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A Winter/Spring Study of Salamanders in a Disturbed, Fragmented Habitat Surrounded by Farm Land

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The continued development of fragmented habitats contributes to the decline of amphibians species, and there needs to be a method for evaluating the potential of these habitats to sustain amphibian populations. The effect of fragmented and disturbed habitats among salamander species is difficult to assess because these caudates are difficult to find and enumerate. One approach for estimating the total population of salamanders is to sample them when they migrate to their winter/spring breeding ponds. One fragmented habitat in Southern Michigan was sampled for breeding salamanders during the winter/spring of 1997. Four species of salamanders were detected at this site; blue-spotted salamanders (*Ambystoma laterale* complex), eastern newts (*Notophthalmus viridescens*), spotted salamanders (*A. maculatum*), and tiger salamanders (*A. tigrinum*). Blue-spotted salamanders (54 adults), and tiger salamanders (two adults). Based on markrecapture methods, the total population of blue-spotted salamanders at this site was estimated to be about 3100 (95% C.I. 2800– 3800). Two of the four species of salamanders detected at this site seem to be thriving, blue-spotted salamanders and eastern newts. Spotted salamanders are vulnerable to natural events that could cause the extirpation of this species from this site. Tiger salamanders, while detected in these woods, are too few to represent a sustainable population.

INDEX DESCRIPTORS: salamanders, fragmentation, migration.

There is evidence that amphibians are declining worldwide from the loss of suitable habitat and from unknown causes (Blaustein et al. 1994, Hecnar and M'Closkey 1996, Pounds and Crump 1994). The continuing formation of fragmented habitats may only exacerbate the decline of amphibians. However, little work has been carried out to study fragmented habitats and their potential support of viable populations of amphibians. It is possible that fragmented habitats of a certain size and quality may indeed sustain amphibian life. The determination of those characteristics of fragmented habitats that are necessary to support and sustain amphibian populations may be helpful for resource managers in determining the size and quality of a wooded area formed as the result of development.

The presence and relative abundance of anurans can be estimated from frog and toad calling surveys. The presence and relative abundance of salamander populations is not as easily ascertained. Many salamander species (e.g. *Ambystoma* spp.) are fossorial or spend their time below the leaf litter/forest debris and are not easy to count or detect (Duellman and Trueb 1994). One method that can be used to estimate populations of *Ambystoma* is to sample the adult populations when they migrate to breeding ponds. This method samples the breeding adult population and is, therefore, only a lower estimate of the total salamander population because juvenile and non-breeding adults would not be counted.

A preliminary survey of breeding salamanders (1996) in one temporary pond in an isolated wooded area showed the presence of three species of mole salamanders; *Ambystoma laterale* complex (blue-spotted salamanders), *Ambystoma maculatum* (spotted salamander) and *Ambystoma tigrinum* (tiger salamander). These woods are surrounded by cultivated farm land on three sides and a road on the eastern border, making this habitat a "wooded island." This initial survey suggested the possibility that a potentially diverse population of salamanders lived in these woods. A second, more in depth, survey was conducted during the winter/spring of 1997 to further characterize the salamander populations that live in this fragmented habitat, and is the subject of this paper.

METHODS

Site Description

This site is located approximately 2 km north of the small town of Bridgewater (southwest of Ann Arbor, Michigan). These woods have been logged three times since the turn of this century: once before World War II, once during World War II to supply wood for the Ypsilanti bomber factory, and once in 1964 to supply rough lumber for the farmers home (land owner, pers. comm. 1997). These woods show clear evidence of logging activity as indicated by the presence of tree stumps, old roads, and tire ruts. Hence, these woods have been heavily disturbed in recent times. An aerial photograph was taken of the site in early spring of 1997 (Fig. 1). The area of the site was calculated using Vernier calipers and a measured length of road adjacent to this site. Section A of Fig. 1 is a cultivated field demarcated by vegetation and was calculated to be 4.49 ha, which is equivalent to the size indicated by the owners of the property (4.45 ha). The total area of this study site was calculated to be 19.7 ha. These woods are of the oak-hickory deciduous forest type with the following trees comprising a majority of the species; silver maple (Acer saccharinum), shagbark hickory (Carya ovata), northern hackberry (Celtis occidentalis), button bush (Cephalanthus occidentalis), dogwood sp. (Cornus sp.), green ash (Fraxinus pennsylvanica), and the white oak (Quercus alba). High and low air temperatures and precipitation were recorded daily for this site.

Pond Characteristics

The site has five ephemeral ponds of sufficient depth to support breeding populations of salamanders (Fig. 1). Pond one is the largest

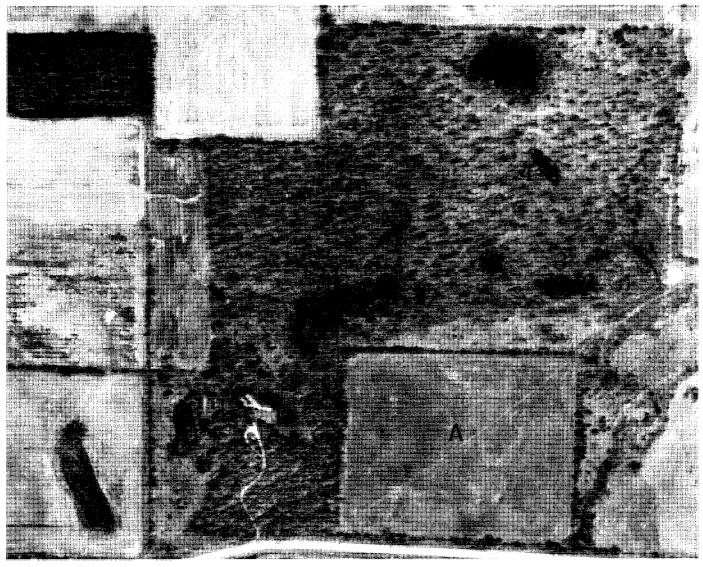


Fig. 1. Aerial photograph of 20 ha wooded lot near Bridgewater, Michigan. The letter A shows the field used to evaluate the method used for estimating the area of the site. The numbers are adjacent to those pond that were studied in this paper. Other potential sites seen in this photograph were to shallow to support salamanders for breeding (≤ 10 cm).

pond on the site with an area of 0.4 ha with a maximum depth of 59 cm. The pH of this pond during the winter/spring breeding season was 6.96. Pond two is the smallest pond studied on this site with an area of 0.010 ha. This pond had a maximum depth of 88 cm and a pH of 7.03. Ponds three, four, and five had areas of 0.1, 0.06, and 0.2 ha, respectively. The maximum depths of ponds three, four and five were 96 cm, 75 cm, and 85 cm, respectively. The pH of ponds three, four, and five during the salamander breeding season was 7.20, 7.24, and 7.34, respectively.

Salamander Traps

Minnow-type traps, with enlarged openings (5-6 cm), were placed in each of the five ponds. The number of traps used per pond (Pond 1, 6 traps; Pond 2, 2 traps; Pond 3, 8 traps, Pond 4, 2 traps; Pond 5, 9 traps) was based on a combination of suitable water depth and area of the pond. For example, while pond 1 is large in area, there is only a limited area that is deep enough to cover the trap. The unbaited traps were set out on March 2, checked daily, and collected on April 11, 1997. In the early part of this study many of the traps could not be recovered because of the formation of ice. These traps could not be checked until the ice thickness was less than two centimeters. A trap night is defined here to mean a single trap was examined after a minimum of one night in the pond. If a trap was ice-bound, a trap night was not counted until the trap was actually sampled. This study had a total of 1023 trap nights and caught 1078 salamanders for an average of 1.05 salamanders/trap/night.

Morphological Measurements

Salamanders from each pond were kept separate and transported to the authors residence for morphological measurements. Salamanders were anesthetized in 0.1% MS-222 (methanesulfonic acid salt of ethyl 3-aminobenzoate, Sigma Chemical Co., Milwaukee, Wisconsin) adjusted to pH 6.5. The total length (snout to tip of the tail) and the standard length (snout to the posterior angle of the vent)

						Pe	ond							
	1	a	1		2	2	1	}		4	5		To	otal
Salamander Species	U	R	U	R	U	R	U	R	U	R	U	R	U	R
Ambystoma laterale complex	63	0	126	3	36	6	468	80	77	15	90	4	79 7	108
Ambystoma maculatum	1	0	7	0	2	0	12	0	0	0	33	0	54	0
Notophthalmus viridescens	0	0	0	0	2	1	37	3	4	1	68	1	111	6
Ambystoma tigrinum	2	0	0	0	0	0	0	0	0	0	2	0	2	0

Table 1. Summary of salamanders caught or recaptured in five temporary ponds (1-5). With the exception of Pond 1, data are reported only for 1997. U = unique captures, R = number of recaptured animals.

^a1996.

were measured using a digital Vernier caliper. This caliper is capable of measurements to 0.01 mm, but in practice these measurements are precise only to 0.1 mm. Anesthetized salamanders were allowed to recover in a 12.0 l bucket with cold, vigorously aerated water. Even with these precautions, some mortality was observed (<10 individuals) among individuals of the blue-spotted salamander complex and the spotted salamander (two individuals). All newts, spotted salamanders and tiger salamanders were measured. Because males are relatively rare in blue-spotted salamander populations (Uzzell 1964) all males were measured while only the first 389 female members of blue-spotted salamander complex were measured. In addition to the above morphological measurements each salamander was examined for any gross deformities, shortened tails, and for the presence of obvious subcutaneous cysts.

Population Size Estimates

The population of blue-spotted salamanders in these ponds was estimated using mark-recapture methods (Donnelly and Guyer 1994). Salamanders caught for the first time had one toe on one foot clipped according to a scheme that identified that salamander as breeding in one particular pond. Unique toe clipping for each salamander, while desirable for certain aspects of mark-recapture studies, was not done because of the number of toes that would have to be clipped to uniquely identify 797 blue-spotted salamanders. During the 40 days these ponds were sampled there was no evidence that any salamanders migrated from one pond to another pond. The computet program used in this study is CAPTURE (Otis et al. 1978, Rextad and Burnham 1991): a program that assumes a closed study population. A closed population assumes that there is no immigration, emigration, or changes in population due to births or deaths. The small number of deaths associated with anesthetizing the animals was not considered significant (ten deaths out of 797 unique blue-spotted salamanders caught). The assumption of no immigration or emigration during the course of this study is probably valid because of the time of the year of this study. No salamanders were observed migrating over open fields, across frozen land, or crossing roads. In addition, there was no indication of salamanders using different ponds. The population of breeding salamanders could not have increased during the course of this study from recently laid eggs. The assumption of no deaths or a constant death rate among breeding salamanders is difficult to measure or estimate. However, it seems plausible that few of the breeding blue-spotted salamanders will die over the short time period of this study (40 days). The CAPTURE program is limited by the maximum number of trapping occasions allowed for any analysis (18 trapping occasions). Since this study trapped salamanders for 40 capture occasions the number of salamanders caught for two days in a row were used for one trapping occasion as input into the program except for the last four trapping occasions which combined three days in a row. The program was run multiple times with different starting dates to see if using different combinations of days had any affect on the population estimates. The results were robust to the starting date and the starting date for combining data had little effect on the population estimates (data not shown).

RESULTS AND DISCUSSION

The initial survey in the winter/spring of 1996 detected the presence of three Ambystoma species of salamanders. Eastern newts (Notophthalmus viridescens) were first found during the summer of 1996 by sweeping pond five with a net. Other salamander species potentially present in these woods include the red-backed salamander (Plethodon cinereus), the small-mouth salamander (A. texanum), and the four-toed salamander (Hemidactylium scutatum) (Harding 1997). Extensive searches of logs and forest debris during the summer and autumn found only blue-spotted salamanders. In the winter/spring of 1997, a comprehensive survey of breeding salamanders was completed, and all five of the ephemeral ponds were sampled over the entire breeding season for Ambystoma sp. salamanders (Harding 1997, Pfingsten and Downs 1989) This survey did not attempt to encompass the entire newt breeding season (which can extend from March through June), and sampling of ponds was terminated when no Ambystoma species were captured for a period of several days. A summary of breeding salamanders caught and recaptured during the preliminary survey in 1996 and the survey in 1997 from these ponds is shown in Table 1.

The daily capture of the three predominant species of salamanders was used to estimate peak migration of salamanders to their breeding ponds (Fig. 2): tiger salamander capture frequency is not plotted since only two were captured during this survey. In addition, the daily high and low air temperatures are shown in the middle panel of Fig. 2. The daily capture of breeding blue-spotted salamanders showed several nodes with a peak migration date around March 21-22. Spotted salamanders showed a peak migration to their breeding ponds on March 29, about a week after the blue-spotted salamanders. The assignment of a peak migration for the eastern newt is tentative since the entire breeding season was not sampled. However, the data in Fig. 2 suggests that newts showed a peak migration to these ponds during April 5-7, about a week later than the spotted salamanders. Also shown in Fig. 2 are the daily capture of each salamander species by gender. The vast majority of blue-spotted salamanders are triploid females (Uzzell 1964). Male newts and male spotted salamanders were present at two and four times the number of females, respectively. A similar male/female ratio has been reported from several locations in Ohio (Pfingsten and Downs 1989) suggesting that there was no bias in trapping males.

Chromosomes of salamanders from the Ambystoma laterale complex

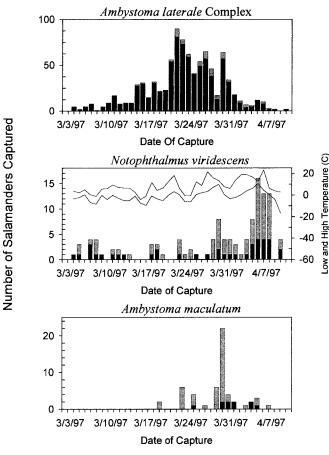


Fig. 2. Daily capture of the three main salamander species found on this site. The dark bars represent the number of females caught; the light, hatched bars represent the number of males caught.

range from diploid to pentaploid. A majority of the female bluespotted salamanders are triploid, but tetraploid and pentaploid female blue-spotted salamander have also been found. A sample of 28 blue-spotted salamanders selected randomly from each pond were sent to Dr. James Bogart (Univ. of Guelph) for chromosome and electrophoretic analysis. Chromosome analysis determined that 22 salamanders were triploid females, four were tetraploid females and two were diploid male blue-spotted salamanders (Bogart 1974, 1989, Bogarr et al. 1985). Electrophoretic analysis of characteristic enzymes showed that triploid salamanders had two sets of laterale chromosomes and one set of jeffersonianum chromosomes (LLJ) while the tetraploids had three sets of laterale and one set of jeffersonianum chromosomes (LLLJ). The diploid males had two sets of laterale chromosomes (LL). Based strictly on morphology, one polypoid male (presumably a triploid) was found. The low frequency of male polyploids in this population (1/797) is similar to that (3/1300) found by Clanton (1934, cited in Uzzell 1964).

There is the possibility that salamanders from a fragmented habitat might show stunted growth, compared ro salamanders in larger habitats, because they would not have similar nutritional resources. Morphological parameters were examined to compare the salamanders at this site with other salamanders from sites nearby. The total lengths and standard lengths of all of newts, spotted salamanders, tiger salamanders and about half of the blue-spotted salamanders are shown in Table 2. Table 2 also shows some of the morphological characteristics of salamanders from other sites in Michigan and from

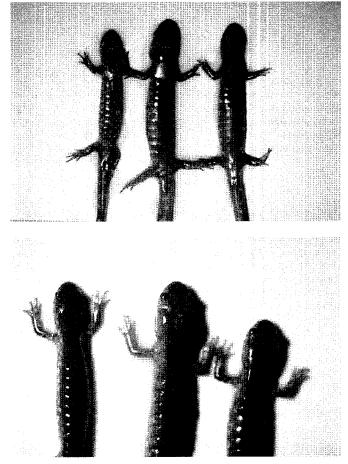


Fig. 3. Photograph of metacercarial cysts of blue-spotted salamanders (*Ambystoma laterale*). The upper panel is a photograph of the ventral surface of three blue spotted salamanders. The lower panel is a photograph of the dorsal surface of three blue-spotted salamanders. The salamander on the left is a normal, diploid female, the center salamander is a triploid female with a heavy infection of *Clinostomum* sp. cercaria, the salamander on the right is a normal, triploid female.

states and provinces bordering Michigan. A strict statistical comparison cannot be made between these sites because many of the data in the literature did not report standard deviations or standard errors of the mean. However, in all cases, the mean standard length and mean total length of each species from locations other than Bridgewater, MI were within one standard deviation of the mean morphological data from Bridgewater (Table 2). This suggests that the morphological characteristics of salamanders in Bridgewater are not significantly different from salamanders found in and around Michigan.

The number of salamanders with deformities (usually multiple feet or toes) were noted (Table 3). In addition, some blue-spotted salamanders had shortened tails, probably due to a predatory attempt. These salamanders were not included in the total length measurements. Many blue-spotted salamanders and a few spotted salamanders had obvious subcutaneous cysts (Fig. 3) that contained white "worms" upon excision. These trematode parasites were identified to the genus level as *Clinostomum* sp. (details to be presented elsewhere). Piscivorous birds (e.g., great blue herons *Ardea herodias*) are the definitive host for these flukes. It is not clear when these salamanders became infected with cercaria from snails, or if these salamanders are a true intermediate host for this parasite. It is possible that these

Location	Species	Sex	Ploidy	SL	TL	Reference
IL, MA, MI	Al	F	3n	72 (76)	126 (76)	Uzzell 1964
OH, ON, WI		Μ	2n	56 (31)	99 (27)	Uzzell 1964
IL, MA, NI	Al	F	3n, 4n	$77 \pm 7 (124)$	*	Lowcock et al. 1992
OH, ON, WI		Μ	2n	$59 \pm 7(208)$	*	Lowcock et al. 1992
ON	Al					
ON	Al					
Bridgewater, MI	Al	F	2n, 3n, 4n	78 ± 7 (389)	138 ± 15 (389)	This study
Bridgewater, MI	Al	М	2n	$63 \pm 3 (53)$	$119 \pm 7(53)$	This study
NW Ohio	Am	F		88 (11)	174 (11)	Pfingston & Downs 1989
NW Ohio	Am	М		86 (40)	166 (40)	Pfingston & Downs 1989
NE Ohio	Am	F		90 (37)	168 (37)	Pfingston & Downs 1989
NE Ohio	Am	М		81 (90)	156 (90)	Pfingston & Downs 1989
Bridgewater, MI	Am	F		91 ± 8 (10)	$159 \pm 14 (10)$	This study
Bridgewater, MI	Am	М		83 ± 8 (40)	$152 \pm 15 (40)$	This study
NE Ohio	Nv	F		41 (21)	88 (21)	Pfingston & Downs 1989
NE Ohio	Nv	М		42 (21)	94 (21)	Pfingston & Downs 1989
Bridgewater, MI	Nv	F		$44 \pm 4 (35)$	83 ± 9 (35)	This study
Bridgewater, MI	Nv	М		$44 \pm 6 (76)$	84 ± 9 (76)	This study
Ohio	At	F		106 (15)	200 (15)	Pfingston & Downs 1989
Bridgewater, MI	At	F		101, 120	74, 214	This study

Table 2. Means of standard length (SL) and total length (TL) ± 1 SD to the nearest mm for Ambystoma laterale (Al), A. maculatum (Am), A. tigrinum (At), and Notophthalmus viridescens (Nv) from Bridgewater, Michigan and from other sites in Michigan, adjacent states and provinces^a. Sample size is given in parentheses.

^aabbreviations: IL = Illinois, MA = Massachussers, MI = Michigan, OH = Ohio, ON = Ontario, WI = Wisconsin. * = not reported.

Table 3. Number (frequency in %) of subcutaneous metacercarial parasitic cysts, deformities, and shortened tails in salamanders in five temporary ponds (1-5) during 1997.

Salamander	Pond							
Species	1	2	3	4	4 5			
cysts Ambystoma laterale complex Ambystoma maculatum	1 (0.8)		3 (0.6)		5 (5.6) 4 (12.1)	9 (1.1) 4 (7.5)		
deformities A <i>mbystoma laterale</i> complex	3 (2.3)		6 (1.3)		4 (5.2)	13 (1.6)		
shortened tails <i>Ambystoma laterale</i> complex	5 (4.0)	3 (8.3)	29 (6.2)	3 (3.9)	4 (4.4)	44 (5.5)		

salamanders were infected by opportunistic cercaria. It may be that frogs (e.g. green frog, *Rana clamitans*) in these ponds are the main intermediate host for this parasite.

This survey marked individuals by using a toe clipping protocol that identified a salamander as coming from a single pond. The capability of estimating total populations from a mark-recapture study depends heavily on the number of recaptured animals (Donnelly and Guyer 1994). An examination of Table 1 shows that in 1997 about 14% of the total blue-spotted salamanders were recaught while only about 5% of the newts were recaptured and none of the other two species were recaptured. The CAPTURE program was used to estimate the total population of blue-spotted salamanders at their winter breeding ponds. It is important to recognize that this analysis does not represent the entire blue-spotted salamander population and probably underestimates the total population because of salamanders not caught in traps during the winter breeding season such as new metamorphs, immature salamanders, or those adults that, for whatever reason, are not breeding during the period of this study. Table 4 shows the CAPTURE estimates for two possible scenarios from each of the five ponds and for the total breeding population. The first scenario is an option in the CAPTURE program that assumes that all of the breeding blue-spotted salamanders have the same chance of being caught on any given trapping occasion (Table 4, null model, m[0]). Fig. 2 shows that the capture frequency of these salamanders had peaks and valleys over the time course of this study suggesting that this assumption was not valid. The second scenario assumes that the chance of capturing breeding salamanders varies from day-to-day and that there is a time-dependence on the frequency of capturing blue-spotted salamanders on each trapping occasion (Table 4, Darroch model, m[t]). This study measured the recapture frequency from individual ponds and from the total population. It is possible to conduct an internal consistency check on

	<u> </u>						
Model	1	2	3	4	5	All Ponds	Total
Null; m(0) EST CI	1700 770–4200	120 70–250	1600 1300–1900	230 160–380	930 410–2300	4580	3300 2800–3900
Darroch; m(t) EST CI	830 530–1300	110 60–220	1500 1300–1900	220 150 –3 50	590 340–1100	3250	3100 2800–3800

Table 4. CAPTURE population estimates (EST) and 95% confidence intervals (CI) of Ambystoma laterale complex populations in individual ponds (1-5) and for the total population.

Table 5. Temperature and precipitation dependence of capturing male (M) and female (F) breeding salamanders (*Ambystoma laterale*—Al, *A. maculatum*—Am, *Notophtbalmus viridescens*—Nv) from Bridgewater, Michigan, assessed using of Spearman's Rank Correlation Coefficients (r_s). P-values are provided in parentheses, with $P \leq 0.05$ considered significant. The daily low and high air temperatures are plotted in Figure 1.

			۲ _s					
Species			Тетре					
	Sex	n	Low	High	Precipitation			
Al complex	М	53	0.550 (0.001)	0.512 (0.001)	0.087 (0.602)			
1	F	744	0.151 (0.367)	0.156 (0.351)	0.001 (0.994)			
Am	М	44	0.417 (0.009)	0.279 (0.090)	0.137 (0.412)			
	F	10	0.240 (0.147)	0.171 (0.303)	0.366 (0.024)			
Nv	М	76	0.342 (0.036)	0.261 (0.114)	0.371 (0.022)			
	F	35	0.276 (0.094)	0.202 (0.224)	0.176 (0.289)			

the data by comparing the total population of salamanders estimated by summing the total salamanders from each pond with the estimated site population total: the two estimates should be the same. The population estimate of blue-spotted salamanders from each pond and the 95% confidence interval for this estimate is shown in Table 4. The computer estimate of the total breeding population and its 95% confidence interval is shown in the eighth column. If one sums the population estimate for each pond (Table 4, column seven) the sum should be about the same as the population estimate for the whole site (Table 4, column eight). The population sum from all of the ponds using the null model is much larger (>95% C.I) than the population estimated for the entire site. This would suggest that the assumptions using the null model were not met. A similar examination of the total population of salamanders calculated by adding up the totals from each pond using the Darroch option (3250 bluespotted salamanders) was about the same as the estimate for the entire population (3100 blue-spotted salamanders) suggesting that this model was the more appropriate model to use for estimating breeding blue-spotted salamanders. Based on the CAPTURE analysis, it would seem that these woods can support about 3000 breeding salamanders. It was not possible to estimate the total population of the other salamander species because the number of recaptured salamanders was too low. Future surveys at this site will use a larger number of traps in order to increase the recapture frequency of spotted salamanders and eastern newts. It is unlikely that there will ever be enough tiger salamanders captured to accurately estimate their total population.

The daily high and low air temperatures and precipitation were measured to test the possibility that these weather parameters could explain some of the variance in the capture frequency of the three main salamander species. These salamanders were sampled at a time of the year that can have subfreezing temperatures that may influence their capture rates. In order to evaluate the effect of these weather parameters on capture rates the number of salamanders caught per day was analyzed using the Spearman's rank correlation for each gender and species of salamander (Table 5, with the exception of tiger salamanders) (Zar 1996). Male blue-spotted salamanders showed a significant positive Spearman's rank correlation coefficient with daily high and low air temperatures that accounted for about 30% and 26% of the daily variance ($r_s = 0.55$; $r_s = 0.512$) in capture frequency, respectively. The positive correlation suggests that they were caught more frequently on warmer days. Male spotted salamanders and male newts also showed a significant positive Spearman's rank correlation coefficient with daily low air temperatures that accounted for about 17% and 12% of the variance in capture frequency ($r_s =$ 0.417; $r_s = 0.342$), respectively. This suggests that these salamanders were more sensitive to the effects of low air temperatures. Male newts and female spotted salamanders showed a significant increase in capture rates with daily precipitation accounting for about 13% and 14% of the variance ($r_s = 0.366$; $r_s = 0.371$) in the daily capture of these salamanders, respectively. It is difficult to interpret these data because only a small fraction of the variance could be accounted for using these weather variables (12-30%) and there was not a consistent relationship with species or gender.

DISCUSSION

This wooded "island" supports four species of salamanders at different populations levels. The data presented here suggest that this habitat supports a population of the Ambystoma laterale complex and Notophthalmus viridescens. The population of A. maculatum has few females (10) and this population could be susceptible to a natural disaster (extreme weather, disease, increase in predators of eggs, larvae, or adults, etc.) that would extirpate this species from these woods. The fact that few tiger salamanders were captured suggests that this species is on the brink of being eliminated from this site. The morphological characteristics of this population of salamanders are similar to those of other populations inside and outside of Michigan, suggesting that these salamanders have adequate nutritional resources to support a normal pattern of growth. These data suggest that a twenty hectare isolated habitat, with appropriate fauna and ephemeral breeding ponds, can support populations of at least two species of salamanders-the blue-spotted salamander and the eastern newt. A similar conclusion is not possible for the other two species present in these woods. This study examined only one site, and more research is needed on other sites in order to have an accurate assessment of what is required to support and sustain amphibian life in fragmented habitats.

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