USING PASSIVE INTEGRATED TRANSPONDER (PIT) SYSTEMS FOR TERRESTRIAL DETECTION OF BLUE-SPOTTED SALAMANDERS (*AMBYSTOMA LATERALE*) *IN SITU*

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Abstract.—Pure-diploid Blue-spotted Salamanders (*Ambystoma laterale*) are the smallest members of the family Ambystomatidae which makes tracking with radio-transmitters difficult because of small battery capacity. Passive integrated transponder (PIT) tags provide another tracking approach for small fossorial animals such as salamanders. We evaluated the use of portable PIT tag readers (PIT packs) to detect PIT tag-implanted pure-diploid Blue-spotted Salamanders *in situ*. We also examined the detection probability of salamanders with PIT tags held in enclosures in wetland and terrestrial habitats, as well as the underground detection range of PIT packs by scanning for buried tags not implanted into salamanders. Of the 532 PIT tagged salamanders, we detected 6.84% at least once during scanning surveys. We scanned systematically within a 13.37 ha area surrounding a salamander breeding pool on 34 occasions (~119 hours of survey time) and detected PIT tags 74 times. We detected 55% of PITs in tagged salamanders and 45% were expelled tags. We were able to reliably detect buried PIT tags from 1–22 cm below the ground surface. Because nearly half the locations represented expelled tags, our data suggest this technique is inappropriate for future studies of pure-diploid Blue-spotted Salamanders, although it may be suitable for polyploid Blue-spotted Salamanders and other ambystomatid species, which are larger in size and may exhibit higher tag retention rates. It may also be prudent to conduct long-term tag retention studies in captivity before tagging and releasing salamanders for *in situ* study, and to double-mark individuals.

*Key Words.—*amphibians; detection; expulsion; PIT pack; PIT Tag; PIT telemetry; retention

INTRODUCTION

The Blue-spotted Salamander (*Ambystoma laterale*) is a pool-breeding species that spends the vast majority of its life in terrestrial habitat adjacent to breeding pools (typically vernal pools adjacent to riparian or lacustrine areas, or within forests; Faccio 2003; Gibbs et al. 2007). It is the northernmost ambystomatid in eastern North America, ranging as far north as the southern tip of Hudson Bay, Canada. On the eastern seaboard of North America, it is found from northern New Jersey to the Canadian Maritime Provinces and Labrador. It is also widely distributed around the Great Lakes, westward to Minnesota and southeastern Manitoba (Klemens 1993).

The vast majority of Blue-spotted Salamander populations in New England are composed of individuals belonging to the "Blue-spotted Salamander complex," which consists of individuals that typically have more than two sets of two chromosomes and in some cases up to five. At least one set of chromosomes in these individuals is from the closely related Jefferson Salamander (*Ambystoma jeffersonianum*). The eastern Connecticut population examined in this study is one of three known pure-diploid populations in the northeastern

United States. This has been confirmed by karyotyping work previously conducted by Bogart and Klemens (1997, 2008) and James Bogart (unpubl. data).

Passive integrated transponder (PIT) tags are small, glass-encased microchips (as small as 1.4 mm × 8.4 mm) that transmit a unique numeric or alphanumeric code on activation through an electromagnetic field generated by a PIT tag reading device. They were first used as a marking method in fisheries studies (Prentice and Park 1983; Gibbons and Andrews 2004), and have been used extensively to mark a variety of amphibians, both anurans and caudates, ranging in size from hellbenders (*Cryptobranchus alleganiensis*; Humphries and Pauley 2000) to metamorph African Clawed Frogs (*Xenopus laevis*; Waldner et al. 2007).

Polyploid Blue-Spotted Salamanders attain a larger size than diploid salamanders (Bogart and Klemens 1997, 2008), which are the smallest of the ambystomatid salamanders. Tracking diploid Blue-spotted Salamanders via radio-transmitters is difficult due to the short battery life of the small transmitters appropriate for use in small salamanders. Advances in PIT technology allow an alternative method of tracking Blue-spotted Salamanders as well as other species of small fossorial animals not

well-suited to radio-transmitters due to size constraints.

In amphibian studies, PIT tag detection has traditionally required the recapture of tagged animals, often via pitfall trap arrays (Regosin et al. 2005; Homan et al. 2007), as the tag typically needs to be close to the reading device (Blomquist et al. 2008). More recently, portable PIT tag readers (PIT packs) have been used to detect tags of implanted amphibians without physically contacting them (e.g., Cabarle et al. 2007; Blomquist et al. 2008). Two studies have highlighted a technique of searching for amphibians in their terrestrial habitat *in situ* (Hamed et al. 2008; Connette and Semlitsch 2012). Although these studies were successful in detecting PIT tag-implanted amphibians, they were restricted to relatively homogenous, forested terrestrial habitat.

We conducted a study investigating the use of PIT packs to detect PIT tag-implanted Blue-spotted Salamanders *in situ* in a range of habitats including forests, agricultural fields, and forested wetlands. The objectives of this study were: (1) to evaluate the use of a PIT pack as a tool to detect Blue-spotted Salamanders *in situ*; (2) to assess the detectability of Blue-spotted Salamanders implanted with PIT tags and held in terrestrial enclosures; and (3) to assess the detection rates of PIT tags by PIT packs as a function of depth and habitat type.

MATERIALS AND METHODS

Research sites.—We conducted our study on a 28.84 ha privately-owned farm (41.6489°N, 71.9612°W) in the Quinebaug River drainage in Windham County, Connecticut, USA, a state where pure-diploid Blue-spotted Salamander is listed as Endangered. Several specimens collected at the site prior to the initiation of this study had been deposited at the American Museum of Natural History in New York, New York, USA. Major land cover types present on the site include hayfields, mixed oak/pine forest (*Quercus rubrum* and *Pinus strobus*), oak savanna, and Red Maple (*Acer rubrum*) forested wetlands. Two breeding pools are present: a 0.24 ha semi-permanent kettle-depression scrub-shrub vernal pool and a 0.15 ha classic kettle-depression open water vernal pool. The former serves as the primary breeding habitat for the population of Blue-spotted Salamanders inhabiting the site. Also present are five human-created permanent ponds ranging in size from 0.03 to 0.24 ha. An ~30 y-old gravel extraction area at the center of the site formed three of these permanent ponds and a 0.34 ha area of wet-meadow.

Capture and tagging.—We captured Blue-spotted Salamanders in an on-site drift fence/pitfall trap array completely enclosing the scrub-shrub kettle depression vernal pool. We installed approximately 2 km of drift fence/pitfall traps at the site and monitored daily from late winter through the late fall/early winter during each year of this study. Upon capture of each individual, we measured snout-to-vent length (SVL; to the nearest 0.1 cm), mass (to the nearest 0.1 g), and implanted each individual with a PIT tag following the methods in Madison et al. (2010). Due to the small size of Blue-spotted Salamanders (average adult SVL and mass are 54.5 mm and 3.7 g, respectively), we used relatively small PIT tags (12 mm, 0.1 g Model HPT12, 134.2 kHz ISO FDXB tag; Biomark, Boise, Idaho, USA). This was the same model PIT tag used in several previous amphibian terrestrial PIT tag detection studies (e.g., Blomquist et al. 2008; Hamed et al. 2008; Connette and Semlitsch 2012).

We anesthetized salamanders using 3.1 mM tricaine methane sulfonate (MS-222) neutralized to pH 7.0 using aqueous NaOH. Following Faccio (2003) and McDonough and Paton (2007), when the righting response and response to touch were completely suppressed, we made a 1 mm incision in the ventral posteriolateral abdominal wall and inserted a single PIT tag (bathed in chlorhexidine and rinsed with well water) into the peritoneal cavity. Due to the small incision, we deemed sutures unnecessary. We rinsed PIT tag implanted individuals with well water and maintained them overnight separately in plastic containers lined with wet paper towels.

In 2008, we captured 16 adult Blue-spotted Salamanders as they exited the scrub-shrub kettle depression vernal pool and implanted these individuals with PIT tags and held in captivity for use in the enclosure scanning trials (discussed below). From 2009 through 2011, we implanted all non-metamorph Blue-spotted Salamanders captured exiting the scrub-shrub kettle depression vernal pool with PIT tags (559 in total) and released the day after surgery directly across from the pitfall trap in which they were captured. We used Vetbond™ tissue adhesive (3M, St. Paul, Minnesota, USA) to seal the incisions of three salamanders, but all sloughed off the adhesive prior to being released. We therefore discontinued our use of Vetbond™.

In situ scanning.—The PIT pack model we used for detection of PIT-tag implanted Blue-spotted Salamanders *in situ* was designed from the constituent components (i.e., removed from original housing) of a Destron-Fearing transceiver (Model FS 1001A-ISO; Digital Angel Co., St. Paul, Minnesota, USA) mounted with battery packs inside a watertight case that was attached to a pack frame (see Hill et al. [2006] and Kurth et al. [2007]; hereafter "backpack scanner"). We used a custom-built ~60 cm diameter oblong antenna connected
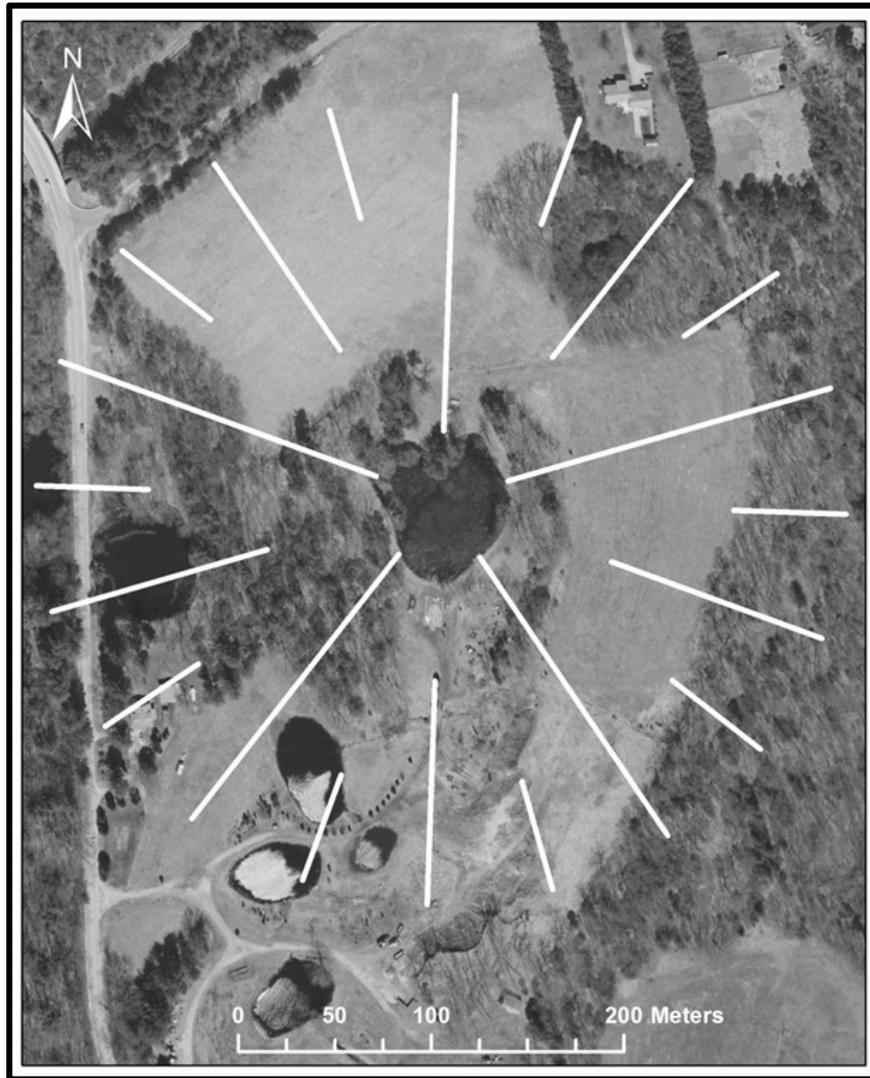
FIGURE 1. Sample schematic of scanning transects for detection of PIT tag-implanted Blue-spotted Salamanders (*Ambystoma laterale*) *in situ*. White lines indicate transect lines. Individual transects are 58, 116, and 174 m in length. (Orthophotography provided by the Connecticut Department of Energy and Environmental Protection).

to the end of a 182 cm straight section of PVC pipe with this backpack scanner.

We conducted 34 in situ surveys on separate dates between 19 April 2009 and 12 August 2011. Using the backpack scanner, we methodically scanned the habitats within 13.37 ha surrounding the breeding pool to detect PIT tag implanted Blue-spotted Salamanders *in situ*. In all years, we began scanning for Blue-spotted Salamanders shortly after they emigrated from the breeding pool (late-April or May). Scanning was conducted until August in 2009 and 2010 and until July in 2011. We conducted scanning transects opportunistically (i.e., as researcher time allowed) only on non-rainy days (during daylight hours) but independent of previous days' weather conditions.

Each survey consisted of scanning along 20 evenly-spaced straight line transects radiating out from the wetland (Fig. 1). We walked each transect using a hand-held global positioning system (GPS) unit (GPSmap 76Cx; Garmin International, Inc., Olathe, Kansas, USA) to ensure proper direction of travel. To allow for sampling a similar proportion of the circumference at various distances from the wetland, five transects 164 m in length started at the drift fence surrounding the pool, five transects 116 m in length began at 48 m from the fence, and 10 transects 58 m in length started at 106 m from the fence. We terminated all transects once the outer limit of a 164 m "buffer" from the fence was reached, as this distance represents the limit of primary terrestrial habitat for ambystomatid salamanders adjacent

to a wetland (see Semlitch 1998; Fig. 1). The average scanning swath was ~2 m wide, which accounts for the following percentages of the circumference surveyed at the designated distances: at fence = 4.4%, 48 m = 1.7%, 106 m = 2.1%, 164 m = 1.5%. Each set of transects covered 0.41 ha (3.07%) of the total 13.37 ha area located within the 164 m buffer from the drift fence (Fig. 1). We rotated succeeding sets of transects 5° clockwise to not repeatedly survey the same areas. If a scanning transect bisected a non-terrestrial landscape feature (i.e., a farm pond, house, or breeding wetland), we did not scan transect segments through those.

Upon detection, we confirmed salamander presence by carefully searching through the leaf litter with our hands. The duff and soil were searched and repeatedly scanned with a hand-held PIT tag reader (Pocket Reader; Biomark, Boise, Idaho, USA) until the implanted salamander or a PIT tag was found. After salamanders were found, we carefully re-covered them with leaf litter. We did not revisit salamander locations to assess salamander presence.

Enclosure scanning trials.—The PIT pack model we used in the enclosure scanning trials (i.e., tag-implanted salamanders) was a Destron-Fearing transceiver with a custom-built ~15 cm diameter antenna attached at the end of a forearm crutch (see Blomquist et al. 2008). During August 2008, we conducted scanning trials in six

3×3 m enclosures: four in oak-pine forest and two in a red maple forested wetland (in areas with saturated soil, but no standing water). We built enclosures using 0.9 m tall construction silt fence. To prevent escape of salamanders, we buried enclosure walls 15−20 cm in the ground and we curved the wall tops toward the interior of the enclosures. On six separate occasions, an observer released an undisclosed number (0–16 total, as chosen by the observer) of salamanders in each of two forest or wetland enclosures. The following day, we scanned the enclosure three times: the first and second by two naïve observers (independently) and the third by the informed observer. Naïve observers scanned a given enclosure until they were confident that all salamanders had been detected within that enclosure. The informed observer then scanned the area to see if more salamanders could be detected than the uninformed observers. Informed and uninformed observers did not rotate roles.

Buried tag trials.—To assess the subterranean detection range of the backpack scanner, we scanned for buried tags within three 10×30 m runways demarcated with pin flags; one each in the forest, red maple wetland complex, and hayfield. We constructed runways in this fashion to mimic *in situ* scanning transects. In each enclosure, we buried five tags (30 total) at each of the following depth classes based on detection depths

TABLE 1. Rankings of logistic regression models of PIT tag detection. K is the number of variables included in the model, and log (L) is the log-likelihood of the model. Models were ranked using change in Akaike's Information Criterion corrected for small sample size (ΔAIC_c) and Akaike's model weights (ω).

Rank	Model	K	Log(L)	AIC_c	ΔAIC_c	ω	Cum. ω
Enclosure Scanning Trials (Tag-Implanted Salamanders)							
1	HABITAT	2	-69.80	143.60	0.00	0.54	0.54
2	HABITAT + OBSERVER	3	-69.17	144.50	0.81	0.36	0.90
3	NULL	1	-72.95	147.90	4.27	0.06	0.96
4	OBSERVER	2	-72.34	148.70	5.09	0.04	1.00
Buried Tag Trials - Runways (Trials 1 and 4)							
1	DEPTH + HABITAT + DEPTH x HABITAT	4	-89.90	192.30	0.00	0.46	0.46
2	DEPTH	2	-94.89	193.80	1.56	0.21	0.67
3	DEPTH + HABITAT + OBSERVER + DEPTH x HABITAT	5	-89.83	194.30	2.03	0.17	0.84
4	DEPTH + OBSERVER	3	-94.82	195.80	3.50	0.08	0.92
5	DEPTH + HABITAT	3	-93.99	196.20	3.93	0.06	0.98
6	DEPTH + HABITAT + OBSERVER	4	-93.93	198.20	5.91	0.02	1.00
7	NULL	1	-122.58	247.20	54.89	0.00	1.00
8	OBSERVER	2	-122.53	249.10	56.85	0.00	1.00
9	HABITAT	2	-122.15	250.40	58.14	0.00	1.00
10	HABITAT + OBSERVER	3	-122.10	252.40	60.14	0.00	1.00

reported by Blomquist et al. (2008): 1–6, 7–12, 13–18, 19–24, 25–30, and 31–36 cm. One observer buried tags in 30 mL polyethylene vials by driving rebar to the desired depth, removing the rebar, gently pushing the vial to the bottom of the hole and backfilling the hole with soil. We used polyethylene vials because the material is known not to interfere with PIT tag detection (Kazyak and Zydlewski 2012). To facilitate tag recovery, we attached strings to the vials and laid them on the ground surface but hid them using dead organic debris and a small amount of soil. A second observer, unaware of the location of buried tags, scanned the runway in three separate passes to detect the tags. The informed observer then made one pass through the area to see if they could detect more tags than the naïve observer.

Data analysis.—For the enclosure scanning trials and the buried tag trials, we used an information-theoretic approach to evaluate competing hypotheses about the factors influencing tag detection (Burnham and Anderson 2002). We used an all-subsets approach to build logistic regression models including the following variables: whether scanning was conducted by an informed or a naïve observer (binary), actual depth of buried tags (discrete), the habitat type that the tags/salamanders were located in (i.e., forest, wetland, hayfield; categorical), and the interaction between depth buried and habitat type. Detection/non detection of PIT tags served as the response variable.

We ranked models explaining tag detection using Akaike's Information Criterion corrected for small sample size (AIC_c) and Akaike's model weights (ω). We used model averaging to derive parameter estimates from all models in each set (Anderson 2008). We

considered variables useful for describing PIT tag detection if the 95% confidence intervals for their odds ratios did not overlap one. For the buried tag trials, we included only the first pass of the naïve observer and the informed observer, as they are statistically independent.

We then calculated proportions and Wilson's 95% confidence intervals (Wilson score interval) with continuity correction for detection of PIT tag-implanted salamanders or buried PIT tags. We used the Wilson's method as other interval estimate methods for proportions tend to exhibit poor coverage and can produce improper intervals (see Newcombe 1998). Note that we used the AIC_c-ranked logistic regression models and associated odds ratios and confidence intervals to determine which variables affect tag detection; proportions and associated Wilson's 95% confidence intervals are provided for descriptive purposes only. We conducted all statistical analyses using the statistical software R version 2.14.2 (R Development Core Team 2012).

RESULTS

In situ scanning.—The total number of PIT-tagged salamanders released and hence potentially available for detection during each scanning event ranged from 290 to 532 individuals. We detected 0–5 salamanders per survey. This amounted to 41 salamander detections, which represented the locations of 36 individual salamanders as 31 individuals were located once, four were located twice, and one was located three times. We never detected any salamander at a single location multiple times and of the five individuals we detected more than once, only two were re-detected within the same year. Salamanders we detected more than once

TABLE 2. Model-averaged parameter estimates (β), standard error, odds ratios, and 95% confidence intervals from logistic regression explaining PIT tag detection rates. SE = unconditional standard error. Bolded parameters considered useful for describing tag detection (i.e., 95% confidence intervals do not overlap one).

Variable	Estimate	SE	Odds Ratio	95% CI Lower	95% CI Upper
Enclosure Scanning Trials (Tag-Implanted Salamanders)					
INTERCEPT	**1.64**	**0.53**	**5.13**	**1.81**	**14.57**
HABITAT (FOREST)	**1.23**	**0.53**	**3.41**	**1.21**	**9.63**
OBSERVER	0.31	0.28	1.37	0.78	2.39
Buried Tag Trials - Runways (Trials 1 and 4)					
INTERCEPT	**3.01**	**0.72**	**20.20**	**4.92**	**82.87**
DEPTH	**-0.13**	**0.03**	**0.87**	**0.82**	**0.93**
HABITAT (WETLAND)	-1.34	1.03	0.26	0.03	2.01
HABITAT (FOREST)	2.34	1.74	10.41	0.34	323.13
DEPTH × HABITAT (WETLAND)	0.06	0.05	1.06	0.97	1.16
DEPTH × HABITAT (FOREST)	-0.11	0.07	0.90	0.78	1.03
OBSERVER (NAÏVE)	-0.13	0.36	0.88	0.43	1.79

were detected 10–41 m from their previous detection locations. We expended ~119 survey hours to detect the 41 individual salamander locations (i.e., 0.34 individuals/h). On average we detected 0.34% of salamanders available for detection during each survey event. On 33 tag detection occasions, representing 48% of total tag detections, we located PIT tags that had been presumably shed by salamanders.

Enclosure scanning trials.—The top-ranked models included habitat type and observer (Table 1). Although there was also strong support for a model containing observer, results of model averaging indicated that the proportion of salamanders detected with the PIT pack was not affected by whether or not an observer knew the number of salamanders released in a given enclosure. Habitat type was found to be a significant predictor of tag detection rate as 95% confidence intervals for the odds ratios did not overlap one (Table 2). We detected 96% (Wilson's 95% confidence interval 89% to 98%) of tag-implanted salamanders in forest enclosures and 80% (Wilson's 95% confidence interval 78% to 92%) in wetland enclosures.

Buried tag trials.—The top-ranked model included depth, habitat, and the interaction between depth and habitat. There was also substantial support of the model including only depth (Table 1). Model averaging indicated however that the depth of buried tags was the only variable affecting tag detection, as the confidence intervals for the odds ratio of this variable did not overlap one (Table 2). We detected 92% (Wilson's 95% confidence interval 81% to 97%) of the tags at the 1–6 cm depth class and 22% (Wilson's 95% confidence interval 12% to 35%) at the 31–36 cm depth class (Fig. 2).

DISCUSSION

In situ scanning.—In our study, we detected 6.84% of PIT tagged *A. laterale* at least once during scanning surveys. There were 0.34 detections per hour of effort in covering an area of 13.37 ha during 119 h of surveying. This is lower than the 9.8% overall detection rate and 1.18 individuals detected/hour reported by Hamed et al. (2008), however their survey area was a 0.25 ha area of floodplain forest in Sullivan County, Tennessee, which they searched in entirety during each scanning occasion. While our study area was considerably larger (i.e., 13.37 ha), we only scanned 3.07% (0.41 ha) of it during each survey (Fig. 1). Structural complexity of the two different terrains may have also affected detection rates, as floodplain forest is flat while our study area was hilly with areas of rock outcrops and/or dense vegetation.

Enclosure scanning trials.—The lower detection rates of PIT tag-implanted *A. laterale* we observed in the wetland enclosures (80%) than in the forest enclosures (96%) was likely due to the greater structural complexity of wetland enclosures. Specifically, the hummock-hollow microtopography in the wetland enclosures resulted in a greater variety of refuge locations (i.e., tree root crevices, clumps of dead vegetation, sedge tussocks) for salamanders within the enclosure. Perhaps more importantly, this microtopography also affected how close the antenna could be to the ground surface. Independent of habitat type, our overall detection rate for implanted salamanders (91%) was markedly higher than for buried tags (57%). This may be attributed to salamanders commonly using surface habitat (e.g., leaf litter or cover objects), and not subterranean habitat (e.g., in small mammal burrows). This finding concurs with observations made by Klemens (1993) and Harding (1997) who both noted that, unlike their more fossorial, sympatric relatives such as Spotted Salamanders (*Ambystoma maculatum*), Blue-spotted Salamanders are commonly found under cover near the ground surface throughout the warmer months.

Buried tag trials.—For our buried tag trials, we were unable to detect buried tags at rates reported by Blomquist et al. (2008), who detected 33 ± 7% of PIT tags buried 43 cm in soil in Maine. Our trials detected 22% (Wilson's 95% confidence interval 12% to 35%) at the 31–36 cm depth class. We speculate this disparity may be due to differences in soil type, subtle differences in the orientation of buried tags in relation to the transceiver antenna, or a combination of both. Our buried tag scanning trials were also not as successful as those of Hamed et al. (2008) who were able to detect an implanted preserved Spotted Salamander (*Ambystoma maculatum*) specimen 27.5 cm underground in all 10 of their location accuracy trials. Note that Hamed et al. (2008) knew the exact location of the implanted salamander and thus could manipulate the orientation of their PIT tag reader antenna until the tag was detected; our scanning trials method more accurately reflects *in situ* scanning conditions. Our results are consistent with those from Cabarle et al. (2007) who reported an effective range of 8–22 cm below ground for the two antennae used in their experiment. This is similar to the conclusion of Blomquist et al. (2008) who state that the effectiveness of the PIT-pack used in their study would be limited for species that burrow deeper than 13 cm.

Detection probability.—We were able to detect *A. laterale* in their diurnal refuges *in situ* using a PIT pack. The majority of salamanders scanned were located under the leaf litter (i.e., not under cover objects) and likely
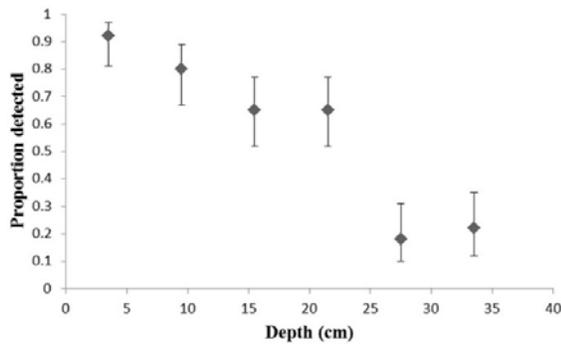
FIGURE 2. Proportions (± 95% confidence interval) of PIT tags detected using the backpack scanner as a function of depth in the soil in three 10 m × 30 m runways. Each depth had 15 tags available for detection. Proportions were calculated from all 12 passes.

would not be detected during traditional presence/absence surveys (see Vonesh et al. 2010), which, for salamanders, consists mainly of searching under cover objects. In a concurrent radio-telemetry study, we also found the majority of salamanders to be located just under the leaf litter. As was reflected in the enclosure scanning trials, detection probability using PIT-scanning surveys likely varies among different cover types, structural complexities, etc. within a study site. For example, a particular hillside in our study area was quite steep and strewn with large boulders and thick, thorny vegetation; detection probability in this area was likely lower than other, more easily traversable portions of the site. This variability in detection probability should be considered, estimated, and accounted for as best as possible when using PIT-scanning surveys for inferential purposes.

Expelled tags.—We found 33 tags when conducting *in situ* scanning. The detection of these tags was an unexpected finding; these were assumed to be expelled by the salamanders they were implanted into as they were found completely clean, absent of any residue of a decaying salamander or fecal matter of a potential predator. Throughout the entire course of the study, we observed no incident of apparent predation of a marked (PIT-tagged or transmittered) Blue-spotted Salamander. Of the 33 expelled tags, we never detected 15 within a salamander. Thirteen tags remained in the salamander for greater than two weeks (as shown by pitfall trapping data), and we recovered five expelled tags at the point of release. It is likely that the tags recovered near salamander release points were expelled from implant incisions. From informal inspection of salamanders held in captivity for the enclosure scanning experiments, and of salamanders observed during *in situ* scanning, we observed that PIT tag implant incisions were completely

healed after two weeks. We therefore speculate that the tags known to be retained by salamanders for at least two weeks were expelled by means other than through their surgical incisions. This phenomenon has been documented in frogs and toads by Tracy et al. (2010), who found that foreign objects can be sequestered and voided from the body cavity via incorporation into the bladder. It is likely that salamanders are capable of this as well. It may therefore be prudent to conduct long-term tag retention studies in captivity before tagging and releasing salamanders for *in situ* study. If this is not feasible, then tag-implanted salamanders should be double-marked (a mark in addition to an implanted PIT tag) so that it will be possible to discern whether a recaptured individual has not yet been implanted, or if it has expelled its tag.

Summary and implications.—The PIT pack can be used to reliably detect tags from 1–22 cm below the soil surface. While we successfully used PIT tag telemetry to locate Blue-spotted Salamanders *in situ*, the apparent tag expulsion rate in pure-diploid Blue-spotted Salamanders was high. These results suggest that the use of this technique may not be effective for quantitative applications where negligible tag loss is assumed. Tag retention may have improved if sutures were used to close implant incisions. This technique may be suitable for polyploid Blue-spotted Salamanders and other ambystomatid species which attain larger sizes and may exhibit higher retention rates. However, these species (e.g., *A. jeffersonianum*) may be found deeper under the soil surface thus reducing their detection rate with this method. A previous study (Homan et al. 2007) involving implanting PIT tags subcutaneously in both Spotted Salamanders and polyploid Blue-spotted Salamanders did not observe any instances of animals apparently expelling PIT tags (Bryan Windmiller, pers. comm.). The use of the method described in this paper seems promising for PIT tag detection of ambystomatid and other fossorial species; however, additional work is needed to assess the effectiveness of various implant methods/locations (i.e., tags implanted into the coelomic cavity or subcutaneously) and long-term retention rates.

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