Street, Oklahoma State University, Stillwater, Oklahoma 74078, USA*
[2]*Oklahoma Biological Survey, 111 East Chesapeake Street, University of Oklahoma, Norman, Oklahoma 73019, USA*
[3]*Corresponding author, e-mail: neil.balchan@okstate.edu*

Abstract.—**Two species of tiger salamanders occur in Manitoba, Canada: the Eastern Tiger Salamander (*Ambystoma tigrinum*; Endangered) and the Western Tiger Salamander (*A. mavortium*; Special Concern). The distributions of the two species presumably are separated by the Red River. A geographic gap of about 30 km separates the nearest locations of the two species, though recent evidence casts doubt on the existence of the gap. We conducted field surveys across southern Manitoba, focusing on the Red River Valley, to investigate the contemporary distributions and the possibility of contact between the two species. We surveyed ponds for larval and adult salamanders and used dip and seine-netting to verify presence and to collect voucher specimens and tissues. Occurrence data from our surveys, coupled with museum and citizen science occurrence records, revealed that the previously reported 30 km gap between the two species is inaccurate. We found that *A. tigrinum* and *A. mavortium* occur within just a few kilometers of each other across the Red River, indicating contemporary distributional contiguity. We found tiger salamanders within a variety of anthropogenic water bodies in clay-rich soils, challenging previous assumptions about their habitat specificity. Evidence of introgression from previous genetic studies, coupled with our geographic findings, suggests that gene flow may occur between the two species. Our results highlight the need for finer-scale genetic sampling to evaluate the extent of hybridization and serve as a first step in guiding conservation planning. We underscore the importance of peripheral populations in evolutionary and ecological research and identify a need for expanded survey and collections-based efforts in the study of Canadian herpetofauna.**

Key Words.—amphibians; Caudata; contact zone; Endangered; larvae; seine; Special Concern; Species at Risk

Introduction

The tiger salamander complex (*Ambystoma* spp.) comprises a group of large-bodied salamanders whose collective distribution spans North America, ranging from central Mexico to southern Canada (Everson et al. 2021). The complex has a complicated taxonomic history, with relationships among many species only recently clarified (Shaffer and McKnight 1996; O'Neill et al. 2013; Everson et al. 2021). Historically, one species, the Tiger Salamander (*Ambystoma tigrinum*), and two subspecies, the Eastern Tiger Salamander (*A. t. tigrinum*) and Gray Tiger Salamander (*A. t. diaboli*) were recognized in Manitoba, Canada (Preston 1982). The two subspecies reportedly occurred on opposite sides of the Red River of the North (hereafter, Red River; Powell et al. 2016). Subsequent systematic investigations revealed deep divergence between eastern and western lineages (Shaffer and McKnight 1996; Irschick and Shaffer 1997), resulting in the recognition of the Gray Tiger Salamander as a distinct species (*A. mavortium*), now referred to as the Western Tiger Salamander, and the common name of *A. tigrinum* as the Eastern Tiger Salamander (Everson et al. 2021).

With this change in our understanding of the systematics of the complex, new attention was focused on the distributions of the two species of tiger salamander in Canada (Fig. 1). Although the Western Tiger Salamander occurs from Manitoba to British Columbia, the Eastern Tiger Salamander has a more restricted range. One vouchered specimen (CMNAR 623) of unverified provenance suggests that *A. tigrinum* historically occurred at Point Pelee, Ontario, but the veracity of this specimen and the accuracy of its collection locality have been questioned (Ngo et al. 2009), and tiger salamanders are not known to occur in Ontario. The only extant populations of *A. tigrinum* in Canada occur in southeastern Manitoba, with voucher specimens (Fig. 2; Appendix) documenting its distribution in the area east of the Red River, extending from the

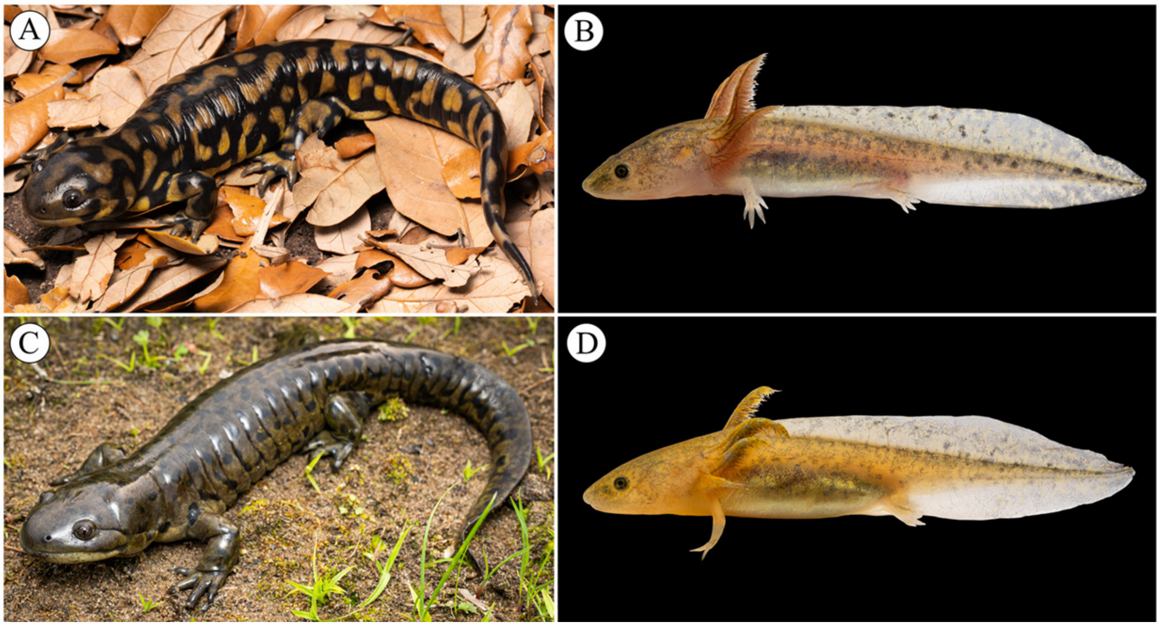

Figure 1. Profile images of adult and larval Eastern Tiger Salamander (*Ambystoma tigrinum;* A, B) and the Western Tiger Salamander (*Ambystoma mavortium;* C, D) from Manitoba, Canada. (Photographed by Owen M. Edwards).

Canada-USA border to as far north as Steinbach (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2013; Fig. 3). Field surveys have confirmed the presence of *A. tigrinum* at several breeding sites (COSEWIC 2013), and two areas of occurrence are known in the Rural Municipality of Stuartburn and the Sandilands Provincial Forest area (Toffelmier et al. 2022; Church 2025).

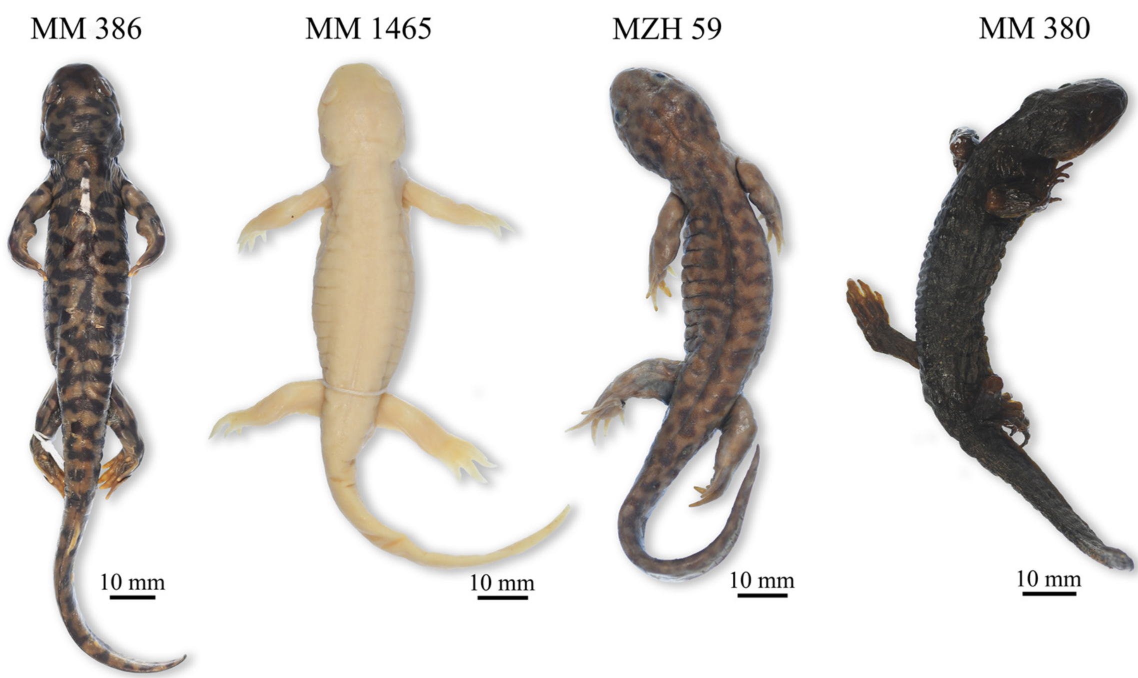

Figure 2. Example voucher specimens from Manitoba, Canada, of Western Tiger Salamander (*Ambystoma mavortium*: MM 386) collected near Erickson in 1970, and MM 1465 (albino specimen) collected near Spruce Woods Provincial Park in 2017, and Eastern Tiger Salamander (*Ambystoma tigrinum*: MZH 59) collected near Steinbach around 1969, and MM 380, collected near Emerson in 1972. (Photographed by Neil R. Balchan).

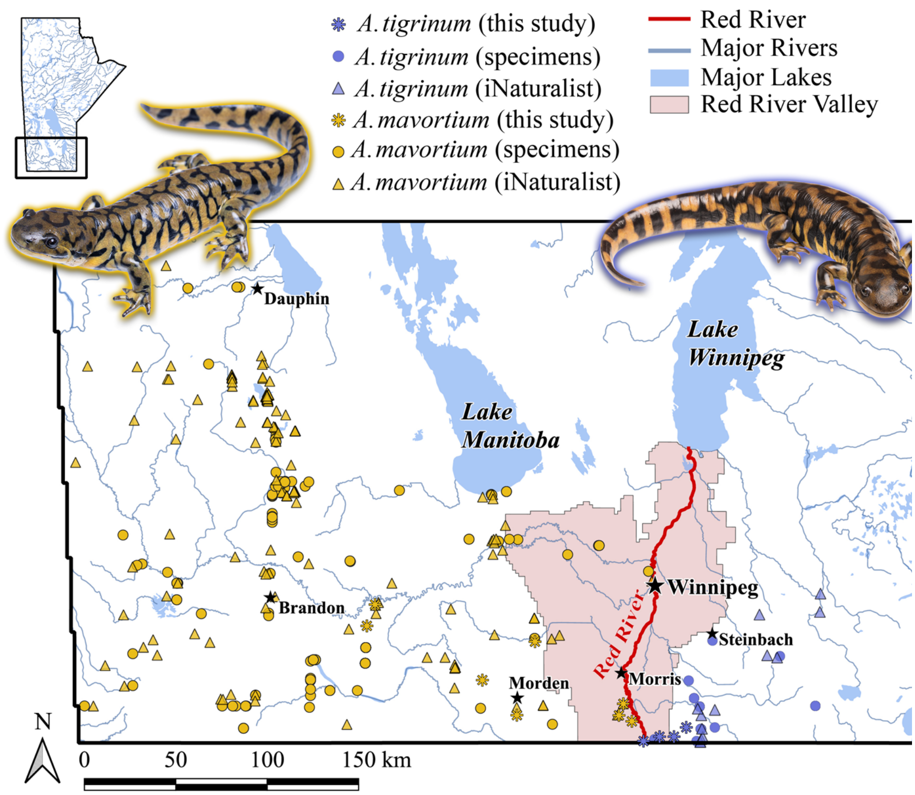

Figure 3. Map of the province of Manitoba, Canada (top left), with the southern region shown in greater detail (bottom right). Points represent known localities for the Western Tiger Salamander (*Ambystoma mavortium*; yellow) and Eastern Tiger Salamander (*Ambystoma tigrinum*; blue). Confirmed locations from this study are indicated with asterisks, voucher specimens with circles, and iNaturalist records with triangles. (Photographs of tiger salamanders by Owen M. Edwards).

Despite confirmed records of *A. tigrinum* in Manitoba, its perceived distribution reflects a significant contraction compared to what voucher specimens and regional biogeography suggest, and it is likely that *A. tigrinum* occupies a broader range in the province than is currently recognized. Although loss of contemporary distribution has been attributed to habitat degradation for some populations (i.e., presumed extirpation from Steinbach about 1969; COSEWIC 2013), recent surveys have not been conducted at many historic localities to verify the extirpation of those populations. Distributional contiguity between *A. tigrinum* and *A. mavortium* is expected, with the Red River posited as a barrier between the two species (Powell et al. 2016); however, the existence of a contemporary contact zone between the two species remains unknown. The assertion in COSEWIC (2013) of a 30 km gap between any records of *A. tigrinum* and *A. mavortium* in Manitoba overlooks a poorly preserved specimen of *A. tigrinum* (Manitoba Museum [MM] 380; Fig. 2) from Emerson, Manitoba (erroneously identified as *A. m. diaboli*) with a collection locality only about 5.3 km east of the Red River. Meanwhile, a specimen of *A. mavortium* (Canadian Museum of Nature Amphibian and Reptile Collection [CMNAR] 11782) collected about 1.1 km west of the river in Winnipeg shortens this gap to only about 3.1 km in distance, which is well within the reported dispersal distance of ≤ 16 km for an individual tiger salamander (Lannoo 1996). Considering this proximity, a straight-line distance of about 55.3 km still separates the nearest specimens of *A. mavortium* (CMNAR 14270) and *A. tigrinum* (MM 380) from one another. Despite the likely presence of tiger salamanders in this area devoid of voucher specimens, recent survey work by the Manitoba Conservation Data Center did not detect *A. tigrinum* further west than Tolstoi (Toffelmier et al. 2022), and targeted surveying for *A. mavortium* within the posited gap has not occurred. *Ambystoma tigrinum* is designated as Endangered (Prairie Population; COSEWIC 2013), and *A. mavortium* is designated as Special Concern (Prairie/Boreal Population; COSEWIC 2012) by the Committee on the Status of Endangered Wildlife in Canada, and both species are listed on the federal Species at Risk Act with these designations under Schedule 1 (https://laws-lois.justice.gc.ca/eng/acts/S-15.3).

Populations at distributional edges or extremes are especially deserving of study, as individuals from these peripheral regions often exhibit morphological (e.g., dispersal-related phenotypes at range edges; Edwards et al. 2025b) or genetic (e.g., variation in genetic diversity; Carvalho et al. 2019) differentiation. Additionally, populations occurring at range edges are most susceptible to the effects of range expansion or contraction (Carvalho et al. 2019), and active monitoring of these regions is important for early detection of distributional shifts. In addition, communities of herpetofauna in Canada may be under unique threats from species introductions, over-exploitation, climate change, UV-B radiation, chemical contaminants, and diseases that are exacerbated within their Canadian contexts (Lesbarrères et al. 2014). A lack of voucher specimens for tiger salamanders has inhibited precise distributional estimates for both species and Designatable Units in Manitoba (COSEWIC 2012, 2013) and hinders our ability to verify the continued persistence of populations of the Manitoban endemic *A. tigrinum*.

We conducted surveys for tiger salamanders in southern Manitoba, coupled with the collection of new voucher specimens, to assess whether contemporary contiguity exists between populations of *A. tigrinum* and *A. mavortium* in the province. In addition, we compiled existing museum and citizen science records to highlight areas that warrant future survey efforts. We discuss the ecology and habitats of both species, with particular attention to the contact zone in the Red River Valley and emphasize the need for future work to characterize gene flow and hybridization dynamics between the two species of tiger salamanders in Manitoba.

Materials and Methods

Study area.—Our study area was in south-central Manitoba, Canada, between 49.0° and 49.7° latitude (Fig. 3). We focused survey efforts on the Red River Valley (Fig. 3), a glacial lake plain characterized by thick beds of colloidal lacustrine clay deposits and gentle slopes flanking both sides of the river (Jacobson 1982; Prochnow et al. 1985). We focused our efforts in this area because the Red River represents the putative distributional boundary separating the two species of tiger salamander and few records of either species exist in the region (Fig. 3). The area experiences frequent severe flooding (Haque et al. 2023), and intensive agriculture has led to extensive habitat degradation and fragmentation, with 98% of the wetlands in the region converted to farmland (Sierra Club, unpubl. report). Most water bodies in the area are artificial, consisting primarily of dugout ponds and drainage ditches, while natural wetlands have become increasingly rare on the landscape.

Surveying procedures.—We conducted field surveys for tiger salamanders from 1 June to 14 June 2025. We selected this period for our surveys because we thought larvae would be present and well-developed and that ponds would still contain sufficient water to support abranchiate adults that linger in ponds after breeding. Our surveys were opportunistic rather than systematic, as we did not follow a minimum survey area threshold to report negative results for surveyed water bodies. The reporting of negative survey results for the related California Tiger Salamander (*A. californiense*) requires sampling of 30% of the pond area (U.S. Fish and Wildlife Service 2003), and because of varying depth, structural complexity, and sampling methods, we were unable to meet a similar percentage area threshold for ponds in which we did not find tiger salamanders. Consequently, we are unable to rule out false absences at some ponds. We note that a presence-only approach was sufficient for our purposes, as our objective was simply to confirm occurrence in potential areas of contact. We identified water bodies likely to contain larvae or aquatic adults with Google Maps, obtained permission from landowners, and surveyed these sites using a seine net (1.2 × 3.1 m) or dip nets.

At sites where we detected tiger salamanders, we recorded the type of water body (e.g., cattle dugout, ornamental pond, roadside ditch) and collected a series of voucher specimens with genomic tissues (Species at Risk/Wildlife Scientific Permit SAR24013; following protocols from Balchan et al. 2023, 2024). For all specimens, we measured snout-vent length (SVL) and tail length (TL) to the nearest 0.1 mm using calipers, and recorded body mass to the nearest 0.1 g using a digital scale. Species identifications were assigned based on morphology (adults) or geographic location relative to the Red River, with individuals west of the river identified as *A. mavortium* and those east of the river identified as *A. tigrinum* (adults and larvae).

Table 1. Water bodies from which Eastern Tiger Salamanders (*Ambystoma tigrinum*) and Western Tiger Salamanders (*Ambystoma mavortium*) were confirmed during our sampling efforts. Ponds are named after the nearest large landmark, and coordinates are abbreviated to a single decimal place to obscure exact collection sites. Abbreviations are RM = Rural Municipality and NRB = Neil R. Balchan Field Series. Superscripts following species names indicate if detections included larvae (L), adults (A), or both (L, A). All dates are in 2025.

Pond ID	Date	Species	Pond Type	Location	Approximate Coordinates	Voucher Series
Morden	1 June	*Ambystoma mavortium* L	Cattle dugout	RM of Stanley	49.1, -98.1	NRB 769-774
Letellier	7 June	*A. mavortium* L	Roadside ditch	RM of Montcalm	49.1, -97.3	NRB 791-793
Ridgeville	9 June	*A. tigrinum* L	Cattle dugout	RM of Emerson-Franklin	49.0, -97.0	NRB 796-801
Saint Joseph	10 June	*A. mavortium* L	Cattle dugout	RM of Montcalm	49.1, -97.4	NRB 802-807
Emerson	10 June	*A. tigrinum* L	Ornamental pond	RM of Emerson-Franklin	49.0, -97.2	NRB 808-813
Fredensthal	10 June	*A. tigrinum* L	Cattle dugout	RM of Emerson-Franklin	49.0, -97.1	NRB 814-819
Spruce Woods	11 June	*A. mavortium* L, A	Cattle dugout	RM of Glenboro-South Cypress	49.7, -99.2	NRB 820-825
Glenboro	12 June	*A. mavortium* A	Cattle dugout	RM of Glenboro-South Cypress	49.6, -99.2	NRB 830
Miller Environmental	12 June	*A. mavortium* L	Ornamental pond	RM of Montcalm	49.2, -97.3	NRB 833-838
Overstoneville	13 June	*A. tigrinum* L	Cattle dugout	RM of Emerson-Franklin	49.1, -96.9	NRB 843-848
Lizard Lake	14 June	*A. mavortium* L	Cattle dugout	RM of Thompson	49.3, -98.4	NRB 850-855
Carman	14 June	*A. mavortium* L	Cattle dugout	RM of Dufferin	49.5, -98.0	NRB 856-861

Occurrence data collection.—To estimate the distribution of tiger salamanders in Manitoba, we compiled locality data (vetted for accuracy by the authors) from vouchered specimens maintained in natural history collections and from citizen science records, with species identifications assigned based on morphology (adults) or geography (adults and larvae). We collected locality data from 237 voucher specimens (Appendix) at the following collections: Canadian Museum of Nature Amphibian and Reptile Collection (CMNAR), Manitoba Museum (MM), North Carolina State Museum (NCSM), Royal Ontario Museum (ROM), Smithsonian National Museum of Natural History (USNM), University of Manitoba Stewart Hay Herpetology Collection (MZH), and University of Texas at Austin Biodiversity Center (TNHC). We downloaded citizen science data from iNaturalist on 26 July 2025, which included all research-grade records from Manitoba of 13 *A. tigrinum*, 330 *A. mavortium*, and one record of *Ambystoma* sp. (iNaturalist observation 130294067) assignable to *A. mavortium* based on external appearance of the photographed animal. We plotted these samples on a map of Manitoba (Fig. 3) to visualize the geographic distributions of the two species in the province and highlight areas lacking records.

Results

Surveys.—We captured either larvae or abranchiate adult tiger salamanders in 12 ponds (Table 1). Both seine and dip netting techniques were effective in capturing salamanders, with the former being more effective in shallow ponds lacking large amounts of aquatic vegetation or debris, while the latter was more useful in deeper ponds with large amounts of vegetation or debris. We found tiger salamanders in several types of water bodies (Fig. 4), including cattle dugouts, roadside ditches, and ornamental ponds, but only if fish were absent or present in low densities (fewer than five individuals per net pull).

Geographic distributions.—We compiled a dataset of 601 occurrence points for tiger salamanders in Manitoba, encompassing both vouchered specimens in public natural history collections, citizen science records from iNaturalist, and our 12 confirmed survey localities (Fig. 3). Voucher specimens (circles, Fig. 3) approximated the distribution of both species accurately, showing a large area of occurrence for *A. mavortium* in southwest and southcentral Manitoba, west of the Red River. Vouchers for *A. mavortium* became less abundant with increasing proximity to the Red River, though one specimen (CMNAR 11782; collected in 1969) was collected near the west bank of the river in Winnipeg, and an absence of vouchers exists in the Red River Valley from approximately Winnipeg to the USA border. Vouchers for *A. tigrinum* were also sparse in the Red River Valley, with most specimens clustering in the Rural Municipality of Stuartburn and a few specimens collected east in the Sandilands Region. One voucher specimen of *A. tigrinum* (MM 380; collected in 1972) was collected near the east bank of the river in Emerson, Manitoba,

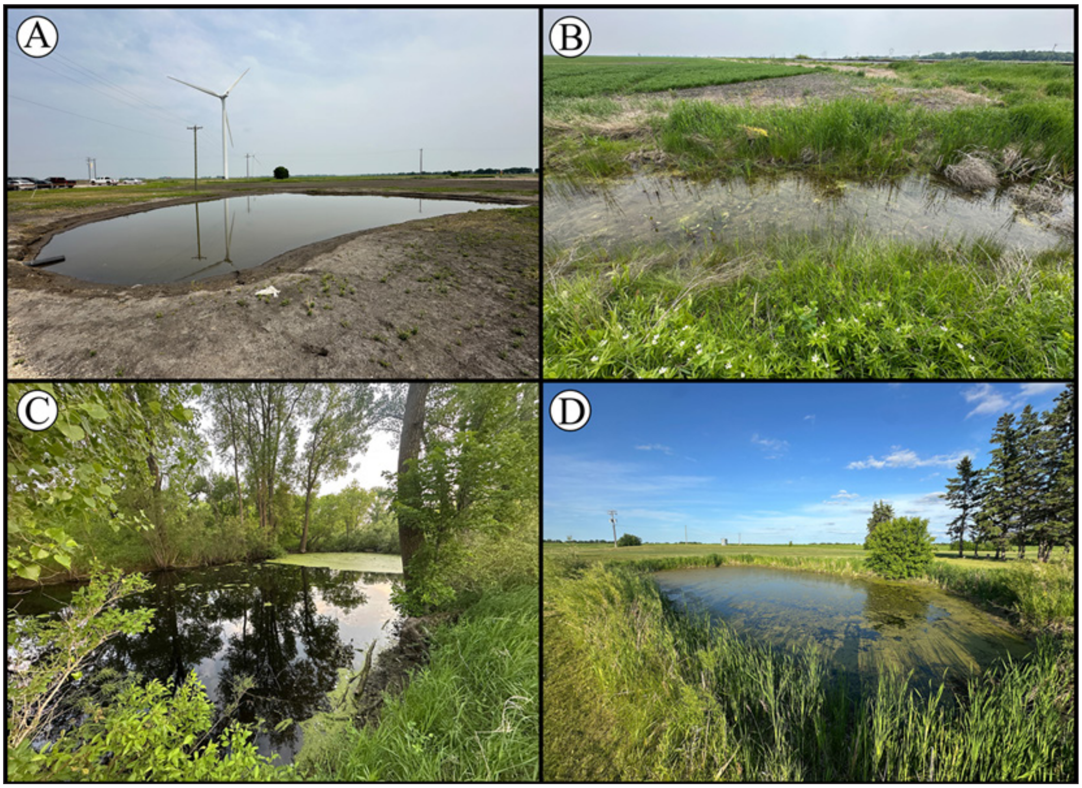

Figure 4. Examples of sites from which tiger salamanders were collected, illustrating the variation among water bodies, including (A) a recently excavated ornamental pond (Miller Environmental), (B) a roadside ditch bordering an agricultural field (Letellier), (C) a wooded cattle dugout (Fredensthal), and (D) an ornamental pond with dense aquatic vegetation surrounded by maintained lawn (Emerson). (Photographed by Owen M. Edwards).

but no vouchers exist north of this locality in the Red River Valley.

Citizen science data from iNaturalist (triangles; Fig. 3) generally mirrored the distributions estimated from voucher specimens, though these data comprised a greater number of overall observations. Citizen science observations showed the same approximate distribution for *A. mavortium*, but provided additional localities near the Red River, where two observations from St. Joseph and St. Jean Baptiste documented this species near the west bank of the river and one observation documented this species on the east bank of the river near Winnipeg. We did not find any citizen science records for *A. tigrinum* near the Red River but did see a large cluster of points in the Rural Municipality of Stuartburn and an extension of the distribution north to approximately Hadashville. Our surveys (asterisks, Fig. 3) revealed that tiger salamanders are present across the purported 30 km gap (COSEWIC 2013) extending over the Red River.

Discussion

Distributional contiguity.—Our surveys indicate that the two tiger salamander species in Manitoba have contemporary distributional contiguity, with extant populations (of each species) present on both banks of the Red River. These findings contradict the existence of a distributional gap between the two species (COSEWIC 2013) and suggest that populations exist in contemporary contact, although syntopic breeding has not been confirmed. The permeability of the Red River as a barrier remains untested, though the river is not known to constrain the distributions of other amphibian species in Manitoba (Preston 1982), and rivers are known to function as semi-permeable barriers for other terrestrial salamanders (Figueiredo-Vázquez et al. 2021). A citizen science record (https://www.inaturalist.org/observations/130294066) of a tiger salamander photographed near the east bank of the Red River (0.17 km distance from nearest riverbank) is identified as *A. mavortium* based on morphology, indicating the river may not serve as a complete barrier between the two species. We contacted the individual who submitted the record to iNaturalist, verifying the identity of the salamander (from additional photographs) and the location of observation, but failed to find larvae in any of the ponds near this site. Although the Red River likely serves as a barrier in some capacity, it is plausible that processes such as overland flooding (Walls et al. 2013) or meander cutoff (Jackson and Austin 2013) facilitate the movement of individuals or populations across this barrier. Human-mediated movement may also result from the transport of salamander larvae between sites for use as bait.

Although our findings verify the contiguity of species distributions, we suggest that habitat degradation potentially decreases the likelihood of contemporary connectivity among some populations. During surveys, we observed a broad area (about 11.5 km stretch) lacking larvae, from approximately Stockport to Dominion City, despite the presence of numerous suitable-looking breeding ponds. This region is predominantly cropland throughout, characterized by frequent tilling and heavy disturbance, and habitat here may be unsuitable for salamanders as a result (Baker et al. 2013; Bartelt et al. 2023). We identified an additional area lacking larvae between approximately Vita and Zhoda, where we did not detect larvae in any of the ponds during our two-week survey period. Unlike the Stockport-Dominion City gap, suitable habitat appeared to be present, and we expect that additional surveying should produce tiger salamanders in this area.

Habitat.—Both *A. mavortium* and *A. tigrinum* reportedly require terrestrial habitats with sandy or friable soils and semi-permanent or permanent bodies of water lacking predatory fish (COSEWIC 2012, 2013). Although these habitat requirements are largely upheld in many areas where tiger salamanders occur (e.g., Church 2025), populations in the Red River Valley appear capable of using habitats that do not strictly conform to these requirements. Soils in the valley are clay-rich vertisols that are not friable (Anderson 2010). The ponds we surveyed in this area exist in vertisol so called gumbo soils that are sticky when wet and have slow rates of water infiltration (Anderson 2010). Consequently, tiger salamanders in the Red River Valley are not restricted to loose, friable soils. Although capable of digging their own burrows (Semlitsch 1983), tiger salamanders also make use of small animal burrows to access subterranean shelter (Kolbe et al. 2002), which may be even more important on vertisol soils where the excavation of deep burrows may be difficult.

The second major habitat requirement, semi-permanent or permanent bodies of water lacking predatory fish (COSEWIC 2012, 2013), is upheld, and we found salamanders in various types of water bodies. We observed an overall lack of salamanders in water bodies containing large or abundant fish, which could potentially operate as population sinks (Furrer and Pasinelli 2016). Many water bodies that appeared otherwise suitable yielded no salamanders during our surveys but contained bullhead catfish (*Ameiurus* sp.), Yellow Perch (*Perca flavescens*), Fathead Minnows (*Pimephales promelas*), and Brook Sticklebacks (*Culaea inconstans*), often in high densities. We occasionally found co-occurrence between salamander larvae and small fish (Fathead Minnows or Brook Sticklebacks), but in these cases, fish existed in very low densities (fewer than five individuals per net pull). These observations suggest that fish populations at these sites may be ephemeral or periodically reduced by winterkill or intermittent drying, though more survey work is required to understand the relationship between fish and salamander presence.

We also emphasize the importance of disturbance and human-made water bodies in areas where suitable ponds may be lacking. One of our positive survey ponds (Miller Environmental) was notable for its recent anthropogenic origin, having been excavated just 9 mo before our July 2025 surveys, in October 2024. Despite the disturbed appearance of this site, we noted high abundances of *Daphnia* in the water column and verified the presence of many larval tiger salamanders. Recently dug ponds like this may represent an ideal spawning location for tiger salamanders as they provide an environment with little to no competition or predation, and they should not be overlooked during surveys due to their fresh or disturbed appearance.

Natural history.—The natural history of tiger salamanders is incredibly flexible among populations and landscapes (Everson et al. 2021), and here we provide a summary of natural history observations gleaned during our fieldwork in Manitoba. Abranchiate adult *A. mavortium* were common to find during surveys, and we captured them in three of our eight ponds, though we suspect they were also present in others. One of us (NRB) has found abranchiate adults in ponds during the summer months (e.g., iNaturalist observation 6621402; https://www.inaturalist.org/observations/6621402; eight adults in a single pond on 11 June 2017), and this is likely a

Figure 5. Adult Western Tiger Salamanders (*Ambystoma mavortium*) being preyed on by (A) a Great Blue Heron (*Ardea herodias*) on 3 May 2021 and (B) Black-crowned Night Heron (*Nycticorax nycticorax*) on 12 June 2023 in wetlands in southwest Manitoba. (Photograph A by Walter Potrebka and B by Tami Malone: used with permission).

common occurrence in Manitoba. Although most spawning adults return to terrestrial habitats following breeding, adults remaining in aquatic habitats likely incur benefits in doing so (Welsh 2015). The aquatic adults that we temporarily held captive during our surveys were distended with food and excreted large amounts of pond snail shell fragments (Gastropoda: Lymnaeidae). Adult salamanders likely remain in aquatic habitats in Manitoba to capitalize on abundant pond snails and other aquatic invertebrates as food sources (Fig. 5), though they may be at increased risk of avian predation in these environments (Seiders and Watermolen 2022). The authors did not find any adult *A. tigrinum* in ponds during surveys, and NRB has never encountered an aquatic adult during approximately 8 y of prior fieldwork in Manitoba.

Implications for gene flow and introgression.—The existence of a contact zone between the two tiger salamander species has major ecological and evolutionary implications, as this area may facilitate genetic exchange. Hybridization is well characterized among amphibians and can run a continuum from one-off events among distant taxa (Edwards et al. 2025a) to stable zones of introgression between closely related species (Smith et al. 2013). In rare cases, hybridization may result in the generation of new species via polyploidy (Schmid et al. 2015). Within the tiger salamander complex, hybridization is well characterized in California between the endemic California Tiger Salamander (*Ambystoma californiense*) and the introduced Western Tiger Salamander (*A. mavortium*; Riley et al. 2003; Ryan et al. 2009), as well as in naturally occurring zones of kleptogenesis involving several *Ambystoma* species in the Great Lakes region (Bogart 2019). Consequently, characterizing the presence and implications of a contact zone between the two tiger salamander species in Manitoba is vital for conservation efforts.

Major questions remain regarding population genetics of the two tiger salamander species in Manitoba, and while our study does not incorporate genetic analyses, we briefly discuss the potential genetic implications of a contact zone. Introgression among tiger salamander lineages is well characterized across broad spatial axes (Everson et al. 2021), yet little data exists to characterize fine-scale gene flow at species boundaries (Pierce and Mitton 1980; Jones and Collins 1992). Toffelmier et al. (2022) detected considerable genomic introgression from *A. mavortium* in the westernmost sampled population of Manitoba *A. tigrinum* (near Tolstoi) and some ancestry from *A. tigrinum* in western Manitoba *A. mavortium*. The potential contact zone between these two taxa in Manitoba, coupled with the knowledge of existing introgression, suggests that contemporary gene flow may still be occurring between tiger salamanders, which raises additional questions and concerns for conservation of *A. tigrinum*. A more detailed analysis of population genetics with fine-scale sampling is needed to better understand the genetic makeup of tiger salamander populations in Manitoba, with particular attention to the contact zone in the Red River Valley.

Conclusions.—We continue to expand on the known distribution of the Endangered Eastern Tiger Salamander, suggesting that the species may have a larger distribution than previously appreciated in Manitoba. Although the Special Concern Western Tiger Salamander is better known in the province, new insights continue to arise regarding its presence and distribution at its eastern range limits in the Red River Valley. Most importantly, the presence of a contemporary contact zone raises new questions regarding the genetic composition of tiger salamander populations and presents important implications for their conservation. While additional survey work is vital in understanding the spatial elements of tiger salamander distributions, a genomic appraisal of tiger salamander population structure is vitally needed to clarify the affinities of populations in the Red River Valley and throughout Manitoba more broadly. Future work attempting this should consider a careful sampling of individuals along the longitudinal transect of the two species in the province, with thorough sampling from the contact zone.

Acknowledgments.—We are grateful for the assistance of Brett Siatecki and Taylor Toffan during surveys and acknowledge the kindness of Neelam Balchan and Ravi Balchan for feeding and housing the authors during this fieldwork. We thank Gabrielle Rowland (Miller Environmental Corporation), Laurie Braun, Lisa Tretiak (Prairie Wildlife Rehabilitation Centre), and Lorraine Mabon (St. Léon Interpretive Center) for providing access to sampling sites and animals, Margaret Docker (University of Manitoba Stewart Hay Herpetology Collection), Randall Mooi (Manitoba Museum), and Marie-Hélène Hubert (Canadian Museum of Nature) for sharing voucher specimen data and facilitating collections visits, and Walter Potrebka and Tami Malone for granting permission to use their photos. Nick Cairns and Erin Toffelmier provided comments that improved the quality of this manuscript. This research was conducted under approved Institutional Animal Care and Use Committee protocols from Oklahoma State University (AUP #22-33) and University of Oklahoma (AUP #2024-0302), and specimen collection occurred under permit from Manitoba Sustainable Development and Indigenous Futures (Species at Risk/Wildlife Scientific Permit SAR24013). NRB was supported by the Natural Sciences and Engineering Research Council of Canada (PGS-D fellowship, award number 587615) and OME by the Oklahoma Biological Survey during this research.

Literature Cited

Anderson, D. 2010. Vertisolic soils of the prairie region. Prairie Soils and Crops Journal 3:29–36.

Baker, N.J., B.A. Bancroft, and T.S. Garcia. 2013. A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. Science of the Total Environment 449:150–156.

Balchan, N.R., O.M. Edwards, K.M. Banks, and J.J. Whitlock. 2024. 206 new county records for amphibians and reptiles in Oklahoma, USA. Herpetological Review 55:374–390.

Balchan, N.R., J.J. Whitlock, O.M. Edwards, and K.M. Banks. 2023. 46 new county records of reptiles and amphibians in Oklahoma, USA. Herpetological Review 54:77–81.

Bartelt, P.E., A.T. DeVries, and R.W. Klaver. 2023. Response of Tiger Salamanders (*Ambystoma t. tigrinum*) to wetland restoration in a midwestern agricultural landscape, U.S.A. Ichthyology & Herpetology 111:571–583.

Bogart, J.P. 2019. Unisexual salamanders in the genus *Ambystoma*. Herpetologica 75:259–267.

Carvalho, S.B., J. Torres, P. Tarroso, and G. Velo-Antoń. 2019. Genes on the edge: a framework to detect genetic diversity imperiled by climate change. Global Change Biology 25:4034–4047.

Church, C. 2025. Tigers on the prowl: determining the distribution and habitat requirements of Eastern Tiger Salamander (*Ambystoma tigrinum*) in Manitoba. M.Sc. Thesis, University of Winnipeg, Winnipeg, Canada. 93 p.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2012. COSEWIC assessment and status report on the Western Tiger Salamander *Ambystoma mavortium* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada. 63 p.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2013. COSEWIC assessment and status report on the Eastern Tiger Salamander *Ambystoma tigrinum* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada. 53 p.

Edwards, O.M., N.R. Balchan, K.M. Banks, B. Zhang, F.A. Machado, M.S. Reichert, and D. Esquerré. 2025a. Genomic and morphological evidence support contemporary three-way interspecific hybridization in ranid frogs. Ecology and Evolution 15:e72035. https://doi.org/10.1002/

ece3.72035.

Edwards, O.M., L. Zhai, M.S. Reichert, C.A. Shaughnessy, L. Ozment, and B. Zhang. 2025b. Physiological and morphological traits affect contemporary range expansion and implications for species distribution modelling in an amphibian species. Journal of Animal Ecology 94:195–209.

Everson, K.M., L.N. Gray, A.G. Jones, N.M. Lawrence, M.E. Foley, K.L. Sovacool, J.D. Kratovil, S. Hotaling, P.M. Hime, A. Storfer, et al. 2021. Geography is more important than life history in the recent diversification of the tiger salamander complex. Proceedings of the National Academy of Sciences 118:e2014719118. https://doi.org/10.1073/pnas.2014719118.

Figueiredo-Vázquez, C., A. Lourenço, and G. Velo-Antón. 2021. Riverine barriers to gene flow in a salamander with both aquatic and terrestrial reproduction. Evolutionary Ecology 35:483–511.

Furrer, R.D., and G. Pasinelli. 2016. Empirical evidence for source-sink populations: a review on occurrence, assessments and implications. Biological Reviews 91:782–795.

Haque, C.E., J.R. Zaman, and D. Walker. 2023. Risk-reduction, coping, and adaptation to flood hazards in Manitoba, Canada: evidence from communities in the Red River Valley. Geosciences 13:88. https://doi.org/10.3390/geosciences13030088.

Irschick, D.J., and H.B. Shaffer. 1997. The polytypic species revisited: morphological differentiation among Tiger Salamanders (*Ambystoma tigrinum*) (Amphibia: Caudata). Herpetologica 53:30–49.

Jackson, N.D., and C.C. Austin. 2013. Testing the role of meander cutoff in promoting gene flow across a riverine barrier in Ground Skinks (*Scincella lateralis*). PLoS ONE 8: e62812. https://doi.org/10.1371/journal.pone.0062812.

Jacobson, M.N. 1982. Soil survey of Clay County, Minnesota. U.S. Soil Conservation Service, Washington, D.C., USA.

Jones, T.R., and J.P. Collins. 1992. Analysis of a hybrid zone between subspecies of the Tiger Salamander (*Ambystoma tigrinum*) in central New Mexico, USA. Journal of Evolutionary Biology 5:375–402.

Kolbe, J.J., B.E. Smith, and D.M. Browning. 2002. Burrow use by Tiger Salamanders (*Ambystoma tigrinum*) at a Black-tailed Prairie Dog (*Cynomys ludovicianus*) town in southwestern South Dakota. Herpetological Review 33:95–99.

Lannoo, M.J. 1996. Okoboji Wetlands: A Lesson in Natural History. University of Iowa Press, Iowa City, Iowa, USA.

Lesbarrères, D., S.L. Ashpole, C.A. Bishop, G. Blouin-Demers, R.J. Brooks, P. Echaubard, P. Govindarajulu, D.M. Green, S.J. Hecnar, T. Herman, et al. 2014. Conservation of herpetofauna in northern landscapes: threats and challenges from a Canadian perspective. Biological Conservation 170:48–55.

Ngo, A., V.L. McKay, and R.W. Murphy. 2009. Recovery strategy for Tiger Salamander (*Ambystoma tigrinum*) (Great Lakes Population) in Canada [Proposed]. Species at Risk Act Recovery Strategy Series. Parks Canada Agency. Ottawa, Ontario, Canada. 28 p. + Appendix.

O'Neill, E.M., R. Schwartz, C.T. Bullock, J.S. Williams, H.B. Shaffer, X. Aguilar-Miguel, G. Parra-Olea, and D.W. Weisrock. 2013. Parallel tagged amplicon sequencing reveals major lineages and phylogenetic structure in the North American Tiger Salamander (*Ambystoma tigrinum*) species complex. Molecular Ecology 22:111–129.

Pierce, B.A., and J.B. Mitton. 1980. Patterns of allozyme variation in *Ambystoma tigrinum mavortium* and *A. t. nebulosum*. Copeia 1980:594–605.

Powell, R., R. Conant, and J.T. Collins. 2016. Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America. 4th Edition. Houghton Mifflin Harcourt Publishing, New York, New York, USA.

Preston, W.B. 1982. The amphibians and reptiles of Manitoba. Manitoba Museum of Man and Nature, Winnipeg, Manitoba, Canada. 128 p.

Prochnow, N.D., N.J. Lunde, W.J. Terry, and D.P. Opdahl. 1985. Soil survey of Cass County area, North Dakota. U.S. Soil Conservation Service, Washington, D.C., USA. 199 p.

Riley, S.P.D., H.B. Shaffer, S.R. Voss, and B.M. Fitzpatrick. 2003. Hybridization between a rare, native tiger salamander (*Ambystoma californiense*) and its introduced congener. Ecological Applications 13:1263–1275.

Ryan, M.E., J.R. Johnson, and B.M. Fitzpatrick. 2009. Invasive hybrid tiger salamander genotypes impact native amphibians. Proceedings of the National Academy of Sciences 106:11166–11171.

Schmid, M., B.J. Evans, and J.P. Bogart. 2015. Polyploidy in amphibia. Cytogenetic and Genome Research 145:315–330.

Seiders, J., and D.J. Watermolen. 2022. Ring-billed Gull predation on Eastern Tiger Salamanders. Bulletin of the Chicago Herpetological Society

57:65–67.

Semlitsch, R.D. 1983. Burrowing ability and behavior of salamanders of the genus *Ambystoma*. Canadian Journal of Zoology 61:616–620.

Shaffer, H.B., and M.L. McKnight. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the Tiger Salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. Evolution 50:417–433.

Smith, K.L., J.M. Hale, M.R. Kearney, J.J. Austin, and J. Melville. 2013. Molecular patterns of introgression in a classic hybrid zone between the Australian tree frogs, *Litoria ewingii* and *L. paraewingi*: evidence of a tension zone. Molecular Ecology 22:1869–1883.

Toffelmier, E., H.B. Shaffer, and P. Des Brisay. 2022. Genetic analysis and species determination of Tiger Salamanders in Manitoba, Canada. Canadian Wildlife Service, Winnipeg, Manitoba, Canada. 14 p.

U.S. Fish and Wildlife Service (USFWS). 2003. Interim guidance on site assessment and field surveys for determining presence or a negative finding of the California Tiger Salamander. U.S. Fish and Wildlife Service, Sacramento, California, USA. 6 p.

Walls, S., W. Barichivich, and M. Brown. 2013. Drought, deluge and declines: the impact of precipitation extremes on amphibians in a changing climate. Biology 2:399–418.

Welsh, K.J. 2015. Occupancy, abundance, and summer ecology of the Western Tiger Salamander (*Ambystoma mavortium* Baird) in the Beaver Hills, Alberta. M.Sc. Thesis, University of Alberta, Edmonton, Canada. 94 p.

Neil R. Balchan (left) is a Ph.D. Candidate in the Department of Biology at Oklahoma State University in Stillwater, Oklahoma, USA, where he currently works on evolution and venomics of the Asian cobras (*Naja* spp.). He received his B.Sc. from the University of Manitoba, Winnipeg, Canada, where he studied Common Garter Snake (*Thamnophis sirtalis*) phenology, and his M.S. from the University of Northern Colorado, Greeley, Colorado, USA, where his thesis work focused on Prairie Rattlesnake (*Crotalus viridis*) and Western Massasauga Rattlesnake (*Sistrurus tergeminus*) venom resistance in Great Plains rodents. His primary research interests are on the evolution and ecology of snakes and snake venoms, but he is broadly interested in all aspects of herpetology, field biology, and natural history.

Owen M. Edwards (right) is a Heritage Zoologist for the Oklahoma Biological Survey at the University of Oklahoma, Norman, Oklahoma, USA, where he currently studies herpetofauna throughout Oklahoma. He received his B.S. in Biology from Eckerd College, St. Petersburg, Florida, USA, where he studied the Gopher Tortoise (*Gopherus polyphemus*), and a M.S. in Integrative Biology from Oklahoma State University, Stillwater, Oklahoma, USA, where he studied the Green Treefrog (*Hyla cinerea*) and Crawfish Frog (*Rana areolata*). Owen is particularly interested in climate-induced range expansions and the factors that set the geographic distributions of amphibians. This photograph was taken at the St. Léon Interpretive Center in St. Léon, Manitoba. The town of St. Léon takes great pride in holding the Gray Tiger Salamander (*Ambystoma mavortium diaboli*) as its local mascot, and the area is famous for mass migrations of salamanders making their way to and from the prairie pothole wetlands near the town. (Photographed by Owen M. Edwards).

APPENDIX

List of referred voucher specimens:

Ambystoma mavortium: CMNAR 1008, 1009, 1145, 11769, 11771, 11772, 11782, 14063, 14270, 15525, 2025, 27, 30072, 32893, 34861, 4509, 4510, 4522, 4523, 4537, 4541, 4574, 4589, 4590, 4597, 4602, 4603, 4617, 4624, 4625, 4634, 4636, 4643, 4657, 4677, 4680, 4688, 4692, 4693, 4696, 4698, 4710, 4711, 4712, 4714, 4717, 4718, 4722, 4729, 4737, 4740, 4741, 4743, 4744, 4746, 4747, 4748, 4749, 4750, 4751, 4752, 4755, 4757, 4758, 4761, 4762, 4763, 4764, 4765, 4766, 4776, 4777, 4779, 4904, 5324, 5331, 5335, 5338, 5685, 5686, 5687, 5688, 5689, 5690, 5691, 5692, 5693, 5694, 5695, 5696, 5835, 5871, 5872, 5873, 6051, 6536, 6632, 894, 9521, 9526, MM 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 376, 377, 379, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 818, 821, 822, 1464, 1465 MZH 32, 35, 36, 37, 38, 45, 46, 50, NCSM 83296, ROM 18725, 18726, 18727, 18728, 18729, 18730, 18731, 18732, 18733, 18734, 18735, 18736, 18737, 18738, 18739, 18740, 18741, 18742, 18743, 18744, 18745, 18746, 18747, 18748, 18749, 18750, 18751, 18752, 18753, 18754, 4044, 4045, 4046, 4047, 4048, 5157, 5158, 5159, 5160, 5161, 5162, 5163, 5164, 5165, 5166, 5167, 5168, 5169, 5170, 5171, 5172, 5173, 5174, 5175, 5176, 5177, 5178, 5179, 5180, 5181, 5182, 5183, 5184, 5185, 5186, TNHC 26898, USNM 35512, 35513, 35514

Ambystoma tigrinum: CMNAR 12182, 12198, 30016, 30975, 30977, 30981, MM 375, 380, 1464, 1513, 1514, 1515, 1516, MZH 59, ROM 17033, 17034, 17035, 17036, 17037, 17038, 17039, 17040, 17041, 17042, 17043, 17044

Abbreviations: CMNAR = Canadian Museum of Nature Amphibian and Reptile Collection, MM = Manitoba Museum, MZH = University of Manitoba Stewart Hay Herpetology Collection, NCSM = North Carolina State Museum, ROM = Royal Ontario Museum, TNHC = University of Texas at Austin Biodiversity Center, USNM = Smithsonian National Museum of Natural History