

Estimating critical habitat based on year-round movements of the endangered Jefferson Salamander (*Ambystoma jeffersonianum*) and their unisexual dependents

S.G. Van Drunen, J.E. Linton, J.P. Bogart, J. McCarter, H. Fotherby, A. Sandilands, and D.R. Norris

Abstract: Habitat protection is a key component of endangered species conservation, but critical habitat designations are often based on limited data or habitat use during only a portion of a species' life cycle. Protected habitat around breeding pools for the endangered Jefferson Salamander (*Ambystoma jeffersonianum* (Green, 1827)) and their unisexual dependents (*Ambystoma laterale* – (2) *jeffersonianum*) is based upon limited movement data from primarily spring and summer seasons. Furthermore, despite their treatment as distinct species under Canada's *Species at Risk Act*, differences in habitat use have not been directly compared in areas where they co-occur. We used radiotelemetry to track *A. jeffersonianum* (JJ) and *A. laterale* – *jeffersonianum* (LJJ and LJJJ) during fall migrations to overwintering sites. We used these data and summarized available movement data from past studies that tracked movements in other periods of the annual cycle to estimate year-round critical habitat. *Ambystoma jeffersonianum* travelled significantly longer distances to overwintering locations than unisexuals. Individuals were more likely to overwinter next to a similar genotype individual than not. Critical habitat encompassing the entire annual cycle of *A. jeffersonianum* extends up to 400–450 m from breeding ponds indicating existing regulatory habitat protections in Canada do not currently protect sufficient habitat.

Key words: overwintering habitat, *Ambystoma laterale* – *jeffersonianum* unisexuals, *Ambystoma* salamander complex, spatial segregation, radiotelemetry.

Résumé : Si la protection des habitats est un élément clé de la conservation des espèces menacées, la désignation d'habitats essentiels repose souvent sur des données limitées ou l'utilisation d'habitats durant une partie seulement du cycle biologique de l'espèce. Les habitats protégés autour d'étangs de reproduction de salamandres de Jefferson (*Ambystoma jeffersonianum* (Green, 1827)) et de leurs dépendants unisexués (*Ambystoma laterale* – (2) *jeffersonianum*) sont basés sur des données limitées sur leurs déplacements principalement pour le printemps et l'été. En outre, malgré le fait qu'elles soient traitées comme des espèces distinctes en vertu de la *Loi sur les espèces en péril* du Canada, les différences d'utilisation de l'habitat des deux espèces dans des lieux où elles coexistent n'ont pas été examinées. Nous avons utilisé la radiotéléométrie pour suivre des *A. jeffersonianum* (JJ) et des *A. laterale* – *jeffersonianum* (LJJ et LJJJ) durant des migrations automnales vers des sites d'hivernage. Nous utilisons ces données et un condensé des données sur les déplacements disponibles tirées d'études antérieures qui ont suivi les déplacements pendant d'autres périodes du cycle annuel, afin d'estimer les habitats essentiels à l'échelle annuelle. Les *A. jeffersonianum* parcourent des distances significativement plus longues vers les lieux d'hivernage que les ambystoma unisexués. Les individus sont plus susceptibles d'hiverner à côté d'un individu de génotype semblable que de génotype différent. L'habitat essentiel qui couvre tout le cycle annuel d'*A. jeffersonianum* s'étend sur des distances de jusqu'à 400–450 m des étangs de reproduction, ce qui indique que les mesures de protection réglementaires actuellement en place au Canada ne protègent pas des habitats suffisants. [Traduit par la Rédaction]

Mots-clés : habitat d'hivernage, *Ambystoma laterale* – *jeffersonianum* unisexués, complexe des salamandres *Ambystoma*, ségrégation spatiale, radiotéléométrie.

Introduction

Habitat disturbance and destruction are dominant drivers of species endangerment across the globe (Dirzo and Raven 2003; WWF 2016), making habitat protection a key component of recovery for species at risk (Taylor et al. 2005). Effective regulatory tools designed to protect species-at-risk habitat require identification of habitat essential to species survival and long-term persistence (Camaclang et al. 2015). Current species-at-risk legislation, such as

the United States' *Endangered Species Act* and Canada's *Species at Risk Act* (SARA), incorporate habitat protections that require identification of what is termed "critical habitat". Critical habitat can be practically defined as the minimum area required to ensure a species' continued persistence in the wild (Camaclang et al. 2015) and protection of these areas is a key component towards conservation of species at risk.

Despite the importance of habitat protection, many species at risk lack clear boundaries that define their critical habitat

Received 30 September 2019. Accepted 28 October 2019.

S.G. Van Drunen and J.P. Bogart. Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada.

J.E. Linton, J. McCarter, and H. Fotherby. Natural Resource Solutions Inc., 415 Phillip Street, Unit E, Waterloo, ON N2L 3X2, Canada.

A. Sandilands. Gray Owl Environmental Inc., 1356 Lockie Road, Branchton, ON N0B 1L0, Canada.

D.R. Norris. Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada; Nature Conservancy of Canada, 245 Eglinton Avenue East, Suite 410, Toronto, ON M4P 3J1, Canada.

Corresponding author: S.G. Van Drunen (email: s.vandrunen@gmail.com).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from [RightsLink](https://www.rightslink.com).

(Camaclang et al. 2015; Bird and Hodges 2017; Walls et al. 2017). For example, in Canada the dominant threat to species at risk is anthropogenic habitat destruction and degradation (McCune et al. 2013), but >60% of recovery strategies developed under SARA do not designate, and therefore protect, critical habitat (Bird and Hodges 2017). Inadequate identification of critical habitat is largely attributed to insufficient data (Camaclang et al. 2015; Bird and Hodges 2017). Even those species with existing critical habitat designations may not be adequately protected because of data limitations in the species information used to define critical areas (Camaclang et al. 2015; Bird and Hodges 2017; Lefebvre et al. 2018).

Pond-breeding amphibians are of special concern and exemplify of conservation issues stemming from data deficiencies. Greater than 40% of all amphibian species globally are listed as threatened (Pimm et al. 2014), but endangered amphibians often receive less conservation attention than other vertebrate species such as birds or mammals (Stuart 2012; Walls et al. 2017). In the US, approximately 80% of endangered amphibians are not listed under their *Endangered Species Act* (ESA) and can face multi-year delays in their protection by endangered species legislation. These delays are often attributed to a lack of basic species knowledge of demographic data and critical habitat (McCune et al. 2013; Lesbarrères et al. 2014; Camaclang et al. 2015; Bird and Hodges 2017; Walls et al. 2017). In this study, we fill knowledge gaps relating to some *Ambystoma* salamanders and provide an improved method for estimating pond-breeding amphibian critical habitat area.

Assessed as endangered at provincial (COSSARO 2011, 2016) and federal (COSEWIC 2010, 2016) levels in Canada, Jefferson Salamanders (*Ambystoma jeffersonianum* (Green, 1827)) and their unisexual dependents (*Ambystoma laterale* – (2) *jeffersonianum*) are examples of species with critical habitat designations based upon limited data. Bisexual *A. jeffersonianum* consist of diploid male and female individuals (i.e., denoted as JJ), whereas Jefferson-dependent unisexuals possess one Blue-spotted Salamander (*Ambystoma laterale* Hallowell, 1856) chromosome complement and up to four *A. jeffersonianum* chromosome complements. Unisexuals are predominately female, reproduce via kleptogenesis (Bogart et al. 2007), and are usually triploids (LJJ), though individuals can range anywhere from diploid (LJ) to pentaploid (LJJJJ) (Bogart 2003; Bogart and Klemens 2008). Unisexuals require the sperm from bisexual males to initiate egg development but usually do not incorporate bisexual genetic material, resulting in gynogenetic offspring that are clones of the unisexual mother. Unisexual *A. laterale* – (2) *jeffersonianum* (LJJ) are found in association with all Canadian *A. jeffersonianum* populations. Because of similarities in life cycle and genetic make-up, *A. jeffersonianum* and LJJ are assumed to identically use the habitat where they co-occur (COSEWIC 2016; Environment Canada 2016; Linton et al. 2018). In Canada, current legislation protects any wetland, pond, or pool used by *A. jeffersonianum* or LJJ for breeding and the surrounding terrestrial area within 300 m. This protected area is presumed to provide suitable habitat for foraging, migration, and hibernation. In addition, any wetland, pond, or pool with suitable breeding conditions within 1 km of a known breeding site, plus the corridor of land between occupied and potential breeding sites, is also protected to ensure adequate habitat availability for migration and dispersal of juveniles (SARA 2002). However, research used to develop these regulations is generally based on small sample sizes, is primarily focussed on spring and summer movements (Table 1), does not incorporate all available movement data, and does not distinguish or directly compare bisexual and unisexual individuals.

Detailed information on habitat use throughout a species' entire life cycle is important for correctly identifying critical habitat in its entirety (Lefebvre et al. 2018). Many taxa, including salamanders, show regular seasonal migration between multiple habitats (Burt 1943). Understanding and incorporating seasonal changes in habitat use within critical habitat designations allow for conser-

vation of all distinct areas associated with supporting different essential life-cycle processes (Bendik et al. 2016; Rice et al. 2017; Lefebvre et al. 2018). Current information for *A. jeffersonianum* and LJJ critical habitat is largely from studies of spring and summer movements (Table 1). Wacasey (1961), Williams (1973), and Douglas and Monroe (1981) all measured *A. jeffersonianum* and (or) LJJ post-breeding movements away from breeding ponds. Bériault (2005) tracked LJJ and blue-spotted-dependent unisexual salamanders (*Ambystoma* (2) *laterale* – *jeffersonianum* or LLJ) from April to June, and Hoffmann et al. (2018) tracked blue-spotted-dependent unisexuals (LLJ and LLLJ) from May to August. Only a single study in Vermont, USA (Faccio 2003), has tracked a combination of five *A. jeffersonianum* and LJJ to their overwintering sites (May to November). Faccio (2003) did not genotype these individuals, but they are thought to contain some *A. jeffersonianum* individuals based on sex (Hoffmann et al. 2018). The historic focus on post-breeding movements has led to an underrepresentation of fall and overwinter habitat use for *A. jeffersonianum* and LJJ critical habitat designations.

Another potential issue with the current description of *A. jeffersonianum* and LJJ critical habitat is the assumption that habitat use is identical for sympatric bisexual and unisexual individuals, which has not been formally tested. Ecological observations suggest that habitat partitioning exists in Ohio, USA, between Small-mouthed Salamander (*Ambystoma texanum* (Matthes, 1855)), Eastern Tiger Salamander (*Ambystoma tigrinum* (Green, 1825)), and their unisexual dependents (Bogart et al. 1987). Lowcock (1994) found spatial segregation in habitat use between *A. laterale* and *A.* (2 or 3) *laterale* – *jeffersonianum* unisexual individuals during metamorph emergence, where bisexuals were captured at higher frequencies along a beaver dam than along the rest of the pond shoreline. Using environmental niche modeling of salamanders surveyed across Ohio, Greenwald et al. (2016) found LJJ were more prevalent in marginal habitat sites (i.e., areas of higher anthropogenic disturbance, cooler temperatures, and higher elevation) than *A. jeffersonianum*, suggesting these unisexuals may inhabit larger geographic areas than bisexuals. Conversely, from treadmill endurance trials and landscape genetic analysis, bisexual *A. texanum* exhibited higher locomotor endurance and dispersed twice as far from their natal wetlands than sympatric unisexual individuals (Denton et al. 2017). Further investigation into potential differences in habitat use between sperm-donor species and their unisexual dependents is required to further our understanding of these species' basic biology and to test the assumptions underlying the critical habitat descriptions used in Canada to inform species protection and recovery efforts.

To assess the efficacy of existing regulatory protections, we addressed three questions related to *A. jeffersonianum* and *A. laterale* – *jeffersonianum* migration distances and habitat use: (1) are there differences between bisexual and unisexual individuals in distance travelled to, time of arrival at, or spatial arrangement in overwintering location; (2) what area around a breeding pond encompasses 95% of a population's overwintering habitat; and (3) combining available information from all seasons in which movement occurs, what area around a breeding pond encompasses 95% of a population? By assessing both bisexual and unisexual adults year-round, this study has the potential to support or improve existing habitat regulations, fill important gaps in ecology, and inform species-at-risk management activities.

Materials and methods

Study site and trapping

Situated on land owned by the Hamilton Conservation Authority, our study pond is located near Dundas, Ontario, Canada (exact location withheld because of the endangered status of these species). The pond is a small (approximately 450 m²), precipitation and groundwater fed vernal pool within a relatively undisturbed area of mature

Table 1. Summary of terrestrial migration distances for Jefferson Salamanders (*Ambystoma jeffersonianum*) and *Ambystoma laterale-jeffersonianum* salamanders from several geographic regions.

Data source	Study location	Species	Tracking method	Tracking season	Sample size	Distance (m)		95% Quantile distance \pm SE (m)
						Mean \pm SD	Range	
Bishop 1941	New York, USA	<i>A. jeffersonianum</i> ^a	—	—	—	—	Up to 1609	—
Wacasey 1961	Michigan, USA	<i>A. jeffersonianum</i> ^a	Hand collection of marked individuals	Post-breeding (May to November)	6	39 ^b \pm 34	21–107	100 \pm 62
Wacasey 1961	Michigan, USA	<i>A. jeffersonianum</i> ^a	Hand collection of unmarked individuals	Post-breeding (May to November)	45	87 ^b \pm 63	8–208	203 \pm 6
Williams 1973	Indiana, USA	<i>A. jeffersonianum</i>	Radioisotope	Post-breeding (summer)	79	219 \pm 117	23–518	466 \pm 27
Douglas and Monroe 1981	Kentucky, USA	<i>A. jeffersonianum</i> ^a	Radioisotope	Post-breeding (spring)	10	250 ^c	—	—
Faccio 2003 ^e	Vermont, USA	<i>A. jeffersonianum</i> ^a	Radiotelemetry	Post-breeding to overwintering (May to November)	8	137 ^d \pm 130	30–431	402 \pm 184
Faccio 2003 ^f	Vermont, USA	<i>A. jeffersonianum</i> ^a	Radiotelemetry	Post-breeding to overwintering (May to November)	5	176 \pm 153	61–431	416 \pm 176
Bériault 2005	Ontario, Canada	<i>A. laterale - jeffersonianum</i>	Radiotelemetry	Post-breeding (April to June)	12 (11 LLJ + 1 LLJ)	206 \pm 135	37–517	483 \pm 119
OMNR 2008 (unpublished study) ^{g,h}	Ontario, Canada	<i>A. laterale - jeffersonianum</i>	Radiotelemetry	Post-breeding (April to August)	41	192 \pm 109	29–536	447 \pm 96
OMNR 2008 (unpublished study) ^{g,i}	Ontario, Canada	<i>A. jeffersonianum</i> ^a	Radiotelemetry	Post-breeding (April to August) ^j	13	—	Up to 720	—
Hoffmann et al. 2018	Maine, USA	<i>A. laterale - jeffersonianum</i>	Radiotelemetry	Post-breeding (May to August)	40 (37 LLJ + 2 LLLJ + 1 unknown)	172 \pm 110	6–403	370 \pm 22

Note: Mean distance values were calculated from raw data when available, otherwise published values are reported (for details on data sources see Summary of data collection methods within the Supplementary material).¹ The distance from a pond's edge that includes 95% of individuals within the population was calculated as the 95% sample quantile estimate \pm SE using Harrell–Davis estimator method.

^aUnknown genotypes, likely a mix of bisexual and unisexual.

^bUnderestimate of movement due to limited search area.

^cUnderestimate because 6 of the 10 individuals were reported moving farther from their pond.

^dUnderestimate due to transmitter failure in some individuals in September or October.

^eDistances for all tracked Jefferson Salamander individuals.

^fDistances of only Jefferson Salamander individuals tracked to overwintering locations.

^gOntario Ministry of Natural Resources (OMNR). 2008. Home range, migratory movements and habitat use of Jefferson salamander complex in Southern Ontario as determined by radio telemetry. Working title; unpublished study.

^hData from tracking conducted in 2007.

ⁱData from tracking conducted in 2008 (raw data unavailable).

^jExact tracking period uncertain, assumed identical to 2007 tracking.

deciduous forest. There are four other small vernal ponds within 300 m of the study pond that also support *Ambystoma* salamander populations (J.E. Linton and J.P. Bogart, personal observation). Within the study pond, approximately one-third of breeding adult Jefferson genotypes are *A. jeffersonianum* (J.P. Bogart, unpublished data) and there are no *A. laterale* inhabiting the area.

We used information and individuals captured during a related study conducted by Natural Resource Solutions Inc. To capture individuals, drift fencing was buried at least 10 cm into the ground to prevent individuals escaping underneath at varying distances around the study pond (Fig. 1) and pitfall traps were positioned on both sides of fences at approximately 5 m intervals. Captured *A. jeffersonianum* and *A. laterale* – *jeffersonianum* were marked with a unique identification code using visible implant elastomer (VIE) tags and a tail clip was collected and stored in 70% ethanol for later DNA analysis. Individuals were weighed and snout-to-vent length (SVL) was measured to the nearest millimetre.

To track salamander movements to overwintering sites, we used individuals captured between 13 and 26 October 2017 ($n = 9 JJ + 8 LJJ$) and between 25 September and 2 October 2018 ($n = 12 JJ + 6 LJJ + 1 LJJJ$). Only individuals weighing 12 g or more were considered for implantation so that transmitters would not exceed 10% of body mass. In an attempt to determine the distance between an individual's breeding pond and overwintering site, we preferentially selected individuals that had been previously captured entering the study pond to breed in the spring of 2016 or 2018. Despite this approach, many individuals of unknown breeding location were also tracked as a result of a combination of low capture rates and time constraints. We present tracking results from both individuals of known breeding origin and the entire sample.

Surgical procedures and genetic testing

Transmitters (model BD-2H, 1.2 g; Holohil Systems, Carp, Ontario, Canada) were implanted in a total of 36 adult salamanders consisting of 21 *A. jeffersonianum* (JJ), 14 triploid *A. laterale* – (2) *jeffersonianum* (LJJ), and 1 tetraploid *A. laterale* – (3) *jeffersonianum* (LJJJ). Surgical procedures generally followed those of Faccio (2003) and Hoffmann et al. (2018), and occurred at the University of Guelph, Guelph, Ontario, Canada. Prior to implantation, transmitters were tested to ensure that they were working and were gas-sterilized at the Ontario Veterinary College, Guelph, Ontario, Canada. All surgical tools were sterilized in ethyl alcohol. Individuals were submerged in a bath of 0.1% solution of MS-222 (tricaine methanesulfonate; buffered to pH 7.0) until they lost the ability to respond to toe pinching or right themselves. A number 15 scalpel was used to make a 12–15 mm incision in the left ventrolateral abdominal wall anterior to the left hind leg. The transmitter was implanted into the lower abdominal cavity and the incision closed with five to eight absorbable sutures (model PDS II, RB-1 taper, size 5-0; Ethicon Inc., Somerville, New Jersey, USA). Individuals were then rinsed with tap water and left on wet paper towel to recover. After recovery from surgery, individuals were released at the study site (usually within 12 to 24 h) under a cover object (e.g., log) within a few metres from their capture location.

Individual genotypes of the tail-tip samples was determined using microsatellite DNA analyses at six polymorphic, tetranucleotide loci (*AjeD75*, *AjeD94*, *AjeD283*, *AjeD346*, *AjeD378*, *AjeD422*) (Julian et al. 2003), which have been used to identify bisexual and unisexual genotypes in previous studies (Bogart et al. 2007, 2009; Bogart et al. 2017). Ploidy was assigned to individuals based on the largest number of microsatellite DNA alleles observed at any locus and the lowest number of chromosomes that could be present based on genome-specific microsatellite DNA alleles.

Radiotelemetry

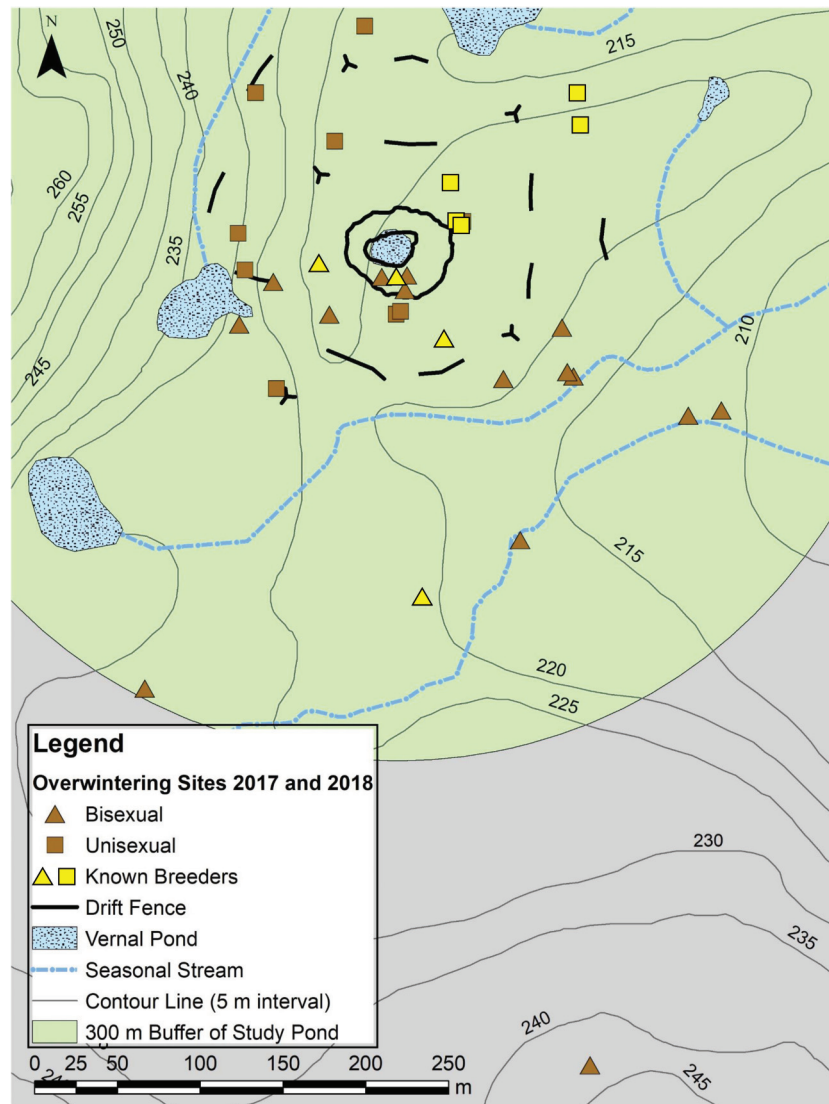
After release, individuals were located on foot using a radio receiver (SRX800; Lotek Wireless, Newmarket, Ontario, Canada) and handheld directional antenna (three-element rigid Yagi by Lotek Wireless (Newmarket, Ontario, Canada) or model RA-1A paddle by Telonics (Mesa, Arizona, USA)). Tracking occurred once per week from 13 October to 22 December 2017 and from 25 September to 21 December 2018. Once an individual's location was identified, a visual search was conducted where leaf litter, smaller cover objects, and the openings of small-mammal burrows were carefully searched by hand with the aid of a flashlight. If the individual was sighted (8% of fixes), then they were visually inspected, weighed, and their incision site was photographed to record postoperative condition. We did not dig up individuals that were underground due to the possibility of injury and the risk of significant habitat disruption. If an individual had moved from their last known location, then their new location was flagged, movement of a metre or less was measured with a metre stick, and otherwise calculated from the UTM coordinates that were recorded using a SXBlue II GNSS GPS (SXblue GPS, Anjou, Quebec, Canada) with ± 1 m accuracy. Flags were positioned 0.5 m north of the individual's location to avoid disturbing or injuring them. The majority of tracked individuals were no longer detectable by the end of each tracking period, likely due to transmitter battery failure. Transmitter duration ranged from 36 to 85 days with a mean (\pm SE) lifespan of 61 ± 3 days.

Of the 36 individuals tagged, one *A. jeffersonianum* and one LJJ died in 2017, apparently due to surgery complications. One other *A. jeffersonianum*'s bare transmitter was found on the ground during tracking in 2018. This individual had been recaptured previously during tracking and its incision site appeared to have healed well. This suggests that this individual was depredated and the tag discarded during consumption, though a search of the surrounding area did not reveal any sign of a predator (tracks, scat, etc.) or salamander remains. These 3 individuals were excluded from all analyses leaving a total of 33 individuals (19 bisexual, 14 unisexual) that were successfully tracked to their overwintering locations. We combined the LJJJ and LJJ individuals together into one group (unisexuals) for all analyses.

All but two individuals (both *A. jeffersonianum*) were located every week of tracking until transmitters expired at the end of each year (summarized in Supplementary Table S1).¹ In 2018, after a significant rainfall event that resulted in large movements (up to 200 m) from about half of the individuals, these two salamanders could not be located in the first week of November. One of these individuals was found the following week approximately 200 m from its last known location. After an extensive search, the second individual was found 4 weeks later approximately 430 m from its last known location. Both individuals were underground when relocated but made small movements (<5 m) in the weeks after being relocated suggesting that they were alive and that the transmitters were not transported by other means (such as a predation event). These two individuals are included in our analyses, although the measure of their total distance moved is likely an underestimate, as any movements occurring in the week(s) that they were not located would not have been recorded. All research was carried out in accordance with the relevant permits and animal care protocols: Wildlife Scientific Collector's Authorizations No. 1086762 and No. 1088920 issued under the *Fish and Wildlife Conservation Act*; Permit No. GU-B-002-17 and No. GU-B-008-18 issued under the *Endangered Species Act*, 2007; Animal Care Protocol

¹Supplementary tables and figures, as well as a Summary of data collection methods, are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2019-0228>.

Fig. 1. Overwintering locations of adult salamanders for which breeding in the study pond (central pond in map) is certain ($n = 9$) or uncertain ($n = 24$). Individuals were known to breed in the study pond because they were captured entering or exiting the pond during pitfall trapping of breeding migrations in the spring of 2016, 2018, or 2019. Bisexual represents Jefferson Salamander (*Ambystoma jeffersonianum*) ($n = 19$ ♀♀) individuals and unisexual represents *Ambystoma laterale* – *jeffersonianum* ($n = 13$ LJJ + 1 LJJJ) individuals. Tracking via radiotelemetry occurred from 13 October to 22 December 2017 and from 25 September to 21 December 2018 at a study site near Dundas, Ontario, Canada. Figure was created using ArcMap version 10.5.0 and was assembled from the following data sources: wetland (pond) features (Land Information Ontario 2017), contours (Land Information Ontario; available from http://geo.scholarsportal.info.subzero.lib.uoguelph.ca/#r/details/_uri@=2471670005, accessed 9 January 2019), and streams (Ontario Hydrographic Network’s watercourse data that were augmented with first-hand knowledge of site characteristics by S.G. Van Drunen; available from http://geo.scholarsportal.info.subzero.lib.uoguelph.ca/#r/details/_uri@=2261318438, accessed 9 January 2019). The drift fence, buffer, and salamander locations are data collected or calculated during the course of our research. Colour version online.



No. 145 issued by the Ontario Ministry of Natural Resources and Forestry Wildlife Animal Care Committee; and University of Guelph Animal Utilization Protocol No. 3673.

Observer accuracy test

To estimate the minimum distance moved that we could reasonably expect to record, we conducted an observer accuracy test. In 2018, observers were given a maximum of 5 min to use the telemetry equipment to locate a test transmitter that was implanted in the ground on a stake within 1 m of their start point. The observer would mark the estimated location of the transmitter with a flag and leave the area while the distance between the transmitter and the flag was measured to the nearest centimetre.

This process was repeated a total of six times per observer ($n = 3$) at two transmitter depths (10 or 20 cm) and for two types of antenna (three-element rigid Yagi (Lotek Wireless) or model RA-1A paddle (Telonics)). Test locations were randomly selected within an area 50 m from the study pond. Observer accuracy was square root transformed to normalize and tested via a general linear model. The factors observer ID, antenna type, and depth of test transmitter, as well as all their interactions were included, but no significant effects were found in any parameter (results not shown). Combined observer mean (\pm SE) accuracy was 13 ± 1 cm (range 1–31 cm) allowing for accurate detection of very small salamander movements.

In addition to the observer accuracy testing, a test transmitter was buried 0.5 m underground and also placed unburied at the bottom of a 10 cm wide, 1.3 m deep soil core shaft, which may be representative of a salamander deep within a vertical mammal burrow. In both cases, the signal could be detected from approximately 25 m away using the Yagi antenna. This suggests that depth of the transmitter did not significantly impede signal detection during tracking, and that lost signals were due to either transmitter failure or movement of an individual outside of the search area.

Survival and general health

Although individuals went largely unseen during tracking because they were underground, survival from release to overwintering location was assumed for all 33 individuals (19 bisexual, 14 unisexual) based on a number of factors. Individuals that were recaptured at least once (19 of 33) had incisions that were healed or healing well. All individuals were seen to make major (≥ 5 m) and minor (0.5 to < 5 m) movements throughout the tracking periods. In addition, six individuals were recaptured during 2018 or 2019 pitfall trapping (identified via their VIE tags). Of individuals tracked in 2017, an *LJj* and *Jj* were caught returning to the study pond to breed in March 2018 and April 2019, respectively, and a *Jj* was caught in October 2018 moving to its overwintering site. Three individuals (one each of *Jj*, *LJj*, and *LJjJj*) tracked in 2018 were captured entering or leaving the study pond during breeding migrations in April 2019.

All but one of these six individuals appeared in good health and their incision sites were well healed. One of the 2018 individuals, when captured in spring 2019, had a small laceration at its incision site, with approximately 1 cm of intestine extruding. This individual was brought back to the laboratory, anesthetized, and the wound sutured closed following the surgical methods above. The individual's transmitter was also removed during this operation. It came out of the body cavity easily and was in pristine condition. After recovering from the surgery, the individual was released near its capture point the same day of capture.

Review of movement studies

Distance data were acquired from studies that tracked *A. jeffersonianum* or *A. laterale* – *jeffersonianum* migrations throughout any portion of the year (spring, summer, fall, winter). Information on individual migration distance from their pond was obtained from six studies (Table 1). In the case of Wacasey (1961) and Williams (1973), raw distance data were extracted from published figures. Distance data from Faccio (2003) was provided by the author (S.D. Faccio, personal communication, 2019). Finally, information from OMNR (2008, unpublished study)² was recovered from a PowerPoint presentation about the project that contained maps of the locations of tracked individuals. A detailed description of our data extraction methods and any previously unpublished raw distance data are outlined in the Summary of data collection methods within the Supplementary material.¹

Statistical analyses

To assess differences in total distance travelled between *A. jeffersonianum* and *A. laterale* – *jeffersonianum*, we used a general linear model. Total distance moved over the course of tracking was log transformed to normalize and predictor variables were genotype (bisexual, unisexual), year (2017, 2018), and the number of weeks that an individual was tracked. This analysis was repeated using log Euclidean distance from release point to overwintering location as the response variable and the results were qualitatively similar to the results from the log total distance

model (results not shown). Mass of individuals was not included as a covariate in the above models because it was significantly related to genotype ($t_{(21,6)} = -2.7, p = 0.01$). To assess the effect of mass on movement per genotype, we conducted simple linear regressions with the response log total distance and the predictor mass for each group (bisexual, unisexual) separately. Results are reported as mean \pm SE unless otherwise noted.

To assess the arrival date of individuals to their overwintering locations, we extracted the date of last significant movement for each individual. Significant movement was considered to have occurred in weeks where an individual moved ≥ 5 m from its previous week's location. Arrival date counts were plotted by genotype and year.

Overwintering site segregation between bisexual and unisexual individuals was assessed using contingency table tests of first-nearest neighbours based upon asymptotic and Monte Carlo critical values (Dixon 1994). Simulated Monte Carlo critical values were based upon 5000 randomized re-labelling of the overwintering locations. A point pattern object of overwintering locations and genotype marks (bisexual, unisexual) was created using the R package "spatstat" (Baddeley et al. 2015) with the Monte Carlo simulations and contingency table analysis conducted using the R package "dixon" (De la Cruz 2008). All analyses were conducted in R version 3.5.0 (R Core Team 2018).

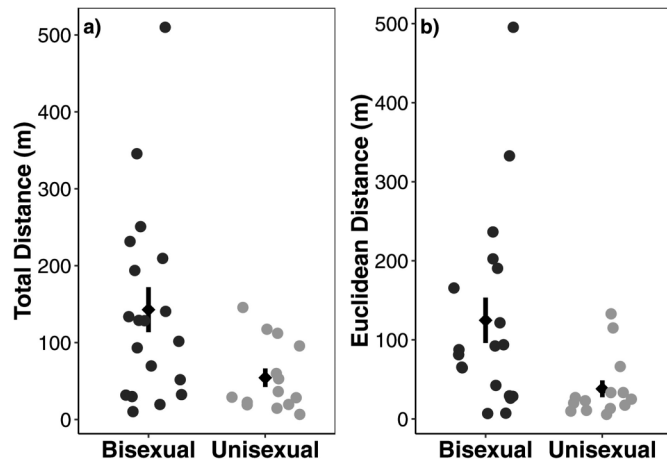
We estimated the distance from the edge of the study pond that encompasses 95% of overwintering locations using 95% sample quantiles and SE, calculated using the Harrell–Davis distribution-free quantile estimator and jackknife standard errors of quantiles (Harrell and Davis 1982). We agree with Hoffmann et al. (2018) that the 95% quantile is a more appropriate distance estimation method than Semlitsch's (1998) 95% confidence interval approach, but Hoffmann et al.'s (2018) approach of using a *t*-score quantile assumes distances travelled are normally distributed, something that is rarely the case (see Supplementary Figs. S1, S2, and S3).¹ The Harrell–Davis estimator is a robust alternative because it is more efficient for small sample sizes and does not require the data to be normally distributed (Harrell and Davis 1982).

Because the breeding location was only known for 9 of the 33 individuals, we used a conservative approach and assumed that individuals of unknown breeding location would use the first-nearest pond to their overwintering site. We calculated the Euclidean distance from overwintering site to the nearest pond for these individuals ($n = 15$ bisexual + 9 unisexual) and combined those distances with the distance from overwintering location to the study pond for the known breeders ($n = 4$ bisexual + 5 unisexual) for a total of 33 distance measures. Distances (summarized in Supplementary Table S1)¹ were calculated in ArcGIS version 10.5.1 (ESRI 2017) using pond-location information from the wetland data set produced by the Government of Ontario (Land Information Ontario 2017) and are effectively the minimum distance individuals would have to travel to reach a breeding pond the following spring. The unknown breeder's distances are likely underestimated of movement, as three of the nine known breeders overwintered closer to ponds other than their breeding location (see Supplementary Table S1).¹ Using this distance data, we calculated 95% quantiles and SE for all individuals combined, as well as for bisexuals and unisexuals separately. Quantile values and SE were calculated using the R package "Hmisc" (Harrell 2019).

For past studies with available raw distance data (Table 1), we estimated Harrell–Davis 95% quantiles and SE. When raw distance data were unavailable, the published range and (or) mean distances moved were reported. Finally, we estimated Harrell–Davis 95% quantile and SE for combinations of all studies for which

²Ontario Ministry of Natural Resources (OMNR). 2008. Home range, migratory movements and habitat use of Jefferson salamander complex in Southern Ontario as determined by radio telemetry. Working title; unpublished study.

Fig. 2. (a) Total distance travelled and (b) Euclidean distance from release point to overwintering location by genotype. Black diamonds with error bars represent the mean (\pm SE) per group. Bisexual represents Jefferson Salamander (*Ambystoma jeffersonianum*) ($n = 19$ JJ) individuals and unisexual represents *Ambystoma laterale - jeffersonianum* ($n = 13$ LJJ + 1 LJJJ) individuals. Tracking via radiotelemetry occurred from 13 October to 22 December 2017 and from 25 September to 21 December 2018 at a study site near Dundas, Ontario, Canada.



there were data (Wacasey 1961; Williams 1973; Faccio 2003; Bériault 2005; OMNR 2008, unpublished study; Hoffmann et al. 2018; our own data), and only studies of bisexual (Williams 1973; bisexual sample from this study) or unisexual (Bériault 2005; OMNR 2008, unpublished study; Hoffmann et al. 2018; unisexual sample from this study) individuals when known.

Results

Fall movement and overwintering sites

Bisexual salamanders travelled significantly farther distances than unisexuals to reach their overwintering location (Figs. 2a and 2b). Total distance was significantly related to genotype ($\beta \pm SE = -0.83 \pm 0.33, t_{[29]} = -2.56, p = 0.02$). Bisexual individuals moved 143 ± 29 m compared with 54 ± 12 m for unisexuals. There was no effect of year ($\beta \pm SE = 0.95 \pm 0.62, t_{[29]} = 1.54, p = 0.13$) and number of weeks tracked ($\beta \pm SE = -0.04 \pm 0.16, t_{[29]} = -0.25, p = 0.80$). Overall, the model including genotype, year, and number of weeks tracked explained 33% of the variation in total distance ($F_{[3,29]} = 4.7, p = 0.009$). There was no relationship between total distance and mass for either bisexual ($F_{[1,17]} = 1.8, R^2 = 0.10, p = 0.20$) or unisexual ($F_{[1,12]} = 0.5, R^2 = 0.04, p = 0.48$) salamanders.

Across both years, there was no clear pattern between genotype in movement patterns and date of arrival to overwintering sites. In 2017, both bisexual and unisexual individuals started to arrive at overwintering locations in late October, but unisexual individuals were active a few weeks later than bisexuals (Fig. 3). This pattern was reversed in 2018 where bisexual salamanders arrived at overwintering sites both earlier and later in the year than unisexuals. In general, salamanders started to arrive at overwintering sites in early October and peak number of arrivals occurred in the first week of November, with a few individuals showing movements into December (Fig. 3).

There was strong evidence of segregation between bisexual and unisexual overwintering locations (Table 2). Observed first-nearest neighbour counts were higher than expected for same genotype pairs. Individuals were more likely than expected to overwinter near a similar genotype individual (Fig. 1) and self-segregation indices were large and positive (Table 3).

Fig. 3. Number of individuals per year and genotype by date at which significant movement ceased (i.e., date of arrival at overwintering site). Significant movement was defined as occurring when an individual moved ≥ 5 m from their previous week's location. Bisexual represents Jefferson Salamander (*Ambystoma jeffersonianum*) ($n = 19$ JJ) individuals and unisexual represents *Ambystoma laterale - jeffersonianum* ($n = 13$ LJJ + 1 LJJJ) individuals. Bars are offset for clarity. Tracking via radiotelemetry occurred from 13 October to 22 December 2017 and from 25 September to 21 December 2018 (start dates denoted by arrows) at a study site near Dundas, Ontario, Canada.

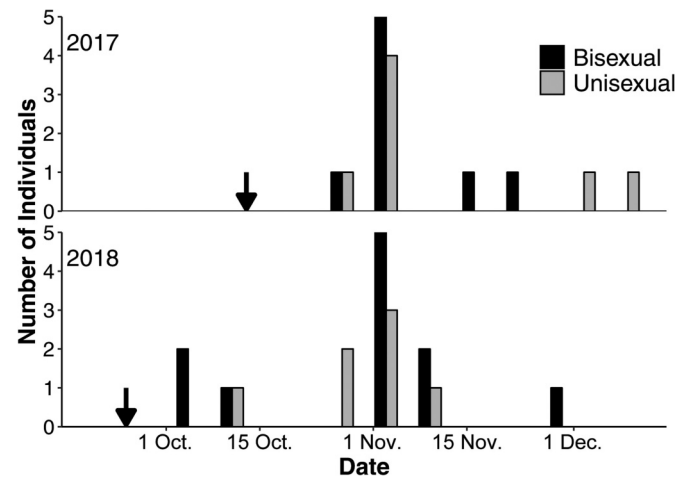


Table 2. Results of χ^2 tests of first-nearest neighbor overwintering locations of adult bisexual Jefferson Salamanders (*Ambystoma jeffersonianum*) and unisexual *Ambystoma laterale - jeffersonianum* salamanders.

	df	χ^2	p-Value asymptotic	p-Value random
Overall segregation	2	13.11	0.001	0.001
From bisexual	1	7.13	0.008	0.006
From unisexual	1	9.12	0.003	0.002

Note: "From" is the label of the point from which the first-nearest neighbor was measured. "p-Value asymptotic" is the p value from the asymptotic χ^2 distribution with the appropriate degrees of freedom for each test. "p-Value random" is the p value from the randomization distribution estimated by 5000 random re-labelling of points.

Critical habitat estimates

The distance from the study pond encompassing 95% of all overwintering locations ranged from 274 ± 79 m for bisexual ($n = 19$), 130 ± 13 m for unisexual ($n = 14$), and 236 ± 51 m when combined ($n = 33$). Combining our data with past studies, the distance (\pm SE) from a pond's edge containing 95% of individuals within a population ranged from 100 ± 62 to 483 ± 119 m (Table 1). The majority of past studies tracked post-breeding migrations of *A. jeffersonianum* and related unisexuals during spring and summer periods, with only Faccio (2003) having tracked individuals ($n = 5$) to their overwintering location. A number of studies did not genotype the individuals tracked and likely contain a mix of *A. jeffersonianum* and *A. laterale - jeffersonianum* within their samples (Table 1). Apart from our work, studies of known genotypes were Bériault (2005), Hoffmann et al. (2018), and the 2007 data from OMNR (2008, unpublished study),² which tracked various *A. laterale - jeffersonianum* unisexuals, and Williams (1973), whose study of *A. jeffersonianum* movement was outside the range of unisexuals (Hoffmann et al. 2018). Combining all available movement distance data resulted in an overall critical habitat distance estimate (\pm SE) of 397 ± 30 m ($n = 264$) for both bisexuals and unisexuals combined, 451 ± 31 m ($n = 98$) for only bisexuals, and 390 ± 40 m ($n = 107$) for unisexuals.

Table 3. First-nearest neighbor counts and related statistics for overwintering locations of adult bisexual Jefferson Salamanders (*Ambystoma jeffersonianum*) and unisexual *Ambystoma laterale-jeffersonianum* salamanders.

From	To	Observed count	Expected count	S	Z	p-Value normal	p-Value random
Bisexual	Bisexual	17	10.69	0.82	2.67	0.008	0.003
Bisexual	Unisexual	2	8.31	-0.82	-2.67	0.008	0.003
Unisexual	Bisexual	2	8.31	-0.94	-3.02	0.003	0.004
Unisexual	Unisexual	12	5.69	0.94	3.02	0.003	0.002

Note: "From" is the label of the point from which the first-neighbor was measured and "To" is the label of the first-nearest neighbor. S is the segregation index and Z is the Z score for testing whether the observed count equals the expected count. The p values are based on the asymptotic normal distribution of the Z statistic and the randomization distribution estimated by 5000 random re-labelling of points.

Discussion

Our results highlight the importance of considering species habitat use throughout the entire annual cycle when defining critical habitat. Although large enough to protect overwintering habitat, the currently regulated terrestrial habitat area within 300 m of breeding ponds in Canada is too small to protect the year-round habitat used by *A. jeffersonianum* and *A. laterale - jeffersonianum*. The majority of critical habitat estimates based on past studies (Table 1) and the estimates derived from our combination of studies encompassed values of 400 m or more. Based on these findings, we conclude that the regulated distance from a pond's edge should be increased to 400–450 m to protect 95% of adult *A. jeffersonianum* and *A. laterale - jeffersonianum* individuals within a given population. These results demonstrate the importance of incorporating habitat use from all life stages when estimating critical habitat, especially for species that migrate between habitat patches throughout their life cycle.

Our recommendation to increase the area of critical habitat for *A. jeffersonianum* and *A. laterale - jeffersonianum* may itself be based on underestimates of the area used by these salamanders. Similar to Denton et al. (2017) who found that bisexual *A. texanum* dispersed twice as far as *A. texanum*-dependent unisexuals, *A. jeffersonianum* travelled, on average, three times farther than unisexuals to reach overwintering locations. Assuming this difference in migration distances holds true throughout the year, then estimates containing a mix of bisexual and unisexual individuals would lead to underestimates of the habitat used by purely bisexual populations. Because the majority of past studies included here did not genotype individuals and likely included an unknown mix of bisexual and unisexual salamanders (Table 1), our estimates of the area essential to protect *A. jeffersonianum* populations is likely biased low.

Critical habitat underestimates may also occur when estimation methods assume pond-breeding amphibian migration distances are normally distributed, for example most salamander movement data included here is non-normal in nature (see Supplementary Figs. S1, S2, and S3).¹ For *A. laterale - jeffersonianum* (LJJ and LLJ), Bériault (2005) estimated a 293 m distance using the t-score 95% confidence interval approach of Semlitsch (1998), and Hoffmann et al. (2018) estimated a distance of 362 m for LLJ and LLLJ unisexuals using a t-score 95% quantile method. Compared with our sample quantile estimates of 483 and 370 m, respectively, it appears that assumptions of normality can result in underestimates of the distance containing 95% of individuals within a population and we therefore recommend using a non-parametric sample quantile estimation method.

In addition to fall migration differences, our results provide evidence that bisexual and unisexual salamander overwintering site selection was spatially segregated, with individuals more likely to occur near similar type individuals than not. This spatial segregation indicates potential differences in microhabitat preference or use. Greenwald et al. (2016) found LJJ were more prevalent in areas of higher anthropogenic disturbance, cooler

temperatures, and higher elevation than *A. jeffersonianum* and attributed this as one mechanism allowing the coexistence of bisexual and unisexual species. Greenwald et al. (2016) suggested that these differences in distribution may be partly attributed to the *A. laterale* genomic component present within unisexual salamanders, as *A. laterale* is generally associated with lower temperature and drier habitat sites. Anecdotal information from our study may support this hypothesis. *Ambystoma jeffersonianum* were more likely to overwinter in low-lying areas with significant groundwater upwelling, whereas *A. laterale - jeffersonianum* preferred mid- to upper-slope locations (S.G. Van Drunen, personal observation; Fig. 1). Regardless of the underlying mechanisms, caution is warranted when making assumptions about the similarity of habitat utilization between bisexual and unisexual salamanders.

There are some limitations to consider that may have affected our radiotelemetry results. First, our sample may be biased towards more active individuals, as these individuals would be more likely to be captured in pitfall traps and therefore tracked. In addition, uncertainty about when individuals started their movement toward overwintering sites could be problematic if bisexual and unisexual individuals have significantly different start dates to their movements, though similarities in overwinter site arrival times may suggest that movement start dates may be equally similar. Finally, differences in sampling periods between years could have influenced the distances tracked. As shown in Fig. 3, four individuals had reached their overwintering locations in 2018 before the first implantations occurred in 2017. This suggests that our 2017 sample may have missed capturing "early" overwintering individuals and selected for individuals that remained active longer into the season when compared with 2018, though distance travelled was not significantly different between years in our model.

Another point to consider is that our small sample size of individuals known to breed in the study pond ($n = 9$) limits our certainty in the accuracy of our distance estimates, because most individuals could be from a different, albeit nearby, breeding pond. The conservative approach used here to account for this likely biases our estimates of critical habitat area low. The issue of uncertain breeding location was partly driven by limitations in the available tracking technology. Capture of ambystomatid adult salamanders is easiest in the spring since individuals make fewer movements during the summer months (Madison 1997; Faccio 2003; Hoffmann et al. 2018). But transmitter size, and therefore battery life, is limited by salamander body size. Individuals could be captured and implanted in the spring, with new transmitters re-implanted throughout the year to enable tracking to overwintering sites, but multiple surgeries may have negative animal-care implications (see Hoffmann et al. 2018). Ideally, individuals would be captured and implanted in the spring with transmitters programmed to activate in the fall. Unfortunately, to our knowledge no appropriately sized programmable transmitter with an internal antenna for surgical implantation exists to date.

Finally, it is not clear whether movements made by blue-spotted-dependent unisexuals are directly comparable with Jefferson-dependent unisexuals. Bériault's (2005) sample includes one *LLJ* and all individuals reported in Hoffmann et al. (2018) were *LLJ* or *LLLJ*. Sperm-host habitat use can vary between bisexual species (Greenwald et al. 2016), which may influence the migrations of the associated sperm-dependent unisexuals. This may not be of concern here, as Blue-spotted Salamanders (*A. laterale*) generally migrate shorter distances than *A. jeffersonianum* (Williams 1973; Douglas and Monroe 1981; Ryan and Calhoun 2014). This suggests including *LLJ* individuals in our sample would at worst result in underestimates of critical habitat area. Because of the likely conservative nature of including *LLJ* or *LLLJ* individuals in our estimates, and due to the limited amount of available information about unisexual movements in general, we believe that it is reasonable to have included the *LLJ* and *LLLJ* data in our analyses here.

Our study is the first to directly measure and compare fall migration and overwintering locations of *A. jeffersonianum* and *A. laterale* – *jeffersonianum* individuals within the same population. This research fills key gaps in basic species biology relating to fall migration distances and increases our understanding of overwinter habitat use. Using this information, we recommend increasing the size of protected critical habitat for *A. jeffersonianum* and *LLJ* unisexual populations. Differences between bisexual and unisexual migration and habitat use also need to be considered during management of these endangered species. Further research to further clarify habitat utilization differences between bisexual and unisexual salamanders, especially within fragmented landscapes, is the next step towards improving our understanding and ability to preserve these species from extinction.

Acknowledgements

We thank employees of Natural Resource Solutions Inc. for assistance with collection of field data: J.V. Turgeon, J. Ferguson, A.M. Cantwell, S. Hofstetter, K.D. Mennie, and G. Schaus. Hamilton Conservation Authority provided land access. Funding was provided by Schlegel Urban Developments Inc. and Waterdown Bay Ltd. in support of their Overall Benefit permits under the 2007 *Endangered Species Act* and a Discovery Grant to D.R.N. from the Natural Sciences and Engineering Research Council of Canada. We thank B. Van Ryswyk, K. Hoffmann, and S.D. Faccio for providing additional information about their respective studies, as well as J. Pisapio for key contributions to the OMNR (2008) unpublished study.²

References

Baddeley, A., Rubak, E., and Turner, R. 2015. Spatial point patterns: methodology and applications with R. Chapman and Hall/CRC Press, London. Available from <http://www.crcpress.com/Spatial-Point-Patterns-Methodology-and-Applications-with-R/Baddeley-Rubak-Turner/9781482210200/>.

Bendik, N.F., McEntire, K.D., and Sissel, B.N. 2016. Movement, demographics, and occupancy dynamics of a federally-threatened salamander: evaluating the adequacy of critical habitat. *PeerJ*, 4: e1817. [26 pp.] doi:10.7717/peerj.1817. PMID:26998413.

Bériault, K.D. 2005. Critical habitat of Jefferson salamanders in Ontario: an examination through radiotelemetry and ecological surveys. M.Sc. thesis, University of Guelph, Guelph, Ont.

Bird, S.C., and Hodges, K.E. 2017. Critical habitat designation for Canadian listed species: slow, biased, and incomplete. *Environ. Sci. Pol.* 71: 1–8. doi:10.1016/j.envsci.2017.01.007.

Bishop, S.C. 1941. The salamanders of New York. N.Y. State Mus. Bull. 324: 5–365.

Bogart, J.P. 2003. Genetics and systematics of hybrid species. In *Reproductive biology and phylogeny of Urodela*. Vol. 1. Edited by D.M. Sever. Science Publishers, Enfield, N.H. pp. 109–134.

Bogart, J.P., and Klemens, M.W. 2008. Additional distributional records of *Ambystoma laterale*, *A. jeffersonianum* (Amphibia: Caudata) and their unisexual kleptogens in northeastern North America. *Am. Mus. Novit.* 2008(3627): 1–58. doi:10.1206/604.1.

Bogart, J.P., Lowcock, L.A., Zeyl, C.W., and Mable, B.K. 1987. Genome constitution and reproductive biology of hybrid salamanders, genus *Ambystoma*, on Kelleys Island in Lake Erie. *Can. J. Zool.* 65(9): 2188–2201. doi:10.1139/z87-333.

Bogart, J.P., Bi, K., Fu, J., Noble, D.W.A., and Niedzwiecki, J. 2007. Unisexual

salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. *Genome*, 50(2): 119–136. doi:10.1139/G06-152. PMID:17546077.

Bogart, J.P., Bartoszek, J., Noble, D.W.A., and Bi, K. 2009. Sex in unisexual salamanders: discovery of a new sperm donor with ancient affinities. *Heredity*, 103: 483–493. doi:10.1038/hdy.2009.83. PMID:19639004.

Bogart, J.P., Linton, J.E., and Sandilands, A. 2017. A population in limbo: unisexual salamanders (genus *Ambystoma*) decline without sperm-donating species. *Herpetol. Conserv. Biol.* 12: 41–55.

Burt, W.H. 1943. Territoriality and home range concepts as applied to mammals. *J. Mammal.* 24(3): 346–352. doi:10.2307/1374834.

Camaclang, A.E., Maron, M., Martin, T.G., and Possingham, H.P. 2015. Current practices in the identification of critical habitat for threatened species. *Conserv. Biol.* 29(2): 482–492. doi:10.1111/cobi.12428. PMID:25472827.

COSEWIC. 2010. COSEWIC assessment and status report on the Jefferson Salamander *Ambystoma jeffersonianum* in Canada. Committee on the Status of Endangered Wildlife in Canada (COSEWIC), Ottawa, Ont.

COSEWIC. 2016. COSEWIC assessment and status report on the unisexual *Ambystoma*, *Ambystoma laterale*, Small-mouthed Salamander-dependent population, Jefferson Salamander-dependent population and the Blue-spotted Salamander-dependent population, in Canada. Committee on the Status of Endangered Wildlife in Canada (COSEWIC), Ottawa, Ont.

COSSARO. 2011. COSSARO candidate species at risk evaluation form for Jefferson Salamander (*Ambystoma jeffersonianum*). Committee on the Status of Species at Risk in Ontario (COSSARO), Peterborough.

COSSARO. 2016. Ontario species at risk evaluation report for unisexual *Ambystoma* (*Ambystoma laterale*). Committee on the Status of Species at Risk in Ontario (COSSARO), Peterborough.

De la Cruz, M. 2008. Métodos para analizar datos puntuales. In *Introducción al Análisis Espacial de Datos en Ecología y Ciencias Ambientales: Métodos y Aplicaciones*. Edited by F.T. Maestre, A. Escudero, and A. Bonet. Asociación Española de Ecología Terrestre, Universidad Rey Juan Carlos y Caja de Ahorros del Mediterráneo, Madrid. pp. 76–127.

Denton, R.D., Greenwald, K.R., and Gibbs, H.L. 2017. Locomotor endurance predicts differences in realized dispersal between sympatric sexual and unisexual salamanders. *Funct. Ecol.* 31: 915–926. doi:10.1111/1365-2435.12813.

Dirzo, R., and Raven, P.H. 2003. Global state of biodiversity and loss. *Annu. Rev. Environ. Resour.* 28: 137–167. doi:10.1146/annurev.energy.28.050302.105532.

Dixon, P.M. 1994. Testing spatial segregation using a nearest-neighbor contingency table. *Ecology*, 75: 1940–1948. doi:10.2307/1941598.

Douglas, M.E., and Monroe, B.L. 1981. A comparative study of topographical orientation in *Ambystoma* (Amphibia: Caudata). *Copeia*, 1981(2): 460–463. doi:10.2307/1444239.

Environment Canada. 2016. Recovery Strategy for the Jefferson Salamander (*Ambystoma jeffersonianum*) in Canada. *Species at Risk Act Recovery Strategy Series*. Environment Canada, Ottawa, Ont.

ESRI. 2017. ArcGIS. Version 10.5.1. Environmental Systems Research Institute (ESRI), Redlands, Calif.

Faccio, S.D. 2003. Postbreeding emigration and habitat use by Jefferson and Spotted salamanders in Vermont. *J. Herpetol.* 37(3): 479–489. doi:10.1670/155-02A.

Greenwald, K.R., Denton, R.D., and Gibbs, H.L. 2016. Niche partitioning among sexual and unisexual *Ambystoma* salamanders. *Ecosphere*, 7(11): e01579. doi:10.1002/ecs2.1579.

Harrell, F.E., and Davis, C.E. 1982. A new distribution-free quantile estimator. *Biometrika*, 69(3): 635–640. doi:10.1093/biomet/69.3.635.

Harrell, F.E., Jr. (Maintainer; with contributions from Charles Dupont and many others). 2019. Hmisc: Harrell Miscellaneous. R package version 4.2-0. Available from <https://CRAN.R-project.org/package=Hmisc>.

Hoffmann, K., Hunter, M., Jr., Calhoun, A.J.K., and Bogart, J. 2018. Post-breeding migration and habitat of unisexual salamanders in Maine, USA. *J. Herpetol.* 52(3): 273–281. doi:10.1670/17-099.

Julian, S.E., King, T.L., and Savage, W.K. 2003. Novel Jefferson salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Mol. Ecol. Notes*, 3: 95–97. doi:10.1046/j.1471-8286.2003.00362.x.

Land Information Ontario. 2017. Land Information Ontario (LIO) File Geodatabase - Additional Layers - Dec. 2017. Wetlands (Ontario coverage). Land Information Ontario, Ministry of Natural Resources, Peterborough. Available from [http://geo2.scholarsportal.info/#r/details/_uri=1613147227\\$OGDE_File_Geodatabase_2013-2015](http://geo2.scholarsportal.info/#r/details/_uri=1613147227$OGDE_File_Geodatabase_2013-2015) [accessed 9 January 2019].

Lefebvre, S.L., Landry-Cuerrier, M., and Humphries, M.M. 2018. Identifying the critical habitat of Canadian vertebrate species at risk. *Can. J. Zool.* 96(4): 297–304. doi:10.1139/cjz-2016-0304.

Lesbarrères, D., Ashpole, S.L., Bishop, C.A., Blouin-Demers, G., Brooks, R.J., Echaubard, P., et al. 2014. Conservation of herpetofauna in northern landscapes: threats and challenges from a Canadian perspective. *Biol. Conserv.* 170: 48–55. doi:10.1016/j.biocon.2013.12.030.

Linton, J., McCarter, J., and Fotherby, H. 2018. Recovery strategy for the Jefferson Salamander (*Ambystoma jeffersonianum*) and unisexual *Ambystoma* (Jefferson Salamander dependent population) (*Ambystoma laterale* – (2) *jeffersonianum*) in Ontario. Ontario Recovery Strategy Series. Prepared for Ontario Ministry of Natural Resources and Forestry, Peterborough.

Lowcock, L.A. 1994. Biotype, genomotype, and genotype: variable effects of

- polyploidy and hybridity on ecological partitioning in a bisexual–unisexual community of salamanders. *Can. J. Zool.* **72**(1): 104–117. doi:10.1139/z94-014.
- Madison, D.M. 1997. The emigration of radio-implanted Spotted Salamanders, *Ambystoma maculatum*. *J. Herpetol.* **31**(4): 542–551. doi:10.2307/1565607.
- McCune, J.L., Harrower, W.L., Avery-Gomm, S., Brogan, J.M., Csergő, A., Davidson, L.N.K., et al. 2013. Threats to Canadian species at risk: an analysis of finalized recovery strategies. *Biol. Conserv.* **166**: 254–265. doi:10.1016/j.biocon.2013.07.006.
- Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., et al. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, **344**(6187): 1246752. doi:10.1126/science.1246752. PMID:24876501.
- R Core Team. 2018. R: a language and environment for statistical computing. Version 3.5.0. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.r-project.org/>.
- Rice, M.B., Apa, A.D., and Wiechman, L.A. 2017. The importance of seasonal resource selection when managing a threatened species: targeting conservation actions within critical habitat designations for the Gunnison sage-grouse. *Wildl. Res.* **44**: 407–417. doi:10.1071/WR17027.
- Ryan, K.J., and Calhoun, A.J.K. 2014. Postbreeding habitat use of the rare, pure-diploid Blue-spotted Salamander (*Ambystoma laterale*). *J. Herpetol.* **48**(4): 556–566. doi:10.1670/13-204.
- Species at Risk Act (SARA). 2002. Species at Risk Act (S.C. 2002, c. 29): an act respecting the protection of wildlife species at risk in Canada. Government of Canada, Ottawa, Ont. Available from <http://laws-lois.justice.gc.ca/eng/acts/S-15.3/index.html> [accessed 2 April 2019].
- Semlitsch, R.D. 1998. Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conserv. Biol.* **12**(5): 1113–1119. doi:10.1046/j.1523-1739.1998.97274.x.
- Stuart, S.N. 2012. Responding to the amphibian crisis: too little, too late? *Alytes*, **29**(1–4): 1246752.
- Taylor, M.F.J., Suckling, K.F., and Rachlinski, J.J. 2005. The effectiveness of the Endangered Species Act: a quantitative analysis. *BioScience*, **55**(4): 360–367. doi:10.1641/0006-3568(2005)055[0360:TEOTES]2.0.CO;2.
- Wacasey, J.W. 1961. An ecological study of two sympatric species of salamanders, *Ambystoma maculatum* and *Ambystoma jeffersonianum*, in southern Michigan. Ph.D. thesis, Michigan State University, Lansing.
- Walls, S.C., Ball, L.C., Barichivich, W.J., Dodd, C.K., Jr., Enge, K.M., Gorman, T.A., et al. 2017. Overcoming challenges to the recovery of declining amphibian populations in the United States. *Bioscience*, **67**(2): 156–165. doi:10.1093/biosci/biw153.
- Williams, P.K. 1973. Seasonal movements and population dynamics of four sympatric mole salamanders, genus *Ambystoma*. Ph.D. thesis, Indiana University, Bloomington.
- WWF. 2016. Living Planet Report 2016. Risk and resilience in a new era. WWF International, Gland, Switzerland.