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Front Cover: The barred grass snake *Natrix helvetica*, which is now considered a full species elevated from *Natrix. n. helvetica*. This taxonomic change led to confusing stories in the British press that suggested we now had four instead of three native snake species. We still have only three species. See article by Uwe Fritz and Carolin Kindler on p.44. (photo: © Jason Steel)

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Preliminary morphometrics, growth, and natural history observations of the short-headed garter snake, *Thamnophis brachystoma* at two urban sites in Erie County, Pennsylvania, USA

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ABSTRACT - We used mark-recapture techniques to study the short-headed garter snake, *Thamnophis brachystoma* at two urban sites (Shannon Road and McClelland Park) in Erie County, Pennsylvania, USA. Mean snout-vent length (SVL) and weight was greater in females than males regardless of age class; whereas relative to total length, tail length was consistently greater in males than in females. Sex ratios did not differ significantly from 1:1 regardless of age-class. At Shannon Road, adults significantly outnumbered juveniles 3.3 to 1. While at McClelland Park, juveniles (N = 63) were nearly twice as numerous as adults (N = 31). Data regarding estimated growth are also reported. The results of the present study conform to previously published data from populations in Pennsylvania and a population near Olean, New York.

INTRODUCTION

The short-headed garter snake, Thamnophis brachystoma (Fig. 1) is relatively small with a recorded maximum length of 578 mm (Lethaby, 2004). It is found primarily in early successional habitats in the Allegheny High Plateau of north-western Pennsylvania and south-western New York, USA (Price, 1978). Introduced populations have been reported in New York (Bothner, 1976), Ohio (Novotny, 1990; Novotny et al., 2011), and Pennsylvania (Price, 1978; McCoy, 1982). In Erie County, Pennsylvania extralimital populations occur in glaciated regions, as far west as 1.5 km of the Ohio-Pennsylvania border (Gray, 2005). The first report of T. brachystoma in Erie County was based on 2 specimens collected from near Corry (Richmond, 1952). Since then, numerous other T. brachystoma populations have been discovered in Erie County. Urban populations within the city of Erie have been attributed to human introduction (Conant, 1975; Price, 1978; McCoy, 1982). Sometime between 1952 and 1970, Neil Richmond inadvertently released a number of T. brachystoma near Erie (Engelder, 1988). In 1970, D.R. Hower collected a specimen (Carnegie Museum of Natural History, CM 53678) 1.6 km south of Wesleyville.

Although *T. brachystoma* (Cope, 1892) was first described about 125 years ago there remain significant gaps in our understanding of the natural history and ecology of this species especially in urban environments. In Pennsylvania, it is of conservation concern due to its uncertain status, limited distribution, and evidence of population declines (Bothner, 1986; Jellen, 2010). For these reasons we chose to study the natural history of *T. brachystoma* at two urban sites in Erie, Pennsylvania, USA that are likely the same population from which Hower collected his specimen. The present paper summarises the morphometrics and growth of *T. brachystoma* from these sites and places these in the context of other unpublished sources regarding the ecology and natural history of this species. This study complements



Figure 1. Adult female Short-headed garter snake, *Thamnophis brachystoma* from the Shannon Road site in Erie, Pennsylvania, USA

previously published data on population size and density, biomass, movement and site fidelity for *T. brachystoma* at the same two sites (Lethaby & Gray, 2015, 2016).

STUDY SITES

There were two study sites, both within the city of Erie, Pennsylvania. They were 1.4 km apart and separated by open green spaces, tree covered areas and some housing (Fig. 2).

Shannon Road

This site (Fig. 3), the property of The Behrend College of Pennsylvania State University, is approximately 5.8 ha, of which ca. 3.6 ha of old field habitat was sampled. Grasses, black-berry (*Rubus* sp.), wild strawberry (*Fragaria virginiana*), and goldenrod (*Solidago* sp.) were common, as were the shrubs red-osier dogwood (*Cornus sericea*) and



Figure 2. Map of the study sites in south-eastern Erie, Pennsylvania, USA. A) McClelland Park. B) Shannon Road. Map courtesy of Google Earth.

northern arrow-wood (*Viburnum recognitum*). Tree species occurring mainly along the perimeter of the sampled habitat included paper birch (*Betula papyrifera*), red maple (*Acer rubrum*), black-locust (*Robinia pseudoacacia*), northern red oak (*Quercus rubra*), basswood (*Tilia americana*), eastern cottonwood (*Populus deltoides*), and willow (*Salix* sp.). Potential earthworm prey at Shannon Road included Allolobophora chlorotica, Aporrectodea sp., Lumbricus rubellus, and L. terrestris. In addition to T. brachystoma two other species of snake were present, Storeria dekayi and Storeria occipitomaculata.

McClelland Park

This site (Fig. 4), the property of the City of Erie, is approximately 22 ha, of which ca. 4.8 ha of old field habitat was sampled. It is within 400 m of Hower's collection site. The dominant herbaceous plants in the sampled area were mugwort (Artemisia vulgaris), grasses and sedges, along with goldenrod (Solidago sp.). Tree species occurring mainly along the perimeter of the sampled habitat included Norway maple (A. *platanoides*), northern red oak (Q. rubra), eastern cottonwood (P. deltoides), and black-locust (R. pseudoacacia). McDannel Run bisects the eastern section of the property, and flows in a northerly direction, eventually emptying into Lake Erie. Potential earthworm prey at McClelland Park included Aporrectodea sp., L. rubellus, and L. terrestris. In addition to T. brachystoma, a single specimen of one other snake species (Thamnophis sirtalis) was observed.

METHODS

During April – August 2011 we used a combination of plywood coverboards (0.61 x 0.61 m) and pre-existing



Figure 3. Cover board in Short-headed garter snake, *T. brachystoma* habitat at Shannon Road site in Erie, Pennsylvania, USA, 14 July 2011

cover objects (primarily flat rocks) and debris to sample *T. brachystoma* at the two Erie County sites. Dimensions of pre-existing cover objects and debris were not obtained. Plywood coverboards were employed at 16 sampling plots at Shannon Road and 7 sampling plots at McClelland Park. Individuals were captured by hand, sexed, and their snout-vent length (SVL) and tail length (tl) measured to the nearest millimeter by gently straightening each snake along a metric ruler. The snakes were weighed with Pesola spring balances, accurate to 0.1 g (snakes \leq 10 g), 0.25 g



Figure 4. Short-headed garter snake, *T. brachystoma* habitat at McClelland Park site in Erie, Pennsylvania, USA, 14 July 2011

(snakes 10 - 30 g), and 1.0 g (snakes larger than 30 g). Sex of mature T. brachystoma was determined by examining the base of the tail. In males, the hemipenes cause the sides of the base of the tail to bulge, whereas in females, the base of the tail is more tapered (Rossman et al., 1996). In male neonates and young ca. 150 mm or less, the hemipenes were manually everted by grasping the snake at mid-tail and rolling the thumb on the ventral surface towards the cloaca. Snakes were considered mature if at least 220 mm SVL in males, and at least 250 mm SVL in females (Pisani & Bothner, 1970; Rossman et al., 1996). In addition to the aforementioned data, we also noted number of dorsal scale rows at midbody, number of pre-oculars and post-oculars, as well as the number of supralabials and infralabials. Any scale anomalies, such as cleft or fused scales, and whether or not the tail was complete was also recorded as an aid to individual recognition. Snakes were individually marked with a portable cautery unit (Winne et al., 2006).

Daily growth rate was estimated by obtaining the difference in SVL between the initial and latest capture, then dividing this by the number of days between capture dates.

Table 1. Summary of mean snout-vent(SVL) length (mm), relative tail length (tl/TL), and weight (g) of immature *T. brachystoma* from Erie, Pennsylvania, USA. Sample size (N). Statistically significant results are marked with an asterisk.

	Mean ± 95% C.I.	range	(N)	t-test (two-tailed)
male SVL	194.4 ± 6.2	159.1 - 218.6	33	
female SVL	209.6 ± 7.9	162.8 - 249.5	43	t' = -3.06, <i>df</i> = 73, <i>P</i> = 0.003*
male tl/TL	0.245 ± 0.003	0.228 - 0.262	32	
female tl/TL	0.226 ± 0.003	0.212 - 0.256	39	t = 9.24, <i>df</i> = 69, <i>P</i> < 0.0001*
male weight	5.1 ± 0.4	3.0 - 7.2	33	
female weight	6.2 ± 0.5	3.4 - 9.5	43	t' = -3.49, <i>df</i> = 73, <i>P</i> = 0.0008*

Only snakes that were recaptured at least 30 days after their initial capture were used. Furthermore, only instances for which the increase in SVL between initial and latest capture was greater than 4% were considered. This was done to exclude individuals that had not grown.

Descriptive statistics used to summarise morphometric data included the mean \pm 95% confidence interval, range, and sample size. Student's t-tests (two tailed, $\alpha = 0.05$) were used to compare SVL, tail length as a proportion of total length (tl/TL), and weight between sexes and within and between sites. Snakes with incomplete tails were not used for some comparisons, such as relative tail length. Prior to performing a t-test, an F-test was used to determine whether variances were homogenous. In the event variances were heterogeneous, a t-test assuming unequal variances was employed (Runyon et al., 1996) and is indicated with t'. Chi-square tests were used to determine if sex ratios and immature to adult ratios were significantly different from a 1:1 ratio ($\alpha = 0.05$). Yates' correction for continuity was used in the calculation of Chi-square tests (Fowler et al., 1998). Data from both sites were pooled to increase sample sizes. With the exception of Chi-square tests, which were calculated by hand, all statistical analyses were performed with Microsoft Excel 2010.

Our data were compared with unpublished data collected during a study of *T. brachystoma* in the vicinity of Olean, New York, USA (Pisani & Bothner, 1970). The data sheets, photomicrographs, and prepared slides of the current study are to be deposited in the Natural History Museum at the Tom Ridge Environmental Center.

RESULTS

Unless otherwise stated the results presented below are from the analysis of data from the Shannon Road and McClelland Park sites combined.

Morphometrics of immature *T. brachystoma*. There were significant differences in mean SVL and weight between immature males and immature females, with females being longer and heavier (Table 1). A significant difference in

Table 2. Summary of mean snout-vent (SVL) length (mm), relative tail length (tl/TL), and weight (g) of adult *T. brachystoma* from Erie, Pennsylvania, USA. Sample size (N). Statistically significant results are marked with an asterisk.

	Mean ± 95% C.I.	range	(N)	t-test (two-tailed)
male SVL	273.4 ± 11.7	227.3 - 336.0	32	
female SVL	315.8 ± 12.8	250.5 - 447.0	50	t' = -4.95, <i>df</i> = 79, <i>P</i> = 0.0001*
male tl/TL	0.256 ± 0.003	0.239 - 0.274	31	
female tl/TL	0.223 ± 0.003	0.186 - 0.240	42	t = 14.53, <i>df</i> = 71, <i>P</i> < 0.0001*
male weight	12.7 ± 1.6	7.8 - 21.6	32	
female weight	23.4 ± 3.5	7.8 - 56.3	50	t' = -5.68, <i>df</i> = 67, <i>P</i> = 0.0001*

Table 3. Comparison of adult *T. brachystoma* snout-vent length (SVL), total length (TL) and relative tail length (tl/TL) between sites in New York and Pennsylvania, USA. Measurements are in millimeters. The mean \pm 95% confidence intervals are listed, followed by sample sizes in parenthesis.

	Pennsylvania from Hulse et al. 2001	New York from Pisani*	Pennsylvania from current study
SVL	♂ 273.1 ±14.6 (N = 102)	266.6 ±5.9 (N = 74)	273.4 ±11.7 (N = 32)
	♀ 325.3 ±14.2 (N = 93)	302.9 ±9.1 (N = 42)	315.8 ±12.8 (N = 50)
TL	♂ 366.8 ±21.0 (N = 93)	358.3 ±8.0 (N = 73)	366.9 ±16.1 (N =31)
	♀ 418.0 ±18.8 (N = 74)	390.6 ±11.2 (N = 42)	398.5 ±15.8 (N =42)
tl/TL	♂ 0.256 ±0.004 (N = 93)	0.256 ±0.003 (N = 73)	0.256 ±0.003 (N = 31)
	♀ 0.230 ±0.004 (N = 74)	0.225 ±0.005 (N = 42)	0.223 ±0.003 (N =42)

*Statistics calculated from unpublished data generously provided by George Pisani

tail length relative to total length was detected between immature males and immature females, with males having relatively longer tails (Table 1). One (3%) immature male and four (9%) immature females were stub-tailed.

Morphometrics of adult *T. brachystoma*. Among adults, significant differences in mean SVL and weight were detected between males and females, with females being slightly larger and heavier (Table 2). A significant difference in relative tail length was detected between adult males and adult females, with males having relatively longer tails (Table 2). Of 32 adult males only one (3%) was stub-tailed, whereas of 50 adult females eight (16%) were stub-tailed.

Sex ratios and immature to adult ratios. The sex ratio of immature male to female specimens was 33:43 (1:1.3), and did not differ significantly from 1:1 ($\chi 2 = 1.06$, df = 1, P = 0.30). The sex ratio of adult male to adult female specimens was 30:44 (1:1.5), and did not differ significantly from 1:1 ($\chi 2 = 2.28$, df = 1, P = 0.13). The ratio of immature to adult *T. brachystoma* was 76:74, almost exactly 1:1 ($\chi 2 = 0.01$, df = 1, P = 0.93). However, when compared between sites, immature to adult ratios were significantly different ($\chi 2 = 25.22$, df = 1, P < 0.01), with Shannon Road having a greater proportion of adults (1:3.3) and McClelland Park having a greater proportion of immatures (1:0.5).

Growth. Growth rate of immature *T. brachystoma* (initial SVL 167.4 – 221.8mm) recaptured 31 - 59 days after initial capture averaged 1.22 ± 0.24 mm per day (range 0.82–1.54, n = 8).

Growth rate of two adult *T. brachystoma*, a male (initial SVL 229.7mm) and a female (initial SVL 337.5mm) recaptured 38 and 86 days after initial capture was 0.4 mm per day and 0.59 mm per day, respectively.

DISCUSSION

The average SVL and TL of adult *T. brachystoma* from the Erie County sites were similar to other Pennsylvania specimens (Hulse et al., 2001). When compared to the published mean SVL for adult males (256.2 mm) and females (273.5 mm) for *T. brachystoma* from the vicinity of Olean, New York (Pisani & Bothner, 1970), those from Erie were considerably larger. However, the smallest mature male and female observed by Pisani & Bothner (1970) were 210 mm and 250 mm SVL, respectively. Reviewing

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the original data for Olean, it was found that six males (mean SVL 171.2 ±19.4 mm) and sixteen females (mean SVL 196.4 \pm 21.2 mm) were smaller than these values, and presumably immature. Therefore, we excluded the data for these specimens in our analysis. When this was done, the average SVLs and TLs were similar between sites (Table 3). When there are body size differences between snake populations this can be attributed to several factors, including geographic variation in climate, availability and quality of prey, inter- and intraspecific competition, and predation intensity (King, 1989). Olean, New York and Erie, Pennsylvania are at approximately the same latitude, 42.0737° and 42.0803°, respectively, and have similar climates. Olean, however is generally cooler than Erie, likely a result of Olean's higher elevation of 432 m compared to Erie's 222 m. Due to the proximity of the two Erie County sites (approximately 1.4 km apart), it is expected that climate would be similar. Both Erie County sites had similar potential earthworm prey, although there may have been differences in prey abundance. Interspecific competition was also potentially different between sites. Thamnophis sirtalis was relatively common around Olean, New York (Pisani & Bothner, 1970). With regards to the Erie County sites, at McClelland Park, where T. brachystoma and a single T. sirtalis were observed, interspecific competition would presumably not be a factor influencing SVL. Conversely, at Shannon Road, other snake species known to feed on earthworms were present. However, the two species, S. dekayi and especially S. occipitomaculata, are primarily predators of gastropods (Lazell, 1976; Hulse et al., 2001). In a sample of 84 scats from 74 Pennsylvania S. dekayi, fortyseven contained only slug remains (e.g., jaws, radulae, and shells), while six contained only earthworm setae. Both slug and earthworm remains were present in seven (Gray, 2013). Although our study did not focus on the effects of predation intensity, differences existed in potential predators of T. brachystoma between sites (see below). Potential predators at the New York sites were not noted (Pisani & Bothner, 1970; Engelder, 1988).

Relative tail length and weight were also similar to previous reports (Barton, 1956; Pisani & Bothner, 1970; Ernst & Gotte, 1986; Mibroda, 2014). Typical of snakes in the genus *Thamnophis*, *T. brachystoma* exhibits significant sexual dimorphism in SVL, TL, and relative tail length (Hulse et al., 2001). Stub-tails were more prevalent in female *T. brachystoma* at both Shannon Road and McClelland Park. While snakes with stub-tails were not specifically noted in Pisani's Olean *T. brachystoma* data, one adult female (347 mm SVL) with a tl/TL of 0.143 likely had a stubtail. The cause of the stub-tails in *T. brachystoma* from our Erie sites was not determined, but could have been due to attempted predation, incomplete ecdysis, or frost bite during hibernation (Harding, 1997; Hulse et al., 2001; Ernst, 2003). In male snakes, a stub-tail may result in a considerable fitness disadvantage and a threefold reduction in mating success (Shine et al., 1999). This may be because the tail tip in Thamnophis is useful in cloacal alignment with the female (Pisani, 1976).

The sex ratios of T. brachystoma at the two Erie County sites were similar to those of Engelder (1988) who reported a sex ratio of 1:1.2 from a presumably introduced population in Horseheads, New York. Whereas Pisani & Bothner (1970) reported male-biased sex ratios in both adults (1.4:1 $[\chi 2 = 4.08, df = 1, P = 0.04])$ and prenatal and neonate $(1.5:1 [\chi 2 = 4.12, df = 1, P = 0.04])$ T. brachystoma from Olean, New York. Differences in behaviour between sexes that result in one sex being more detectable to researchers may reduce the accuracy of sex ratio estimates (Parker & Plummer, 1987). For instance, males may move about more often in spring in search of mates, and females may become more sedentary when gravid. Additionally, gravid female T. brachystoma may require higher temperatures than they can attain via thigmothermy, and thus may bask in the open more frequently than males (Kozubowski, 1980). When cover objects are used, this behaviour could make females less likely to be found under cover objects.

Differences in age-structure among sites may result from variation in predator intensity, or in accessibility and quality of hibernation sites (King, 1989). Blarina brevicauda (northern short-tailed shrew), Microtus pennsylvanicus (Meadow Vole), and Peromyscus leucopus (White-footed Mouse) are known or suspected predators of snakes in general (Fitch, 1975; Ernst & Ernst, 2003), and T. brachystoma specifically (Hummer & Roen, 2008). All three species were observed frequently (B. brevicauda N =10; *M. pennsylvanicus* N = 5; *P. leucopus* N = 15) at Shannon Road, but not at McClelland Park. This apparent lack of potential small mammalian predators at McClelland Park may partially explain the greater proportion of immature snakes to adults at this site. Furthermore, population size, density, and total biomass of T. brachystoma were all greater at McClelland Park (Lethaby & Gray, 2015). Each site had a single observation of mortality involving a T. brachystoma that had been crushed under a cover board. Mortality due to automobiles was not observed at either site.

Growth rate data for *T. brachystoma* are sparse. Barton (1956) reported that neonates approximately doubled their length in the first year, reach sexual maturity during their second, with growth rate steadily decreasing with age. Therefore, the greater rate of growth in juveniles compared to adults was expected. The growth rate of a captive *T. brachystoma* was estimated as 75.6 mm/year during approximately the first 2.5 years; growth rate during approximately 3 subsequent years was 16.1 mm/year (Gray, 2011). Both juveniles and adults of *T. brachystoma* in the present study had greater estimated growth rates than an Erie County, Pennsylvania population of *Storeria dekayi*.

Juvenile male and female *S. dekayi* grew on average 16.2 mm/month and 25.2 mm/month, respectively (Gray, 2014) while adult male and female *S. dekayi* grew on average 8.8 mm/month and 3.0 mm/month, respectively. This is likely due to the difference in size between the two species, *S. dekayi* being somewhat smaller. Additional study of the growth rate of *T. brachystoma* would greatly increase our knowledge of possible differences between sexes within and between populations.

The data presented here augment our knowledge of the morphology, growth, and natural history of *T. brachystoma* in urban habitats. These habitats are subject to profound alterations, indeed McClelland Park has been developed into a public dog park (O'Neill, 2015), almost certainly impacting the dynamics of that snake population. The data collected in this study provide a baseline against which future changes in urban populations may be detected and compared with rural populations.

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Natural history of the Peaks of Otter salamander (*Plethodon hubrichti*) along an elevational gradient

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ABSTRACT - The Peaks of Otter salamander, *Plethodon hubrichti*, is a montane species found at elevations above 442 m within a 117 km² area of the Blue Ridge Mountains in central Virginia, USA. In areas of this species' range where the Eastern Red-backed salamander *Plethodon cinereus*, a potential competitor, is absent, surface-active salamander density was hypothesised to decrease at lower elevations due to increased temperatures and lower humidities, which may adversely affect salamander reproductive output and survival rates. Eggs/female, % gravid females, surface-active salamander density, temperature and relative humidity were recorded from nine sites ranging in elevation from 488 to 1143 m. Survival rates and growth rates were estimated at three of these sites. Surface-active salamander densities, survival rates, growth rates, eggs/female and reproductive output decreased with elevation. Decreases were correlated with increases in temperature and a decrease in relative humidity associated with decline in elevation. Other habitat factors such as % canopy closure did not decrease with elevation at the study sites. Peaks of Otter salamanders had greater dehydration rates and lower critical thermal maxima than the wider-ranging Eastern red-backed salamanders, which reflects their adaptation to montane environments. These results support the importance of conserving mature hardwood forests, particularly at lower elevations, which represent marginal environments for montane species of salamanders.

INTRODUCTION

The Peaks of Otter salamander (*Plethodon hubrichti*) is one of several endemic salamander species found in the state of Virginia, USA (Kramer et al., 1993; Petranka, 1998). It is a montane species with a very limited distribution, being found only in mature, deciduous forests at elevations greater than 442 m within a 117 km² area of the Blue Ridge Mountains in central Virginia (Pague & Mitchell, 1990). Elevation may limit the distribution of P. hubrichti in areas where it is allopatric with a potential competitor, the Eastern Red-backed salamander (P. cinereus), due to physiological factors such as intolerance to the higher temperatures and lower humidities associated with lower elevations. Such intolerance was suggested by Thurow (1957), who observed that *P. hubrichti* had a lower tolerance for increased temperatures and evaporation rates than did P. cinereus, a species with a broad distribution over a wide range of elevations (Petranka, 1998). Arif et al. (2007) considered the entire distribution of P. hubrichti to be limited primarily by abiotic factors. Using 19 bioclimatic variables such as maximum temperature of the warmest month and precipitation of wettest month, they were able to correctly predict 80% of known localities for this species.

Amphibians living at high elevations tend to produce fewer clutches per year, larger absolute clutches and larger eggs relative to those living at lower elevations (Morrison & Hero, 2003). Resource availability is a critical factor in regulating reproductive frequency as was experimentally demonstrated with the four-toed salamander, *Hemidactylium scutatum*, under various food resource levels (Harris & Ludwig, 2004). For *P. cinereus*, resource constraints at high elevations (over 1220 m) were correlated with smaller body size, less frequent reproduction, but greater tail fat storage relative to body size when compared to conspecifics at low elevations (Takahashi & Pauley, 2010). Resource allocation to growth and reproduction, in this case, may be restricted at high elevations because of the shorter growing season and greater allocation of energy to storage.

For montane-adapted *Plethodon* species, low elevations may be the most important constraint upon their distributions (Hairston, 1951, 1981; Kozak & Wiens, 2006). Kozak & Wiens (2006) considered that many montane sister Plethodon and Desmognathus taxa in the Appalachian Mountains are isolated from each other because they cannot tolerate abiotic conditions in the intervening lowlands. Abiotic conditions in these lowland areas may exceed the physiological tolerances of montane salamanders. Bernardo & Spotila (2006) demonstrated that limited physiological tolerance to warm temperatures of two montane species of Desmognathus (D. carolinensis and D. ocoee) restricted their ability to disperse through warmer, drier valleys. Understanding the factors that limit the range of P. hubrichti will help conservation efforts by enabling us to avoid forest management practices that contract this species' distribution.

Here the effects of decreasing elevations, along with corresponding increases in temperature and decreases in relative humidity, were examined on the natural history of *P. hubrichti* in allopatry with its potential competitor *P. cinereus*. *P. hubrichti* surface-active (SA) salamander density, eggs/female, % gravid females, and growth and survival rates were hypothesised to decrease with declining elevations because of physiological limitations associated with their montane existence. In addition, this montane

species was hypothesised to have lower critical thermal maxima (CTM) and greater dehydration rates than the wider-ranging *P. cinereus*.

MATERIALS AND METHODS

Demographics and environmental variables vs. elevation field methods

On 28 April 2007, two teams of six to eight people collected SA P. hubrichti during the daytime, on a cool day following rain so that all sites were moist and cool throughout the day, by carefully turning over rocks and logs at five allopatric sites along an elevational gradient (518, 655, 762, 991, and 1052 m; slope directions were west for all sites except 1052 m site, which faced south). Two teams were used so that all sites could be collected on the same day. The sites searched varied from 577 to 1445 m² and depended upon the overall number of salamanders found at each location since the goal was to collect at least five adult females per site. P. hubrichti were placed in zip-lock bags along with moistened paper towels for reproductive output measurements. The bagged salamanders were then placed in a cooler with ice and transported to the lab where they were weighed (± 0.01) g; Scout Pro SP202, Ohaus Corp., Pine Brook, New Jersey, USA); measured (snout-vent length, SVL) using calipers (CD-6"CSX, Mitutoyo Corp., Aurora, Illinois, USA) and the salamander-stick method (Walston & Mullin, 2005); and examined for sex determination (Fig. 1) and the number of large, volked eggs (Sayler, 1966) using a non-destructive, visual method to examine the body cavity with a fiber optic light (Fig. 1; Gillette & Peterson, 2001). Animals were returned to their collection locations the following week.



Figure 1. Candling method for determining salamander sex and number of eggs per female. The picture on the left shows a male *P. hubrichti* (testes and vas deferens visible). On the right is a female *P. hubrichti*, with eight large yolked eggs (three in one oviduct and five in the other). Photograph by A. Fredrickson.

In 2008, additional allopatric areas were surveyed for high and low elevation sites (488, 579, 1128 and 1143 m; slope directions for sites were north, west, summit and summit, respectively) along with the original sites surveyed in 2007, thus raising the total to nine sites ranging from 488 to 1143 m. Surface-active salamanders were counted on standardised plot sizes of 20 x 50 m at all sites except the lowest elevation site (488 m) where, because of the topography and expected low SA salamander density, a 25 x 100 m plot was established. Surface-active salamander density (SA salamander count/size of area surveyed) was used as a surrogate measure of population size since SA salamander counts correlated well with population estimates in previous mark-recapture studies, including one involving P. hubrichti (Smith & Petranka, 2000; Reichenbach & Sattler, 2007; Gifford & Kozak, 2012). At all sites, metal rods were hammered into the ground at the four corners of each plot, and flags were used to visibly mark the corners and sides of the plot. Salamanders were counted and collected on three dates (12, 26 April and 20 Sept 2008) by two teams of eight to ten people on cool days following rain so that all sites were moist and cool throughout the day. Two teams were used so that all sites could be collected on the same day since the fraction of the salamander population that is surfaceactive is known to vary significantly with time since it last rained (Kramer et al., 1993; Reichenbach & Sattler, 2007; O'Donnell et al., 2014). The 488 m site was the only site that was not surveyed on 26 April 2008 due to time constraints, so data for only two collection periods were available for this site (12 April and 20 Sept 2008). Collection, counting and reproductive output methods followed those used in 2007. In 2008, only salamanders collected on 12 April 2008 were used for reproductive output measurements so that the same salamander was not inadvertently used twice in the yearly assessment.

A regression model was used to characterise the relationship between mean surface active salamander density and elevation. Log₁₀ transformation was used whenever data needed to be normalised and variances stabilised as determined by normality and residual plots associated with regression models. All regression models were significant at $\alpha = 0.05$. Number of eggs per female against elevation, female mass, and SVL were characterised using a multiple regression model. Data from 116 gravid female P. hubrichti (range of 2 to 19 gravid females per site with a mean of 10) were used to fit this model. A regression model was also used to relate % gravid females to elevation. For the low elevation sites (518 and 655 m in 2007 and 488, 518 and 579 m in 2008), where low numbers of SA salamanders were found, data were combined across sites in order to estimate % gravid females and then the associated elevations were averaged. The size of the smallest female with eggs was considered to be the minimum size for female sexual maturity. Of the 200 total mature female P. hubrichti found, the number per site used to determine % gravid females ranged from 6 to 34 with a mean of 18. Chi-square analysis was used to see if ratios of male to female salamanders captured at each site deviated with elevation from an expected value of 1:1. Pearson's correlation was used to determine if there was a significant relationship between gravid salamander mass and elevation. Analyses were done using either SYSTAT (SPSS Inc., Chicago, Illinois, USA) or EXCEL (Microsoft Corp., Redmond, WA, USA) using alpha = 0.05 for all tests. Relative egg production was calculated using regression models for SA salamander density, % gravid females, and number of eggs per female for elevations within the range of this study. Estimates were calculated for number of eggs produced per 1000 m² (area of the study sites in 2008 except for the 488 m site) by multiplying the output from these models. These are relative estimates of egg production that can be used to compare sites. They would be underestimates of what the salamander population actually produces since SA salamanders represent only a fraction of the salamanders in a given population (Kramer et al., 1993; O'Donnell & Semlitsch, 2015).

In 2008, at the nine sites where SA salamander density was measured, the following habitat variables were recorded: (1) Percent canopy closure was recorded based on measurements taken at 2-m intervals along two 50-m transects within each plot. A densitometer was used (Geographic Resource Solutions, Arcata, California) at 25 ground-level points per transect to determine whether the canopy was open or closed at each point. Percent canopy closure was then determined for each plot; (2) Percent ground cover was evaluated with a 0.5 m quadrant with 36 hazard points that was laid on the ground (tall herbaceous vegetation projected through the grid of the quadrant and was not counted) at three locations along the same two transects (5 m from each end and in the middle of each transect giving us a total of six locations for percent ground cover per site). At each hazard point, ground cover was recorded as leaves, rock, bare soil, branches, or vegetation. Percentages were then calculated for each category; (3) Leaf litter depth in cm was recorded within each 0.5 m quadrant used to measure percent ground cover by using a ruler to measure from the top of the thickest layer of leaves to the mineral soil. Median leaf depth was then calculated for each site. Correlations were conducted between the median values for the habitat variables and median SA salamander density at each site to determine if these variables, as well as elevation, were significantly related to SA salamander density. Medians were used because of the skewed distributions for the habitat variables.

Temperature and RH were measured during the 2010 field season using temperature and RH Ibuttons (Model DS1923, Maxim Integrated, San Jose, California) placed at the nine study sites as well as other sites used in additional surveys for *P. hubrichti* (n = 18). A 3-h time interval was set for readings, and temperature and RH were recorded from 30 May 2010 to 9 September 2010. Mean night temperatures and RH (from 2000 to 0600 h) were calculated and then the relationship between mean night temperature and night RH with elevation was characterised using regression models. The RH values at two lower elevation sites (518 and 579 m), which averaged 97% RH, were within 45 m of a stream and had lots of understory vegetation. These conditions apparently created high RH at these two low elevation sites because values recorded here were greater than those recorded at the highest elevations. In addition, they did not follow the linear decrease of RH and elevation decline seen at the 16 other sites. For these reasons they were considered outliers and were dropped from the data used to fit the

regression model of RH and elevation. Dropping these two low elevation sites restricted the RH model's predictive capability to elevations greater than 655 m. For a limited number of sites (n = 8), where SA salamander densities were available along with temperature and RH data, a multiple regression model was used to estimate SA salamander density using mean night temperature and RH.

Survival vs. elevation field methods

In 2010, salamander survival was recorded in the field at three different elevations (518, 655 and 991 m). For this study 30 large plastics tubs (80 x 45 x 43 cm) were modified by drilling 6.4 cm diameter holes in the sides and top to allow for ventilation and 1 cm holes in the bottom for water drainage, so that tubs would mimic field moisture and temperature conditions. Holes were covered inside the tubs with fiberglass window screen to prevent salamanders from escaping and outside with a sturdier plastic screen (1 cm mesh size) to prevent other animals from damaging the more delicate interior window screens. Fiberglass window screen was held in place with cable ties inserted through holes drilled in the plastic tubs and silicone was used to seal the edge of the screen against the wall of the tubs. The sturdier plastic screen was also fastened to the outside by cable ties. Ports made of 13 mm diameter polyvinyl chloride (PVC threaded cap on a male threaded connector) were added to the side of each tub so that food could be added to the tubs without removing the lids (Fig. 2).



Figure 2. A) Tub for P. hubrichti, súrvival study showing screening which covered the 6.4 cm diameter holes drilled in the sides and top so the tub environment would mimic field moisture and temperature conditions. B) Tub being set up in the field. Tub was placed in a hole dug into the forest floor. PVC tube visible by student's hand is one of two artificial burrows provided for the salamanders. C) Tub ready for salamanders showing rocks and leaves placed in tub as surface cover for salamanders. port **PVC** threaded through which food was introduced into the tub once the lid was sealed in place visible at left corner. Photographs by N. Reichenbach.

On 17 April 2010, 10 tubs were buried to a depth of 30 cm in the field at three different elevations. The soil dug out of each hole was placed back into the tub. As the soil was being added back, two 13 mm diameter PVC pipes were

placed at an angle in the tubs. These tubes were intended to function like burrows in the soil that were disrupted by the digging activities. These artificial burrows gave salamanders access to areas below the soil surface. Five "handfuls" of dead leaves were added along with five flat rocks to the soil surface of each tub as cover objects for the salamanders.

On 24 April 2010, salamanders were collected near the locations where the tubs were buried and taken to the lab to be weighed. On 1 May 2010, two P. hubrichti were placed in each tub along with four large and four small worms and about six small crickets. The smallest available salamanders (mean mass 0.34 g, range 0.11-0.93 g) were used since density in the tubs (2 salamanders/0.36 m² or 5.5 salamanders/m²) was slightly greater than the recorded density of 4.5 salamanders/m² for P. hubrichti from a nearby location (Kramer et al., 1993). Salamanders were not marked but since individual growth rates were going to be assessed over the course of the study, the two animals placed in each tub were either of similar mass (e.g., 0.36 and 0.37 g) or had easily-distinguishable masses (e.g. 0.17 and 0.38 g). When salamanders were weighed at the end of the experiment, the larger of the two final masses was simply paired with whichever animal had the larger initial mass. If only one salamander survived and the initial masses were not similar, those data were excluded from growth rate calculations.

For one tub at each elevation, temperature and RH Ibuttons (Model DS1923) were placed inside and outside the tub using structures that looked like three-legged stools. Each "stool" was made of a plastic cap with three short pieces of PVC pipe for legs. The Ibutton was velcroed to the underside of the cap so that it could be easily removed to download data and to protect it from direct contact with water. Temperature-only Ibuttons (Model DS1921, Maxim Integrated, San Jose, California) were also placed under one rock on the soil surface and below the surface at the base of one of the artificial burrows in each tub. Temperature and RH data were recorded every 3-h and mean night air temperature and RH were calculated (2000 to 0600 h), as well as mean temperature beneath the rock and at the base of the artificial burrow for the entire day. Night air temperatures and RH were calculated since this is the period when P. hubrichti is normally active and exposed to surface conditions (Kramer et al., 1993). Temperatures were averaged across the entire day for the rock and base of artificial burrow since salamanders could be in or under those structures for the entire day.

To seal the salamanders in the tub, silicone was applied to the top edge and then a sheet of fiberglass window screen was laid over the entire open top of the tub. The plastic lid was then snapped in place and cable ties threaded through small holes, drilled through the lids and top edge of the tub, were used to secure the lid.

Six small worms were added through the side ports of each tub on a monthly basis. During one of these trips, 29 May 2010, one tub lid at the 991 m site was damaged due to apparent black bear (*Ursus americanus*) activity. The tub was examined and no salamanders were found at the surface. Two new *P. hubrichti* were then captured and placed in the repaired tub. Masses were not recorded for these

animals, but the general SVLs were larger than the original salamanders and hence would be distinguishable should the originals be found at the end of the experiment when the entire tub contents were examined.

On 10 September 2010, all tubs were opened and examined for salamanders. The soil surface was examined first followed by removal of the rocks. The soil was then removed, one handful at a time, and finally the PVC pipes (i.e. artificial burrows) were examined for salamanders. The position of live salamanders was noted as follows: (1) out on the surface; (2) under a rock; (3) in an artificial burrow; or (4) in the soil. Each salamander found was also weighed. Number of salamanders found alive versus not found (i.e., presumably dead) was compared across elevations using chi-square analyses while growth rates (g/d) were correlated with elevation.

Physiology lab methods

In spring 2007, CTMs were recorded during the day using the method described by Hutchison (1961) for 20 adult male P. hubrichti and 20 adult male P. cinereus from three locations (allopatric P. hubrichti site - 655 m elevation; allopatric P. cinereus site - 762 m; and one sympatric site - 1268 m) that had been acclimated to 20 °C for one week. Individual animals were placed in a 2 L round-bottom flask with 300 ml of continuously aerated water, at their acclimation temperature, and then heated with an electric heating mantle at a rate of 1 °C per minute until the signs of thermal distress were noted (i.e., onset of spasms which marked the inability of the salamander to escape the thermal conditions as described by Hutchinson (1961)). Water temperatures were measured continuously using a mercury thermometer graduated to 0.1 °C. Deep body temperatures were not measured because the heating rates used prevented any measurable lag between water and salamander temperatures (Spotila, 1972). Once visual signs of distress were observed, water temperature was recorded and this was considered to be the CTM. Salamanders were revived in water at lower temperatures and returned to their original collection sites within a week of recording CTMs. Analysis of Covariance (ANCOVA) was used to compare CTMs for the two species using elevation as the covariate.

In 2008, dehydration rates were determined during the day using the method described by Spotila (1972) for 29 male and nongravid female P. hubrichti (0.49 to 1.85 g) and 29 male and nongravid female P. cinereus (0.47 to 1.85 g), all collected from one location (1183 m elevation) in the fall and acclimated to 20 °C for one week. Salamanders were weighed (Scout Pro SP202) after blotting them dry with paper towels and voiding their bladders by gently pressing on the dorsal side of the pelvic girdle. A wet spot on the paper towel was evident when the bladder was voided. Salamanders were then placed individually in small nylon screen enclosures that minimised coiling. Screen enclosures were secured using clips and placed in desiccators with anhydrous CaSO₄, and then the entire apparatus was placed in an incubator at the acclimation temperature. Salamander masses were checked periodically, so they would not lose more than 15% of their initial mass, and they were weighed again after one full hour in the desiccator. At the end of the experiment, salamanders were rehydrated in containers partially filled with water and then returned to their original collection site within one week. Respiratory surface area was determined using the formula for plethodontids from Whitford and Hutchinson (1967), and dehydration rates were expressed as mass loss per respiratory surface area per hour (mg/cm² * h). Dehydration rates for the two species of salamanders were compared using ANCOVA with initial mass as the covariate.

RESULTS

Demographics and environmental variables vs. elevation The multiple regression model comparing the number of eggs per female *P. hubrichti* to elevation and female mass was as follows (Fig. 3): eggs/female = 509.815 + 349.409 elevation (\log_{10} m) - 59.281 elevation (\log_{10} m)² + 2.278 mass (g) [1] (F = 15.0, df = 3,112, P < 0.001, r² = 0.29). The number of eggs per female *P. hubrichti* increased directly with mass and elevation to a maximum of 12 eggs per female at 1000 m and then decreased slightly above 1000 m. The number of eggs per female ranged from 1 to 12 with a mean of 8.5 (95% CI 8.2–8.9).

There was an inverse relationship between % gravid females and elevation (Fig. 4): % gravid females = 112.73 - 0.0571 elevation (m) [2] (F = 21.3, df = 1, 9, P = 0.001, $r^2 = 0.71$). Percent gravid females ranged from 62 to 83% at low elevation sites (488–762 m) to 40 to 61% at high elevation sites (991–1143 m). The minimum SVL for a gravid female (i.e. size at sexual maturity) was 45.6 mm.

Surface-active salamander density for *P. hubrichti* in allopatry ranged from a low of 0.0007 to 0.0032 *P. hubrichti*/m² at the low elevation sites (488–518 m), then increased to a maximum of 0.072 *P. hubrichti*/m² at the 1052 m site followed by a decrease between 0.024 to 0.044 *P. hubrichti*/m² at the highest elevation sites (1128–1143 m) (Fig. 5): SA salamander density (log₁₀ number/m²) = -174.2 + 116.1 elevation (log₁₀ m) - 19.5 elevation (log₁₀ m)² [3] (*F* = 11.9, $df = 2,6, P = 0.008, r^2 = 0.80$).

Of the habitat factors measured at each site, elevation was significantly related to median SA salamander density (r = 0.736, n = 9, P = 0.02), while all other habitat variables (% canopy closure, % rocks, % branches, % bare soil, % leaves, and leaf litter depth) were not significantly related to SA salamander density (P > 0.05).

Combining regression models for number of eggs/ female, % gravid and SA salamander density produced relative estimates of egg output across the study sites that ranged from a low of 5 eggs per 1000 m² at 518 m to a high of 148 eggs per 1000 m² at 991 m. Egg production decreased to 73 eggs per 1000 m² as elevation increased to 1128 m (Fig. 6). For these calculations, 50% of the SA salamander density was considered female since there was no significant deviation from a 1:1 sex ratio across the sites ($\chi^2 = 10.8$, df = 7, P = 0.15). Female mass was also held constant at the mean (1.81 g) for the regression model for eggs/female since the correlation between female salamander mass and elevation did not show any significant relationship (r =-0.024, n = 116, P = 0.80).



Figure 3. Multiple regression model output relating the number of eggs per female to mass (g) and elevation (m) for *P. hubrichti*. Colour bands represent ranges for eggs/female.



Figure 4. Relationship between percent gravid females and elevation (m) for *P. hubrichti*.



Figure 5. Relationship between surface-active (SA) salamander density $(\log_{10} \text{ number/m}^2)$ and elevation $(\log_{10} \text{ m})$ for *P. hubrichti*.

Elevation and mean night temperatures during the active season were inversely related while mean night RH was directly related to elevation. Night temperatures ranged from a high of 20 °C at the low elevation sites to a low of 16.5 °C at the high elevation sites: Mean night temperature (°C) = 23.13 - 0.0049 elevation (m) [4] (F = 180.8, df =

1,16, P < 0.001, $r^2 = 0.92$). Using the model, a decrease of 1 °C was calculated with increase in elevation of 204 m for elevations ranging from 518 to 1268 m. Mean night RH during the active season ranged from 88% at low elevation sites to approximately 94% at high elevation sites: Mean night RH (%) = 81.62 + 0.0082 elevation (m) [5] (F = 9.8, df = 1,14, P = 0.007, $r^2 = 0.41$). The model showed a 1% increase in RH with increase in elevation of 120 m for elevations ranging from 655 to 1268 m.

The model for predicting *P. hubrichti* SA salamander density using night temperature and RH was as follows (Fig. 7): SA salamander density (number/m²) = 0.050138 – 0.05273 mean night temperature (°C) + 0.000586 mean night temperature (°C) * mean night RH (%) [6] (F = 8.14, $df = 2,5, P = 0.027, r^2 = 0.76$; model prediction valid for mean night temperatures between 16 to 20 °C and night RH between 86 to 94%). Surface-active salamander densities ranged from a low of 0.004 *P. hubrichti*/m² (86% and 20 °C for mean night RH and temperature, respectively) to a high of 0.09 *P. hubrichti*/m² (94% and 16 °C for mean night RH and temperature, respectively).

Survival vs. elevation

When the tubs were opened in September 2010, surviving salamanders (n = 37) were found under rocks (48.7%), in artificial burrows (32.4%), in the soil (13.5%) and on the surface (5.4%).

The number alive versus dead (i.e. not found) was not homogeneous across the three elevations tested ($x^2 = 10.7$, df = 2, P = 0.005). The number alive out of the ones placed in the tubs was highest (17/20 = 0.85) at the high elevation site (991 m) and then decreased to 13/20 (0.65) at 655 m and was the lowest at the 518 m site (7/20 = 0.35). All 29 salamanders that had initial and final mass measurements that could be linked to specific salamanders showed positive growth rates ranging from 0.0009 to 0.0047 g/d, and growth rates increased with increase in elevation (r = 0.49, n = 29, P = 0.007).

Temperatures from Ibuttons at sites with tubs decreased with increase in elevation. Mean night air temperatures decreased from 20.6 to 18.1 °C for elevations ranging from 518 to 991 m, respectively. Temperatures under the rocks and at the base of the artificial burrows (measured over the entire day) in the tubs also decreased with an increase in elevation (518 to 991 m) from 20.8 to 18.5 °C and 19.6 to 17.1 °C, respectively. Mean night air temperatures inside and outside the tubs were very similar (518 m site inside and outside tub temperatures 20.7 and 20.6 °C, respectively; 655 m site the means were equivalent at 20.4 °C; comparison could not be made at the 991 m site because the Ibutton placed in the tub malfunctioned). Elevational trends, as was done with night temperatures, could not be done for RH because of the failure of the Ibutton inside the tub at the 991 m site and because the low elevation site (518 m) was near a stream and had an abundance of understory vegetation that likely contributed to RH being similar to the 991 m site. Outside night RH means were 94, 87 and 97% for the 518, 655 and 991 m sites, respectively. For the 518 and 655 m sites, the tubs averaged 5% higher RH inside the tub than outside most likely due to reduced air flow inside the tubs



Figure 6. Relative egg output per 1000 m² calculated for *P. hubrichti*, using models developed in this study which related elevation (m) to surface-active salamander density, eggs/female, and % gravid females.



Figure 7. Multiple regression model output for surface-active (SA) *P. hubrichti* (number/m²) versus mean night temperature (°C) and relative humidity (RH, %).

from the screening placed over the holes. Therefore decrease in survival rates with decline in elevation was primarily correlated with increase in temperature and not change in RH.

Critical thermal maxima and dehydration rates

For CTM, there was no significant interaction between elevation and species (F = 2.25, df = 1,36, P = 0.13) so the interaction term was dropped from the ANCOVA model. In the reduced model, *P. hubrichti* had a significantly lower CTM relative to *P. cinereus* (CTM least square means 33.3 and 34.4 °C for *P. hubrichti* and *P. cinereus*, respectively, F = 52.5, df = 1,37, P < 0.001). CTMs for both species also significantly increased with a decrease in elevation (F = 10.3, df = 1,37, P = 0.003).

For dehydration rates, there was no significant interaction between mass and species (F = 0.1, df = 1,54, P = 0.73) so the interaction term was dropped from the ANCOVA model. In the reduced model, *P. hubrichti* had a significantly greater dehydration rate relative to *P. cinereus* (dehydration rate for a mean salamander mass of 2.3 g was 16.3 and 15.2 mg/ cm² * h for *P. hubrichti* and *P. cinereus*, respectively, F = 5.1, df = 1,55, P = 0.027), and dehydration rates increased significantly as masses decreased (F = 28.7, df = 1,55, P < 0.001).

DISCUSSION

In this study, SA salamander density for P. hubrichti in allopatric areas was hypothesised to decrease at lower elevations due to increased temperatures and lower RH which may adversely affect montane salamander survival Surface-active salamander density and reproduction. peaked between elevations ranging from 900 to 1100 m and then decreased rapidly with decrease in elevation. A small decrease in SA salamander density also occurred at elevations greater than 1100 m. Potential causal factors for these SA salamander density decreases, primarily with decline in elevation, included habitat (poor habitat quality), temperature (thermal stress from high temperatures), RH (water stress from dehydration when RH is too low), and potentially differing predator and prey communities with change in elevation. Differences between habitat variables (depth of leaf litter, % canopy closure, etc.) measured at sites from a range of elevations were not significantly correlated with SA salamander density and therefore did not explain the decreases with elevation. Temperature and RH were correlated with elevation and did relate to SA salamander density as seen in the multiple regression equation [6]. Along the elevational gradient in the Peaks of Otter area, with decline in elevation, it got warmer (1 °C with every decline of 204 m) and drier (1% decrease in RH with a decline in elevation of 120 m). Temperature increases with decreasing elevation were more consistent than RH decreases with decrease in elevation due to factors affecting RH such as proximity to streams and transpiration from understory vegetation.

Temperature increases and RH decreases at lower elevations might cause added physiological stress for a montane salamander like P. hubrichti, with its lower CTM and higher dehydration rates, relative to a widespread species like P. cinereus (this study). Spotila (1972) showed that these physiological parameters, as well as others he measured, indicated that some species, like P. yonahlossee and P. jordani, were physiologically adapted to life at high elevations. The kind of physiological stress mentioned above may be due to the impact that warmer and drier conditions found at lower elevations place on a salamander's foraging efficiency. Going down the side of a mountain, where it gets warmer and drier, may affect salamander foraging efficiency in a manner similar to what happens over time as the forest dries following a rainfall event. When the forest is very moist, such as immediately following a rainfall event or during light rains, some plethodontids, including P. hubrichti, climb vegetation (Jaeger, 1978; Kramer et al., 1993) where they forage more efficiently than conspecifics in the litter on the forest floor (Jaeger, 1978) and/or avoid ground-dwelling predators while foraging (Roberts & Liebgold, 2008; McEntire, 2016). Foraging on vegetation occurs only for short periods of time and is apparently limited by desiccation (Jaeger, 1978). After a rainfall event, as the forest floor dries, plethodontids remain increasingly under cover objects like rocks and logs and may consume even fewer prey than do salamanders in forest litter (Jaeger, 1972, 1980). And finally, between rainfall events, the forest

floor, including areas under rocks and logs, may become so dry that salamanders are forced to retreat underground where little feeding occurs (Heatwole, 1962; Fraser, 1976; Feder, 1983).

In a similar fashion, considering the effects of elevation on temperature and RH, the optimal elevation sites might have the greatest number of days where environmental conditions allow salamanders to forage optimally on vegetation and/or avoid ground-dwelling predators while foraging. Seventy-six percent of P. hubrichti found at an optimal elevation site (1100 m) were observed at night on vegetation (Kramer et al., 1993). At lower elevation sites, salamanders might rarely have environmental conditions that allow them to forage on vegetation and, consequently, they forage more frequently in forest litter or under rocks and logs where foraging efficiency is reduced. Eventually, at certain low elevations, varying based on local conditions such as site aspect and proximity to streams, environmental conditions at the surface might force P. hubrichti to remain underground most of the time, thus creating an unsustainable energy budget (Spotila, 1972; Gifford & Kozak, 2012). This physiological stress, from reduced foraging efficiency with decline in elevation, may have translated into reduced reproductive output with decline in elevation as noted in this study. If arboreal foraging is primarily related to predator avoidance, then restricted arboreal foraging at low elevations may result in decreased survival rates due to increased predation. Metabolic depression at low elevations may also be involved in decreased reproductive output and survival rates. Two montane species of Desmognathus had an inverse relationship between metabolic depression and elevation, and low elevation populations of these species were likely living near the limit of their physiological tolerances (Bernardo & Spotila, 2006). For elevations above the optimum, a shorter active season may have contributed to decreases in SA salamander density and relative egg output.

Reproductive output (eggs/1000 m²) was affected by SA salamander density, % gravid females, and eggs/ female. Salamanders at the higher elevation sites, up to the optimum elevation, produced more eggs/female, a trend similar to that noted for other amphibians with increase in elevation (Morrison & Hero, 2003). However with increase in elevation, % gravid females decreased similar to that seen with P. cinereus (Takahashi & Pauley, 2010). This was different from what was hypothesised. At the higher elevation sites in this study, reproduction was primarily biennial as suggested by % gravid females ranging from 40 to 60%. At the lower elevation sites, in contrast, at least some of the salamanders produced eggs annually since %gravid females ranged from 67 to 80% (Takahashi & Pauley, 2010). No reduction in body size was noted for gravid P. hubrichti with elevation, similar to what was seen with many other plethodontids (Adams & Church, 2008). The overall effect with decline in elevation, even with more frequent reproduction, was a decrease in reproductive output.

Survival rates and elevation showed a similar trend to SA salamander density and elevation, with a rapid decrease in survival rates with decline in elevation. At the highest elevation (991 m), the survival rate recorded in the caged experiment of 0.85 was comparable to those noted for free ranging *P. hubrichti* at a 1035 m elevation site (mean = 0.993, 95% CI 0.988–0.997; Kniowski & Reichenbach, 2009).

Understanding the reasons why the Peaks of Otter salamander has such a restricted distribution can contribute greatly to its conservation. Previous work has shown that, in the core of its range of, where elevations are optimal (> 650 m), P. hubrichti dominates the salamander community (94.8% of salamander species) and can be found at high population densities (mean = 2.7/m²; Reichenbach & Sattler, 2007). The range of *P. hubrichti* is located primarily in National Park and National Forest lands, and this helps protect the core of its range. It is along the perimeter of this species' distribution, however, that extreme care must be exercised in forest management. These lands include ones managed by the National Park and National Forest as well as private citizens. This research stresses the importance of conserving mature hardwood forests, particularly in lower elevation areas which represent more fragile salamander habitats. Reduction of forest canopy increases temperatures and decrease RH (Homyack et al., 2011), which subsequently would decrease salamander densities even more at low elevations.

This study could also be applied to timbering effects on P. hubrichti. Sattler & Reichenbach (1998) and Reichenbach & Sattler (2007) found that while densities of *P. hubrichti* in clearcut sites showed significant decreases, densities at shelterwood cut sites did not differ significantly from those at non-timbered reference sites. This indicated that shelterwood cuts provided an alternative to clearcuts that did not harm populations of P. hubrichti. These shelterwood cut sites were all located at elevations considered to be optimal for P. hubrichti (>650 m). Small increases in temperature and decreases in RH due to the shelterwood cuts might still be within acceptable ranges for P. hubrichti at optimal elevations. If these same shelterwood cuts had been conducted in lower elevation areas, however, the already sparse populations of P. hubrichti might have been adversely affected since the forest canopy would have been opened, likely resulting in increased temperatures and decreased RH (Homyack et al., 2011). These increased temperatures and decreased RH might exceed or be closer to the tolerable limits for P. hubrichti, which could either extirpate them from these low elevations or reduce densities. Therefore, if shelterwood cuts are planned for low elevation areas within the range of P. hubrichti, research should be done since it would be hypothesised that this form of timbering would adversely affect P. hubrichti populations at these elevations. A set of long-term monitoring sites should also be set up for P. hubrichti, particularly in the perimeter areas of its range. These could be monitored annually or every two years to look for long-term trends in population densities. Ibuttons could also be placed at these sites to measure temperature and RH. If SA salamander density changes are noted, these could be potentially correlated with changes in temperature or RH and/or any changes in land management practices.

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Nesting characteristics of three turtle species along a wetland matrix in western Pennsylvania, USA

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ABSTRACT - We studied nesting activities and measured afternoon nest-temperatures of the midland painted turtle (*Chrysemys picta marginata*), common snapping turtle (*Chelydra serpentina serpentina*), and eastern box turtle (*Terrapene carolina carolina*) from a wetland matrix during May–November 2013 at the Powdermill Nature Reserve in western Pennsylvania, USA. Nesting turtles were encountered during a 36-day period (2 June–8 July). The aquatic turtle species nesting season spanned 17 days (2–18 June). In general, nests were located in areas lacking extensive vegetation and near wetlands. Across all species, successful nests constituted 15% (n = 5), abandoned nests 53% (n = 18), and depredated nests 32% (n = 11). Nest losses to predation were highest for *C. s. serpentina* at 75% (6/8 nests). Mean nest temperatures were lowest for *C. p. marginata* and *T. c. carolina*. Nest successes were highest for *C. s. serpentina* at 42% (19/45 eggs) and hatchlings emerged from these nests by late August. By the first freeze in November, 40% (4/10 eggs) of *T. c. carolina* eggs hatched and hatchlings remained in the nest to overwinter, whereas none of the eight *C. p. marginata* eggs had hatched by then. Our results from a single site are comparable to findings from other regions and for Pennsylvania generally. Our findings also provide the basis for examining responses in nesting phenology to environmental perturbations, most relevant being climate change.

INTRODUCTION

Reports documenting the nesting ecology of individual furtle species in North America are common (e.g., Congdon et al., 1987). However, reports on the nesting characteristics of turtle communities are more rare (Ernst & Lovich, 2009). Studies on nesting in turtle communities can provide insights into the local factors that influence interspecific variation in hatchling emergence, and in turn can lead to a better understanding of broad patterns underlying nesting ecology across the ranges of various turtle species (Lovich et al., 2014).

Six turtle species occur in Westmoreland County, Pennsylvania, USA (Hulse et al., 2001; McCoy, 1982; Russell et al., 2014). Four of these have been documented from the Powdermill Nature Reserve (PNR) in south-eastern Westmoreland County: midland painted turtle (Chrysemys picta marginata), common snapping turtle (Chelydra serpentina serpentina), eastern box turtle (Terrapene carolina carolina), and North American wood turtle (Glyptemys insculpta) (Meshaka et al., 2008). At PNR, the aquatic species (C. p. marginata and C. s. serpentina) have held the focus of a long-term demographic study from hoop-net trapping (Hughes et al., 2016), and the primarily terrestrial species (T. c. carolina and G. insculpta) have been monitored for over 50 years to ascertain long-term trends in population ecology (Miller, 2004). However, the nesting ecology of turtles at PNR, which is located near the northeastern edge in the geographic ranges for these species in the US (Ernst & Lovich, 2009), has not been studied.

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We set out to ascertain various nesting characteristics for three turtle species (*C. p. marginata*, *C. s. serpentina*, and *T. c. carolina*) including nesting activities, hatching success, and diel-nest temperatures from June–October 2013 from a wetland matrix at PNR. Our primary aim was to document the phenology and associated abiotic variables of nesting in a turtle community during a single season at PNR. Our findings not only offer a useful comparison to studies on nesting in these species from different localities (e.g., Christens & Bider, 1987), but also will serve as baseline information on turtle nesting to understand the effects from future environmental perturbations in the northern Allegheny Mountains of western Pennsylvania.

MATERIALS AND METHODS

Study site

Powdermill Nature Reserve is an 856.2 ha field station located in the Ligonier Valley along the western flank of Laurel Hill in the northern Allegheny Mountains of southeastern Westmoreland County, western Pennsylvania, USA ($40^{\circ}10^{\circ}N$, $79^{\circ}16^{\circ}W$; 400 m elevation). Established as a field station in 1956 for the Carnegie Museum of Natural History by Dr. M. Graham Netting, PNR habitats consist of mixed forests, open fields, artificial ponds, natural wetlands, and mountain streams (Morton & Speedy, 2012). The mesophytic forests on PNR are dominated by oaks (*Quercus*), maples (*Acer*), and beeches (*Fagus*) (Utech, 1999). Several longterm ecological studies of the resident fauna of PNR are ongoing and include birds (e.g., McDermott & DeGroote,

2016), snakes (e.g., Meshaka, 2010; Dahlin et al., 2016), turtles (e.g., Hughes et al., 2016), and amphibians (e.g., Meshaka, 2009; Meshaka & Hughes, 2014). To attract migratory birds for PNR's banding program (Powdermill Avian Research Center), artificial ponds were created near the northern boundary of PNR in the 1960s and active management of these ponds continues today. Associated with this wetland matrix is an extensive network of mistnet lanes for the bird-banding program. Net lanes have been utilised in the morning for 3-6 days/week April-August from 1961 - present. Three turtle species (C. p. marginata, C. s. serpentina, and T. c. carolina) have been regularly encountered digging nests and laying eggs in the net lanes by members of the bird-banding staff over at least the last three decades (Robert C. Leberman, pers. comm.). We chose this area to study the nesting ecology of these turtle species because the trails have exposed soils amenable for digging and the consistent observations of turtles nesting along the trails.

Nesting ecology

The first author walked an approximately 1.4 km transect throughout the lanes of the wetland matrix and an adjacent field twice daily, once in the morning (0700-1000 hr) and another in the evening (1800-2100 hr), during the primary nesting season (late-May to late-June) for these turtle species in Pennsylvania (Hulse et al., 2001). During transects, the first author looked for signs of recent nesting activity (e.g., scratching or digging in soil, abandoned nests, etc.). The status of nests was categorised as active with eggs, depredated with eggshell remnants, or abandoned before eggs were deposited. If the turtle species was not directly observed, we assigned species to depredated and active nests based on proximity of the nest to wetlands and the amount and shape of eggs or shells, which varies among species (Hulse et al., 2001). Nests near wetlands (< 5 m) with numerous (> 10 eggs) spherical eggs were assigned to C. s. serpentina; nests near wetlands (< 5 m) with few (<10 eggs) elliptical eggs were assigned to C. p. marginata; and nests away (> 5 m) from wetlands with few (< 8 eggs) elliptical eggs were assigned to T. c. carolina. Other types of nesting events (e.g., abandoned nests) could not be reliably assigned to species.

When nests or nesting turtles were encountered, we recorded the time, date, GPS coordinates, general canopy cover, and a qualitative assessment of the microhabitat within a 2-m circle of the site. Turtles found digging or laying were monitored from a safe distance until all eggs were laid and the nest was filled by the turtle. Turtles were then captured and given individual identities (see Hughes et al., 2016). We recorded body measurements of females, including weight to the nearest 1 g, carapace length and width, and plastron length to the nearest 1 mm of turtles.

Active nests were gently excavated, and the eggs were carefully removed. We measured various features of the nest chamber, including the depth to first egg, depth to last egg, chamber width, and size of chamber opening. We determined clutch size to be the number of eggs in the nest. We measured clutch mass to the nearest 1 g by placing eggs in a tared container attached to a digital spring scale. Each egg was measured for width and length to the nearest 0.1 mm with Vernier calipers. Eggs were carefully placed back into the nest in the order that they were removed and nests were covered with dirt.

To monitor nests for thermal profiles throughout the season, we placed a plastic fence (ca. 1 m high) around active nests to deter predators and to avoid being trampled by the banding staff. We measured temperatures of active nests from initial nest discovery until all nests were excavated on 4 November 2013. Approximately once a week at midday, we measured the temperature (°C) inside the nest at two depths, one near the middle and another at the bottom of the nest using the probe of a quick-reading Fluke 51II thermometer. The depths varied based on the overall depth of each nest. We also recorded soil-surface temperature directly above the nest with a Pro-Exotics PE-2 infrared temperature gun and monthly average air temperatures were downloaded for Laurel Summit (https://www.ncdc.noaa.gov/), which is ca. 10 km from our site.

RESULTS

A total of 34 events associated with nesting were encountered, including 18 abandoned nests, 11 depredated nests, and five active nests with eggs (Fig. 1). Of the 11 depredated nests, six were assigned to C. s. serpentina, three to T. c. carolina, and two to C. p. marginata. Six turtles were encountered in the course of nesting over a 36-day period (2 June–8 July): C. s. serpentina on 2 June (n = 2) and 5 June (n = 1); C. p. marginata on 19 June (n = 1); and T. c. carolina on 10 June (n = 1) and 8 July (n = 1). An additional nest of C. s. serpentina with just five eggs was found on 5 June—a large root system at the base of the nest seemed to physically prohibit the presence of additional eggs-and depredated on 9 June. Across all species, nest sites were constructed along net lanes or a gravel road (Fig. 1). Nest-site selections for C. p. marginata and T. c. carolina were in areas with more extensive canopy cover or adjacent to taller vegetation than nests of C. s. serpentina.

Selected clutch and nest characteristics are presented in Table 1. For *C. s. serpentina*, 42% of the eggs hatched (19/45 eggs), and these hatchlings emerged naturally from the nests on 27 and 29 August. For *T. c. carolina*, 40% of the eggs hatched (4/10 eggs), which represented 100% of the eggs from one nest. All four hatchlings of *T. c. carolina* were still in the nest at the time of excavation and thus likely to overwinter in the nest. None of the eggs for *C. p. marginata* hatched (0/8 eggs) from a single nest by the excavation date. Mean carapace length for *T. c. carolina* from four hatchlings was 25.9 mm and from five *C. s. serpentina* hatchlings was 26.9 mm (Table 1).

Five active nests with eggs were monitored for thermal profiles: *C. s. serpentina* (n = 2 nests), *T. c. carolina* (n = 2 nests), and *C. p. marginata* (n = 1 nest). Mean temperature at the soil surface was higher than both depths, and mean temperatures closer to the center of the nests were higher than mean temperatures near the bottom of the nests (Tables 2-4). Mean nest temperatures at both depths and at the soil surface were generally highest among those of *C. s. serpentina* than for *T. c. carolina* and *C. p. marginata* (Tables 2-4).



Figure 1. The study area at Powdermill Nature Reserve (PNR), Westmoreland County, western Pennsylvania, USA. Enlarged view of wetland matrix, mist-net lanes, and nest sites (bottom). Circles indicate nests with eggs that were monitored for thermal profiles, squares abandoned nests, and triangles depredated nests.

DISCUSSION

In general, the nesting characteristics (e.g., clutch size) of the three turtle species we examined at PNR were consistent with other studies on these species conducted in Pennsylvania (e.g., Bieber-Ham, 2011; Ernst, 1966, 1971; Hulse et al., 2001) and elsewhere (Ernst & Lovich, 2009). Afternoon nest temperatures of C. s. serpentina were higher than those of T. c. carolina and C. p. marginata. Our qualitative observations and quantitative temperatures indicate that C. s. serpentina nested in sparsely vegetated open areas, where exposure to direct sunlight was high. In contrast, T. c. carolina and C. p. marginata nests were adjacent to thick vegetation or near the edge of tree lines, where exposure to sunlight was comparatively lower. The deeper and generally south-facing nests of C. s. serpentina had consistently higher afternoon nest temperatures than the comparatively shallower nests of T. c. carolina and

barsely vegetated nlight was high. *inata* nests were dge of tree lines, *ively* lower. The C. s. serpentina C. s. serpentinaC. s. s

C. p. marginata. Our results are consistent with previous

findings on the vital role that nest-site selection plays in regulating nest temperatures over other factors such as nest

depth (Bodie et al., 1996). Differences in nest-site selection

are an important source of intrapopulation variation in nest

temperatures (Riley et al., 2014) and nest sites on south-

facing slopes have been shown to increase hatching success

(Schwarzkopf & Brooks, 1987). Moreover, overwintering

in the nest with respect to hatchling survival has been shown

to be a largely unsuccessful strategy in northern populations

	Chelydra serpentina serpentina (2 nests)	<i>Terrapene carolina carolina</i> (2 nests)	Chrysemys picta marginata (1 nest)
Clutch size	22.5 ± 3.5 eggs (20–25 eggs)	5 ± 1.4 eggs (4–6 eggs)	8 eggs
Clutch weight	280 ± 42.4 g (250–310 g)	55 ± 7.1 g (50–60 g)	70 g
Egg weight	12.5 ± 0.1 g (12.4–12.5 g; n = 45)	11.3 ± 1.8 g (10–12.5 g; n = 10)	8.8 g
Egg dimensions	27.4 ± 0.9 mm (26–29 mm; n = 45)	L: 36 ± 2.2 mm (33–39 mm; n = 10) W: 21.7 ± 0.7 mm (21–23 mm; n = 10)	L: 31 ± 1.1 mm (29–32 mm; n = 8) W: 17.8 ± 0.5 mm (17–18 mm; n = 8)
Hatchling carapace length	26.9 ± 1.6 mm (25.2–28.6 mm; n = 5)	25.9 ± 0.4 mm (25.5–26.2 mm; n = 4)	-
Nest depth	110 ± 14.1 mm (100–120 mm)	65 ± 7.1 mm (60–70 mm)	55 mm
% nests depredated	75% (6/8 nests)	60% (3/5 nests)	66.6% (2/3 nests)
% hatching success	42% (19/45 eggs)	40% (4/10 eggs)	0% (0/8 eggs)
Overwintered in nest	No	Yes	-

Table 1. Selected nest characteristics for three turtle species from Powdermill Nature Reserve, Westmoreland County, western Pennsylvania, USA. Means are presented \pm one standard deviation with the ranges and sample sizes in parentheses.

Table 2. Monthly mean nest and air temperatures for the common snapping turtle (*C. s. serpentina*) from Powdermill Nature Reserve, Westmoreland County, western Pennsylvania, USA. Hatchlings emerged naturally on 27 and 29 August 2013. Means are presented \pm one standard deviation with the ranges in parentheses and sample sizes below.

	C. s. serpentina (2 nests)								
Month	Soil surface	Depth 1	Depth 2	Air					
June	30.6 ± 7.9 C° (21.4–40.6 C°) n = 8	27.8 ± 5.1 C° (23.2–36 C°) n = 8	26.7 ± 4.3 C° (22.8–33.8 C°) n = 8	16.7 C° (4.4–26.1 C°)					
July	29.5 ± 3.6 C° (24.7–35.8 C°) n = 6	26.9 ± 2.8 C° (23.2–30.3 C°) n = 6	26.1 ± 2.6 C° (22.4–29.4 C°) n = 6	19.1 C° (8.3–27.8 C°)					
August	32.3 ± 3.7 C° (27.3–36.1 C°) n = 4	28.4 ± 1.7 C° (27–30.8 C°) n = 4	27 ± 1.1 C° (26.1–28.5 C°) n = 4	17 C° (7.8–25 C°)					
September	-	-	-	14.2 C° (3.3–26.7 C°)					
October	-	-	-						
Overall	30.6 ± 5.6 C° (21.4–40.6 C°) n = 18	27.6 ± 3.7 C° (23.2–36 C°) n = 18	26.5 ± 3.2 C° (22.4–33.8 C°) n = 18	12.9 C° (-13.9–27.8 C°)					

Table 3. Monthly mean nest and air temperatures for the eastern box turtle (*T. c. carolina*) from Powdermill Nature Reserve, Westmoreland County, western Pennsylvania, USA. Nests excavated on 4 November 2013. Means are presented \pm one standard deviation with the ranges in parentheses and sample sizes below.

T. c. carolina (2 nests)							
Month	Soil surface	Depth 1	Depth 2	Air			
June	27.3 ± 7.2 C° (21.2–37.3 C°) n = 4	26.6 ± 4.7 C° (22.6–32.6 C°) n = 4	26.2 ± 4.8 C° (22.2–32.4 C°) n = 4	16.7 C° (4.4–26.1 C°)			
July	26.6 ± 1.7 C° (24.5–28.3 C°) n = 5	26.4 ± 1.4 C° (24.9–28.5 C°) n = 6	25.9 ± 1.7 C° (24.4–28.3 C°) n = 6	19.1 C° (8.3–27.8 C°)			
August	26.1 ± 1.9 C° (23.1–28.5 C°) n = 6	25.2 ± 1 C° (23.5–26.3 C°) n = 6	23.9 ± 1.2 C° (22.4–25.8 C°) n = 6	17 C° (7.8–25 C°)			
September	21.4 ± 1.5 C° (19.2–23.6 C°) n = 8	19.9 ± 1.4 C° (17.9–22.2 C°) n = 8	18.8 ± 1.8 C° (16–21.1 C°) n = 8	14.2 C° (3.3–26.7 C°)			
October	15.4 ± 4.4 C° (8.5–21.6 C°) n = 10	14.3 ± 4.1 C° (8.2–20.5 C°) n = 10	13.8 ± 3.9 C° (8.2–19.1 C°) n = 10	9.8 C° (-2.8–26.1 C°)			
Overall	21.9 ± 5.9 C° (8.5–37.3 C°) n = 33	20.9 ± 5.7 C° (8.2–32.6 C°) n = 33	20.1 ± 5.7 C° (8.2–32.4 C°) n = 33	12.9 C° (-13.9–27.8 C°)			

al., 2001). For the aquatic species, the nesting season was shorter than the terrestrial species. A relatively extended nesting season is consistent with other populations of *T. c. carolina* (Wilson & Ernst, 2005), whereas *C. p. marginata* and *C. s. serpentina* typically exhibit a narrower nesting season lasting 2–3 weeks (Ernst & Lovich, 2009; Hulse et al., 2001). Incubation time for *C. s. serpentina* at PNR of 85–87 days is slightly less than the average in Pennsylvania of 90 days (Hulse et al., 2001), yet longer than eggs incubated at constant temperatures in the laboratory (Yntema, 1978).

Data on nesting success and the factors influencing survivorship from the egg to hatchling are important to understanding reproductive strategies. Nest depredation among species in our study was comparable to other studies that have shown it can range from 0-94% in some years (Christens & Bider, 1987; Congdon et al., 1987; Congello, 1978; Petokas & Alexander, 1980). Predation is a major source of nest failures in turtles (Ernst & Lovich, 2009); however, several studies have shown that nests fail for other reasons unrelated to predators, such as environmental fluctuations (e.g., Tinkle et al., 1981). We found that none of the C. p. marginata eggs hatched before the first freeze in November, which is generally consistent with larger samples demonstrating that hatching success in this species can be very low (Christens & Bider, 1987). We suspect that inadequate temperatures were reached for the embryos to fully develop before the first freeze because this particular nest location was adjacent to tall vegetation. Four hatchling T. c. carolina were found in the nest upon excavation in November, suggesting that these individuals were likely to overwinter in the nest, which has been observed in T. carolina from New York (Madden, 1975). However, overwintering in the nest by T. carolina was not observed in Maryland by Kipp (2003) or from another site in New York by Burke & Capitano (2011). Gibbons & Nelson (1978) suggested that species with elevated levels of uncertainty in reproduction or habitat will be more likely to delay emergence (i.e., overwinter). This phenomenon has been recently considered to be normal of temperate zone turtle species (Gibbons,

2013) and can be common in populations throughout the ranges of *C*. *p*. *marginata* and *C*. *s*. *serpentina* (Lovich et al., 2014).

Aside from the small adult population sizes of these turtle species at PNR (Miller, 2004; Hughes et al., 2016), we note two salient factors adversely affecting the nesting ecology at this wetland matrix that the impacts of which may be mitigated through active management. First, a significant reduction in areas with adequate sun exposure for egg development due to overgrown vegetation could be cleared and returned to a more open habitat (Tesauro & Ehrenfeld, 2007). Second, the high number of nests lost to mesopredators may be mitigated through an annual harvest of small mammals, turtle nest screening (Ratnaswamy et al., 1997), or aversive conditioning (Conover, 1990).

Our findings provide a solid base for future studies aimed at examining the long-term dynamics of nesting ecology at PNR. The nest-temperature profiles and patterns in nest-site selection we found are not likely to remain stable in the face of predicted global climate changes (e.g., Mainwaring et al., in press). Consequently, our baseline data will allow us to test the extent to which the nesting ecology at PNR is affected by future environmental changes, for which climate-related phenological changes have already been documented in some of PNR's avian taxa (McDermott & DeGroote, 2017).

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		C. p. marginata (1 nest	ts)	
Month	Soil surface	Depth 1	Depth 2	Air
June	30.9 ± 9.5 C° (24.2–37.6 C°) n = 2	27.6 C° n = 1	30.3 ± 4.1 C° (27.4–33.2 C°) n = 2	16.7 C° (4.4–26.1 C°)
July	24.6 ± 3.1 C° (21–26.5 C°) n = 3	26.5 ± 1.9 C° (24.3–28 C°) n = 3	26.1 ± 1.7 C° (24.4–27.8 C°) n = 3	19.1 C° (8.3–27.8 C°)
August	25.2 ± 4.1 C° (21.6–29.7 C°) n = 3	25.3 ± 2.6 C° (23.4–28.3 C°) n = 3	24.3 ± 1.6 C° (23.3–26.1 C°) n = 3	17 C° (7.8–25 C°
September	22.3 ± 1.5 C° (20.5–24.2 C°) n = 4	21.4 ± 1.6 C° (19.4–23.3 C°) n = 4	19.9 ± 1.5 C° (18.4–21.2 C°) n = 4	14.2 C° (3.3–26.7 C°)
October	16 ± 5.3 C° (8.5–22.7 C°) n = 5	15.3 ± 4.6 C° (8.7–20.9 C°) n = 5	14.5 ± 4 C° (8.8–19.1 C°) n = 5	9.8 C° (-2.8–26.1 C°)
Overall	22.4 ± 6.4 C° (8.5–37.6 C°) n = 17	21.6 ± 5.6 C° (8.7–28.3 C°) n = 16	21.3 ± 6.1 C° (8.8–33.2 C°) n = 17	12.9 C° (-13.9–27.8 C°)

Table 4. Monthly mean nest and air temperatures for the midland painted turtle (*C. p. marginata*) from Powdermill Nature Reserve, Westmoreland County, western Pennsylvania, USA. Nest excavated on 4 November 2013. Means are presented \pm one standard deviation with the ranges in parentheses and sample sizes below.

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The distribution and local density of the critically endangered frog *Conraua derooi* Hulselmans, 1972 in Togo, West Africa

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ABSTRACT - *Conraua derooi* is a Critically Endangered frog, endemic to the hilly forest region between Ghana and Togo, West Africa. Field surveys were conducted in the Togolese areas, considered to be potentially suitable for *C. derooi* in November 2015 and November 2016. The species was not encountered in several potentially suitable sites. However, *C. derooi* was recorded in two areas, Missahohe and Danyi Yikpa, including two new sites, one rediscovered site, and three confirmed sites within these areas. In total we recorded 522 adult individuals. The number of observed frogs ranged from 14 to 150 individuals × km⁻¹. Due to heavy habitat alteration the known populations are isolated. Some conservation considerations are presented.

INTRODUCTION

Ogo slippery frog, Conraua derooi (Fig. 1) was described from the forest around Missahohe in south-western Togo (Hulselmans, 1972). In 1980, a further small population of the species was discovered north of the type locality in the Danyi Yikpa area (Bourgat et al., 1996). Records of C. derooi from Togo and Ghana, identified as Conraua alleni (a species-complex which occurs from western Ghana to Sierra Leone and Guinea; Mark-Oliver Rödel et al., unpubl. data), prior to the description of C. derooi, were published by Schiøtz (1964) and Lamotte & Perret (1968). Surveys conducted from 2000 to 2005 on the Ghanaian part of the Ghana-Togo highlands failed to detect the species (Rödel & Agyei, 2003; Leaché et al., 2006). Segniagbeto et al. (2007) summarised the known sites from Togo but they did not find any new sites. Finally Kouamé et al. (2007) presented new records from the Atewa Range in Ghana; Hillers et al. (2009) confirmed the persistence of C. derooi at several known sites along the Togo-Ghana border. Preliminary results indicate, however, that the Atewa records may represent an undescribed species (Rödel, unpubl. data). In summary, C. derooi has a very restricted distribution, with all known Togolese populations found along the Camalo torrent in the forests of Missahohe and Yikpa (Segniagbeto et al., 2007, 2013).

Typical habitats of the species are torrents in hilly forested landscapes. Previous studies (Rödel & Agyei, 2003; Hillers et al., 2009 and Segniagbeto et al., 2013) have shown that available habitat for this species is highly fragmented and



Figure 1. Adult C. derooi from Camalo I, south-western Togo.

degraded, as natural forest ecosystems being transformed into coffee and cocoa plantations. As a result and because of its Extent of Occurrence, *C. derooi* has been assessed as Critically Endangered, Criteria B2ab(iii), by IUCN (Rödel & Schiøtz, 2004). In addition, the species should be considered a priority for conservation on the basis of its evolutionary distinctiveness and global endangerment (Isaac et al., 2012). In order to obtain up-to-date scientific data on the distribution and conservation status of the *C. derooi* populations in Togo, we conducted field surveys in November 2015 and November 2016 in areas considered, based on habitat, as potentially suitable for the species. In this paper, we present data on the number of observed individuals and some hitherto unknown sites.



Figure 2. Map of Togo, showing the two study areas with presences of *C. derooi*. The new localities discovered during the present surveys are presented in red colour. Locality 1 was rediscovered after it was firstly discovered in 1980.

STUDY AREA

The study area was within the forest zone in south-western Togo (Figure 2; ecological zone IV according to Ern, 1979). In this area, different sites were surveyed, these included the forest of Assoukoko, the forest of "les Deux Béna", the forest of Assimé, Yikpa, Missahohe and Mount Agou (Table 1). Overall, this comprises hilly area with altitudes of up to 972 m asl. Localities in these areas usually have very steep slopes (Addra et al., 1994). The climate is transitional subequatorial (Papadakis, 1966; Trochain, 1980), being characterised by a prolonged rainy season from March to October, with decreasing amounts of rainfall in August or September. The survey periods were selected on the basis of logistical opportunities, in order to avoid periods of heavy rains that would have caused difficulties in exploration of some sites in remote areas of the forest. It is the wettest area in Togo, with an average annual rainfall amounting to 1,300–1,500 mm. This area is characterised by the presence of several rivers spread over three water-sheds, with the basin River Volta source being the most important in terms of size. The main water-courses are: Assoukoko and its tributaries Yégué and Koflo; Wawa and its tributaries Kpon (also called Gbanhou), Obéka, Ové and Gonobe; Danyi and its tributaries Tonon, Iwé, Sassa and Agoumatsa. The vegetation varied between the sites investigated (see Table 1 for site names). Geographic coordinates of the various presence sites are not provided for conservation reasons (Lindenmayer & Scheele,

2017), but are available on request from the authors.

Djiguégué consists of a mosaic of semi-deciduous, more or less degraded, dense forests, a few small gallery forests, savannas, fallow land and crops (see Figure 2 for its location within Togo).

Assoukoko is similar to Djiguégué in that the site is a mosaic of semi-deciduous forests, dry dense forests, wooded savannas, fallow land and crops. In accessible parts of the semi-deciduous forests, coffee and cocoa is cultivated. The forests in this area gradually shift from semi-deciduous on valley floors, to dense dry forests on upper slopes, and finally transition into wooded savannas at the top.

The area of Akloa (Badou) includes patches of heavily degraded forest, dominated by cash crops (mainly coffee and cocoa), growing under forest canopy. Fruit such as *Persea americana*, *Citrus* spp., *Musa sapitum* and *Musa paradisiaca* is also cultivated in these agroforestry systems. Atigba (Danyi) comprises agroforestry systems (mainly coffee), some narrow gallery forests and wooded savanna.

The vegetation in Dany Yikpa resembles that in the Assoukoko area, i.e. comprising a mosaic of semideciduous forests, dense dry forests and wooded savannas; the undergrowth of accessible semi-deciduous forests is cultivated with coffee and cocoa.

The Missahohé forest comprises a mosaic of mesophilic or semi-deciduous forests (Akpagana, 1989) and savannas. It is heavily impacted because of human settlements and the cultivation of both, cash crops and food crops.

MATERIALS AND METHODS

Two field surveys, on 1-11 November 2015 and 8-13 November 2016, were carried out in the localities mentioned above. Each study site was visited twice. Prior to field work we conducted interviews in the nearby villages (n = 21 villages). In each village, 4 to 12 local persons were interviewed. All interviewers were males, and the same was for interviewees, as only men collect frogs in Togo. Interviewees were asked about the presence of the species by presenting them photos of the frogs and by imitating their advertisement call (high-pitched whistles). With the support of two local guides, and based on the results of the interviews, potential sites of the species were identified. Small waterfalls in forested areas, streams with a width of about two to three meters and streambeds with rocks or pebbles (mainly quartzites) were targeted as survey sites, as these habitat features were already seen to be associated with C. derooi populations in the past (e.g., Segniagbeto et al., 2013). All identified sites were surveyed between 7 and 10 pm. Air temperature was not recorded because it is very constant at the study areas, and because all surveys were made in the same period of the year (November, i.e. end of the wet season). The name-places and the distance walked in each site are presented in Table 2. The field team consisted of 3 people (GHS and two students). Surveys were undertaken with the team proceeding slowly upstream, until walking became impossible because the terrain became too difficult to traverse. Flashlight and head torches were used for exploring the sites. The length of the stream which could be investigated varied between sites from 200 m to 1.8 km (Table 2). At each site, all the observed frogs were counted. Adults and subadults were pooled in our counts. All members of the team walked slowly and parallel to each other, thus ascertaining that no specimen was counted twice. Tadpoles and newly metamorphosed individuals were observed but not counted. However, their presence was always recorded.

Density of *C. derooi*, at each site, was determined as the number of adult individuals that were observed per km of transect. Four times per year, interviews with, and examination of collected animals by, reptile farms in Lomé were carried out in order to monitor the current trade of species. During these surveys, the dealers were interviewed about the collected animals, and any details of interest (apparent abundance, locality of capture, destination country, etc.) were recorded.

RESULTS AND DISCUSSION

In total, 23 independent sites were visited and in 21 of them a total of 138 local people were interviewed (Table 1). Overall, in six distinct localities (including four localities were also interviews were made) we recorded 522 *C. derooi* individuals, 349 in 2015 and 173 in 2016 (Table 2). A greater number of *C. derooi* were observed in the southern part of Togo's ecological zone IV compared to the northern part (Fig. 1). Our surveys did not reveal the presence of the species in Diguengue, Assoukoko, Akloa, Danyi Atigba and Mount Agou, despite all sites being surveyed twice (once

Table 1. Summary of areas and localities investigated for *C. derooi* in the forest area of Togo, where also interviews with local people were conducted.

Area investigated	Localities	State of record	Number of people interviewed
	Yégué	No record	8
	Assoukoko	No record	9
Adele area	Dikpéléou	No record	6
	Diguengue	No record	12
	Akloa	No record	10
Akloa area	Wobè	No record	6
	Tomégbé	No record	4
	Danyi Atigba	No record	7
Danyi area	Yikpa Dzigbé	Presence ascertained	4
	Yikpa Anyigbé	Presence ascertained	11
	Kouma Tokpli	No record	6
	Agomé Tomégbé	No record	6
	Kamétonou	No record	5
	Agomé Yoh	No record	8
Kloto area	Kouma Konda	Presence ascertained	7
	Anédi	Presence ascertained	5
	Kouma Adamé	No record	4
	Kouma Tsamé	No record	6
Agou mountain	Kébo Dzigbé	No record	6
area	Kébo Dogbadzi	No record	4
	Kébo Kpéta	No record	4

in each year of study). Based on its habitat features, the mountain side at Agou looked particularly suitable for the species. However, the species was not detected. Based on our interview data and subsequent field surveys, the local communities appeared to often confuse *C. derooi* with the widespread, abundant, and similarly aquatic *Hoplobatrachus occipitalis*. It should be noted that, in Djiguégué and Akloa, the hilly areas were extremely difficult to access and the area had many small waterfalls within a forest environment. Interviews with people from some villages in this area indicated the potential presence of the species. The respective areas thus should be re-surveyed and this will entail the use of specialist climbing equipment.

The presence of the species was confirmed in just two forest regions which are known to have supported the species in the past: Missahohe and Danyi Yikpa (Hulselmans, 1972; Bourgat, 1979; Kulo, 1980; Hillers et al., 2009; Segniagbeto et al., 2013). More in detail, the species was recorded at six distinct sites within these two main areas, two out of which were newly discovered during the present investigations and one was rediscovered after 1980 (Fig. 2). In other three sites, the presence of the study species was confirmed (Fig. 2). The greatest number of C. derooi (30.65% of the total number of encountered adult individuals per km of transect; see table 2), were observed at the Zoto site, in the Missahohe forest, followed by Douane, also situated in Missahohe forest (Table 2). All sites where the species was present were surrounded by unsuitable habitat (forestry operations and coffee and cocoa cultivation in the former forest). Our **Table 2.** Summary of Togolese localities where C. derooi was recorded, including survey dates and number of observed (No.)individuals. Abundance is defined as number of individuals \times km-1. Tadpoles were observed at all sites.

Localities	First surve	ey (2015)	Second Survey (2016)		Transect length (km)	no. frogs x km ⁻¹ (year 2015)	no. frogs x km ⁻¹ (year 2016)
	Date	No. of <i>C. derooi</i>	Date	No. of C. derooi			
Yikpa	2 Nov	30	9 Nov	25	1.8	16.66	13.88
Camalo I, Missahohe	4 Nov	70	12 Nov	45	1.3	53.85	34.61
Zoto, Missahohe	8 Nov	120	10 Nov	40	0.8	150	50
Douane, Missahohe	8 Nov	50	10 Nov	20	0.5	100	40
Camalo, II Missahohe	9 Nov	14	12 Nov	8	0.2	70	40
Anedi, Missahohe	10 Nov	65	11 Nov	35	0.45	144.4	77.77



Figure 3. Small waterfall, habitat of *C. derooi* in Danyi Yikpa, south-western Togo.

surveys indicate that the Togolese population of *C. derooi* is highly fragmented.

Our observations confirm the known habitat preferences of this species (Fig. 3), which is associated with torrents. At Camalo II, some individuals were also found, under the water, in rock crevices or by being dug from the mud. Adults, especially calling males, were observed sitting on rocky substrates outside of the water. Some individuals were observed 10 m above torrents within the spray zone of waterfalls.

The habitats of all C. derooi populations are seriously threatened by logging and agricultural encroachment. The few remaining forests throughout the hilly forest area along the border between Togo and Ghana are being converted into plantations (mostly coffee and cocoa) at increasing rates. According to the FAO (2011), 43.6% of forest ecosystems in Togo have been destroyed since 1990. The increasing production of charcoal by the local populations, following the recent fall in the global prices for coffee and cocoa, is accelerating the ongoing process of forest fragmentation and degradation. What is left of these forests today is practically confined to forest islands in hard-to-reach areas and along watercourses. Because of the rapid population growth, these forest relics are increasingly damaged as well. Even in the Missahohe Forest, which is a protected area, the habitats of C. derooi are heavily degraded (Segniagbeto et al., 2013). Indeed, although legally protected at the country scale, this forest is heavily subjected to illegal timbering, hunting and other resource exploitation activities.

Another threat to the populations of C. derooi is the exploitation of adults for domestic consumption and the international food trade. To date, the level of exploitation has not been quantified and requires further study. In neighboring Bénin, a dramatically increasing exploitation of frog populations for regional and international food market has been documented (Mohneke et al., 2010). In addition, other Conraua species are exploited for meat in West and Central Africa (e.g. Conraua goliath, see Herrmann et al., 2005). There is also indication that the C. derooi population at Yikpa is exploited for local consumption. The interviewed population of this small village (10 people, all men), situated between Togo and Ghana, indicates that the species is well known and interviewees reported that frogs are sold for 100 CFA per frog (= 0.17 \$ or $0.15 \in$) to local households. This population and those of the Missahohe Forest are furthermore exploited, especially to supply the demand for frog meat from the Chinese workforce in Togo. In addition, interviewed collectors at the various sites also acknowledged that they collect *C. derooi* for Togolese reptile farms which export frogs for the international pet trade. This exploitation was confirmed by two of the nine reptile farms that were surveyed (Mare and Toganim). Indeed, about 50 (range: 15-100) C. derooi individuals per year were exported from Togo to the European Union between 2010 and 2015 (Segniagbeto, unpublished data). Nonetheless, this export has now been stopped due to the recommendation of the senior author to the 'Direction des Ressources Forestrières' of the Togolese Republic (year 2015) and to the collectors as well.

However, given that habitat alteration is presumably the main threat for C. *derooi* in Togo, we advise that urgent measures should be taken to protect and (where necessary) restore the remaining habitats of this species in Togo.

Because of the very few known sites, the high fragmentation of the range, and the rampant habitat loss, we consider that this species should be still considered as CR by IUCN. In this regard, it should be mentioned that *C. derooi* has been subjected to conservation initiatives in Ghana, coordinated by Caleb Ofori-Boateng and internationally supported by various donors (e.g. see http://www. saveourspecies.org/projects/amphibians/conservation-critically-endangered-togo-slippery-frog-conraua-derooi; lastly accessed: 20 August 2017). Thus, it would be very important that similar international attention should be attributed to the Togolese populations as well.

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Successful reproduction of the mole salamander Ambystoma talpoideum in captivity, with an emphasis on stimuli environmental determinants

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ABSTRACT - Generating and promoting evidence-based husbandry protocols for urodeles, commonly known as newts and salamanders, is urgently needed because most of the up-to-date ex situ programs are focused on frogs and toads than Urodela. Data on biology, life history, ecology and environmental parameters are lacking for many species and are needed to establish suitable husbandry and breeding conditions in captive environments. Two adult females and two adult males, of the mole salamander *Ambystoma talpoideum* successfully reproduced in captivity. It was found that reproduction of this species depends on various complex stimuli: including natural photoperiod 12:12, rainwater (acidic to neutral pH) and an aquarium full of various debris. Additionally high temperature variations ranging from 2 °C to 17 °C (a decrease followed by an increase) between November and February showed that it is possible to breed adults in aquariums provided the right stimuli are applied at the right moment of time in winter. *A. talpoideum* shows an explosive breeding mode as previously reported for the whole genus *Ambystoma*.

INTRODUCTION

Since the 1980s, the current global amphibian extinction crisis has been discussed and acknowledged (Wake, 1991; Raffaëlli, 2013; Pasmans et al., 2014; Hernandez, 2016a,b, 2017). Habitat destruction and overexploitation of the urodela group for human consumption and terrarium hobbyists accounted for 54% of the population decline observed since 1980 (Hernandez, 2016). 46% were suffering from the disease: chytridiomycosis or "chytrid" caused by two devastating fungus, Batrachochytrium dendrobatidis (Bd) and Batrachochytridium salamandrivorans (Bs). No solution has been found to date to save infected populations in the wild, and contamination continues all over the world (Pasmans et al., 2014). Many assessment programs were established but efforts are insufficient due to a large global extinction at an international scale. Moreover, North and Central America as well as Asia are the most severely affected and threatened places in the world (Raffaëlli, 2013; Hernandez, 2016a). Thus, maintaining in captivity (ex situ) small amphibian populations of potentially threatened or endangered populations providing from these regions is becoming essential today (Raffaëlli, 2013; Pasmans et al., 2014; Hernandez, 2016a,b). However, the husbandry requirements for keeping newts and salamanders in captivity are complex. For many species requiring captive breeding programs the husbandry requirements are just unknown because too little is known about their ecology and habitat. Some salamanders simply fail to breed in captivity and hence it is essential to collect every appropriate parameters and protocols that would successfully lead to a result. All these data are needed for conservation purposes (Hernandez, 2017). In this paper one of the first successes in breeding the mole salamander Ambystoma talpoideum in captivity is described

with an emphasis on the environmental determinant stimuli involved. These data may assist in improving breeding these salamanders under artificial conditions.

A. talpoideum is endemic to the south-eastern and central United States, from southern Carolina to northern Florida and east to eastern Texas as well as south-eastern Oklahoma. The range extends north in the Mississippi valley to southern Illinois. There are a number of disjoint populations in Virginia, North and South Carolina, Georgia, Alabama, Tennessee and Kentucky (Shoop, 1964; Petranka, 1998). It inhabits woodlands and forested habitats including upland coniferhardwood forests, pine flatwoods and bottomland hardwood forests and is often found in expansive floodplains in areas near gum and cypress ponds (Shoop, 1960; Semlitsch, 1983; Petranka, 1998). The mole salamander is listed as Least Concern by the IUCN while it is a species of special concern in North Carolina and Tennessee. Special permits are required to conduct any activity involving this species in these states. Populations are threatened by habitat destruction or its intensive degradation. This fossorial species shows an important geographic variation involving differences in the mode of egg lying or deposition and its history life suggests that these groups are genetically separated (Petranka, 1998; Raffaëlli, 2013). Atlantic Coastal Plain populations deposit eggs singly in ponds whereas Gulf Coastal Plain populations lay egg in small clusters that are often placed on the same twig (Semlitsch & Walls, 1990). The species is known to show maturity at a total length of 8-12 cm (TL) and may live up to 6-8 years in its natural habitat (Raymond and Hardy, 1990; Williams & MacGowan, 2004). In natural conditions A. talpoideum breeds in winter from December to the end of March when temperatures are above freezing (Hardy & Raymond, 1980).

METHODS

On April 2016, 4 adult specimens comprising two females and two males were acquired in a pet store from Paris, animals originating from a special importation from South Carolina. A. talpoideum is a stocky salamander with a short body and a large head, with a total length (TL) of about 10-13 cm (see Figs. 2 & 3). The four adult specimens were obtained in Paris in January 2016 from the pet trade and maintained in a terrestrial terrarium (Exo Terra) in a porch between 16-22 °C up to October 2016 and with a natural circadian rhythm. The terrarium measured 90x45x45 cm (LxWxH) with no lamp, the substrate consisted of 30 cm of leaf litter and humus from pine and oak forests. Moss (Kindbergia praelonga) and oak leaves were placed in the terrarium to provide refuges and a stable humidity of about 75 to 85 %. Small crickets (Acheta domestica) and earthworms (Dendrobaena veneta) were the main food supply. The salamanders were found to be nocturnal with little activity. However, on October 2016, mole salamanders increased nocturnal activity and were sprayed with water twice a day. They were then placed in an aquatic aquarium of about 60x40x40 cm furnished with debris, e.g. gravel, rocks, oak leaves and branches and placed in the garden at temperatures between 2-17 °C for 4 months. The water depth of the aquarium was about 25-30 cm. All water used was rainwater obtained from empty tanks or water collected in a natural spring. Substrate used was gravels, rocks as well as oak leaves and branches. Elodea sp. were also present. No filter was used. Water was acidic to neutral with a pH from about 6.3 to 7.8 during the reproduction period. At the beginning of February 2017, temperatures dropped to 2 °C at night and 8 °C during daytime. The aquarium was sheltered under a covered porch to avoid the intense morning freeze which gave temperatures of around 14-18 °C which were gradually increased. Partial water changes of 5-10 % were performed every two-three weeks.

Food comprising earthworms (Dendrobaena veneta) and small aquatic invertebrates such as Chironimidae, Gammarus spp., Asellus aquaticus and Lumbricus terrestris all supplied weekly. On February 11th at 9h00 in the morning, eggs were found singly deposited on branches and on leaves of Elodea sp. (see Fig. 1). To avoid predation from the adults, eggs were removed from the aquarium one day after the egg laying and maintained under identical parameters at 16-18 °C in a separate tank of 60x45x45 cm until hatching an air pump (Tetra whisper 10") was used during all the protocols to oxygenate water. Oak leaves were added to avoid fungus such as Saprolegnia spp. Adults were left aquatic all year. Adult males did not show any aggressive behaviour. Larvae were raised at 18-25 °C from February to July in small tanks of about 40x40x40 cm containing about 10-20 larvae each and fed with mosquitoes larvae, Gammarus sp., zooplankton and Chironimidae. They became terrestrial at 4-5 months and were maintained in a wet atmosphere in the same kind of tanks than the one described for the adults. Six were still aquatic in August and showed paedomorphism, which is commonly known for this species (Petranka, 1998; Raffaëlli, 2013).



Figure 1. Life cycle of *A. talpoideum* in captivity: **A.** Eggs (2 days). **B.** Eggs with embryo (10 days) **C.** Young larvae with external gills after hatching (22 days). **D.** Larva with legs (30 days).

RESULTS

In late December 2016, males were observed more active and showed colourful blue spots on the body and a large blue-grey line on the tail. On a wet February 9th, 2017 at temperatures between 6.8-7.2 °C one male engaged in a nudging ritual with a female. The male repeatedly encircled the female, and then deposited two spermatophores on rocks and branches. Two days later on February 11th at a temperature of 12.5° C, the female laid 63 eggs singly attached to plants and branches (Fig. 1). The second female laid 71 eggs on February 19th at 11.8 °C with a second laying observed on March, 6th at 14.3 °C of 65 eggs. During 2016-17, the two females therefore deposited a total of 199 eggs. However, egg viability until hatching amounted to 85.9 % giving a total of 171 larvae.

One of these was consumed by congeners and a high rate of cannibalism was subsequently observed when more than 20-30 larvae were housed in the same tank. Another group of larvae was maintained under the same conditions than the adults at 17-22 °C with a diet consisting of *Enchytraeus*, *Gammarus*, *Chironomus* and mosquitoes larvae. For this group, the larval phase lasted approximately 120 to 150 days (Figs. 1 & 2). High larval mortality was observed when summer temperatures increased to 32 °C. A total of 38 animals were raised to the sub-adult stage.

DISCUSSION

The females in this study deposited a total of 199 eggs with egg viability 85.9 % indicating this species is useful for breeding programmes. Attention to food variety may be a key factor in captive reproduction with dietary supplements such as vitamins added to food before the reproductive period. This has been previously reported for the Salamandridae family (Hernandez, 2016b, 2017). This study confirms that the southern Carolina populations



Figure 2. A. Larvae near metamorphosis after 60 days; B. Larvae near metamorphosis after 110 days; C. Adult male after one year.

deposit eggs singly on vegetation and rocks a reproductive mode that corroborates the origin of the adults, which is most of the time unknown for salamanders imported through the pet trade. The origin of the salamanders is important for conservation purposes in captivity (Pasmans et al., 2014; Hernandez, 2016a,b). Additionally, many of the larvae here showed paedomorphism as previously reported in this species (Petranka, 1998; Jackson & Semlitsch, 1993; Doyle & Whiteman, 2008; Raffaëlli, 2013; Walls et al., 2013).

However, the reproductive behaviour documented here is not yet complete and more data is required, for example long-term observations of reproduction. Certain species of salamander exhibit breeding behaviours and phenology wildly out of sync with wild populations and it would be interesting to know the extent of behavioural divergence of captive animals differ from wild counterparts. A new study including patterns of migration, courtship and oviposition in relation with the wild populations would be useful. Important stimuli identified for A. talpoideum reproduction could be further investigated and include a natural photoperiod 12:12; rainwater use (acidic to neutral pH) in an aquarium full of debris (such as branches, rocks and oak leaves; see Fig. 3); and high temperature variations from 2 °C up to 17 °C with temperatures in December close to freezing (2-4 °C). Adults housed in an aquarium at the right time in winter and where all the reported stimuli are present could successfully be bred. This choice of parameters may be useful for ex situ programs involving other related taxas such as A. maculatum,

A. opacum and A. tigrinum that occur in sympatry in southeast America. Finally, it has been shown that A. talpoideum presents an explosive breeding mode as previously reported for the whole genus Ambystoma.

Generating and promoting evidence-based husbandry protocols for Urodela is urgently required since to date most of the ex situ breeding programs concern frogs and toads. For many species data on biology, life history, ecology and environmental parameters are lacking but are needed to establish appropriate husbandry conditions. Moreover, some salamanders fail to thrive and breed in captivity. This is especially true for salamanders originating from the neotropical and tropical zones. The observations reported in this paper indicate captive breeding of A. talpoideum depends on various and complex stimuli that must mimic natural conditions as much as possible. The results obtained are potentially useful to inform captive breeding programmes. However, it should be emphasised that captive bred populations of amphibians do not necessarily protect genetic diversity and are not themselves useful stock for reintroduction to nature. This problem is a major concern in terms of conservation.

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Colonisation of epiphytic ferns by skinks and geckos in the high canopy of a Bornean rainforest

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ABSTRACT - Nest site availability limits the fitness and survival of skinks and geckos, particularly in the canopy of tall tropical rainforests. We document the systematic colonisation and nest use of epiphytic bird's nest ferns (*Asplenium* spp) by the gecko *Hemiphyllodactylus typus* and the skink *Lipinia* cf. *vittigera*. As part of a controlled experiment we placed 32 ferns of similar sizes in the high canopy of a lowland dipterocarp rainforest in Sabah, Malaysian Borneo. Half of these ferns, sampled after six months, contained eggs. The remaining ferns, sampled after 12 months, contained both eggs and adults. Our results demonstrate the importance of epiphytes in providing a resource for reptile populations in the rainforest canopy.

INTRODUCTION

Reptiles play distinct ecological roles in tropical rainforests. Whilst the ecology of skinks and geckos is known for some rainforest species (Vitt et al., 1997; Vitt & Zani, 1997; Vitt et al., 2005; Akani et al., 2002; Teixeira et al., 2003; Huang, 2011), many species remain relatively understudied, particularly those associated with the high canopy. Understanding the ecology of these animals is becoming increasingly important in the face of climate change and habitat disturbance (Huang & Pike, 2011; Wanger at al., 2010).

Nest site availability is known to limit the fitness and survival of skinks and geckos (Ineich, 2010). In the canopy of tropical rainforests, epiphytic habitats are important as refuges for reptiles and amphibians (Huang & Pike, 2011; Scheffers et al., 2014). This study reveals the importance of epiphytic ferns as nest sites in the high canopy of a tropical lowland dipterocarp forest. Bird's nest ferns (*Asplenium* spp) (Yatabe & Murakami, 2003) are abundant at all heights throughout the canopy of Old World tropical forests (Fayle et al., 2009), and have been shown to support large numbers of invertebrates (Ellwood, Jones & Foster, 2002; Ellwood & Foster, 2004). The observations reported here are part of a larger experiment investigating the colonisation of bird's nest ferns by insects and other arthropods.

MATERIALS AND METHODS

The observations were made at Danum Valley in Sabah, Malaysian Borneo (4°58'N, 117°42'E, altitude ~170 m). This 43,800 ha area of undisturbed lowland dipterocarp forest experiences a wet equatorial climate, of low seasonal variation, with an average rainfall of 231.9 mm per month, and 2785.4 mm per year. Daily temperatures are on average 26.7 °C, with mean highs of 31 °C and lows of 22.5 °C (Reynolds et al., 2011). We removed the existing fauna



Figure 1. A. Asplenium bird's nest fern in the high canopy B. Lipinia cf. vittigera C. eggs D. H. typus

from 32 ferns by flushing the existing roots with water, and collecting invertebrates as they emerged from the soil. Fern root soil was then standardised by repacking the roots with soil collected from other ferns, bound with a 2 mm nylon fishing net, resulting in a root ball with a diameter of 20 cm. Eight of these standardised ferns were placed into each of four *Parashorea tomentella* trees adjacent to the Danum Valley Field Centre. The tree crowns chosen for the experiment lacked existing epiphytes, or foliage such as lianas, and the branches of the upper crowns where ferns were attached were between 32-61 m in height (Fig. 1A).

We sampled 16 ferns (four ferns from each tree) after six

Table 1. Number of eggs, adult skinks (S) and adult geckos (G) after 6 and 12 months of colonisation.

		6 months	12 m	onths	
	FERN	eggs	adults	eggs	adults
TREE 1	F1	3	0	0	0
	F2	1	0	0	0
TREE 2	F1	0	0	0	S 1
	F2	0	0	0	0
TREE 3	F1	0	0	1	G 3
	F2	0	0	1	S 1
TREE 4	F1	10	0	0	G 1
	F2	0	0	0	G 1

months and the remaining 16 ferns after 12 months. Ferns were removed from the branch and placed immediately into large plastic bags. These were transferred directly to the laboratory, where they were sampled exhaustively for the presence of eggs and adults (Fig. 1B, C, D). Photographs of each adult were taken to assist identification before releasing them (Zug, 2010). Although the species from which the eggs were derived could not be confirmed, it is presumed that they were produced by the adults sampled from within the same ferns.

RESULTS

Rapid colonisation of empty ferns confirms the high demand for epiphytic habitats for skinks and geckos to lay eggs in the high canopy. After six months, three of the 16 ferns contained eggs, but no adults (Table 1). After 12 months, two of the 16 ferns contained eggs, and five of the ferns contained adults (Table 1). Following consultation with regional experts, the adult geckos were identified as the Indo-Pacific gecko, *Hemiphyllodactylus typus* (Bleeker, 1860), and the skinks tentatively identified as the yellow striped tree skink, *Lipinia* cf. *vittigera* (Boulenger, 1894). In total, across all 32 ferns we collected 16 eggs, two adult skinks and five adult geckos.

DISCUSSION

The Indo-Pacific gecko is a small gecko (snout-vent length ca. 35 mm) with a widespread distribution across south-east Asia and Oceania (Zug, 2010). There is a distinct lack of ecological knowledge surrounding this genus, almost certainly linked to its secretive nature and difficulty of observation (Holden et al., 2013). This species was documented colonising myrmecophyte 'ant plants' in Bako National Park in Sarawak (Janzen, 1974), but to our knowledge this is the first account of colonisation of Asplenium ferns in the upper canopy of a primary rainforest. The yellow striped tree skink is an arboreal skink known to occur at Danum Valley (Das & Austin, 2007), but until now its presence in the high canopy was unconfirmed. Based on our observations, we suspect that the bird's nest fern may be just one of a number of epiphytes that allow these species to persist in the rainforest canopy of Danum Valley and probably elsewhere.

Nest site availability is thought to be the biggest limiting factor for arboreal skink and gecko populations

(Ineich, 2010). Epiphytes in general have been shown to provide nest sites for a range of gecko species including *Lepidodactylus buleli* (Ineich, 2008), *Gehyra vorax* (Ineich ,2010), *Woodworthia chrysosireticus* and *Mokopirirakau granulatus* (Henwood et al., 2014). In the Philippines, bird's nest ferns in particular were shown to provide cool, moist microhabitats for *Platymantis* arboreal frogs within the relatively hot and dry canopy (Scheffers et al., 2014). Elsewhere, bats have been shown to use bird's nest ferns as a roost (Tan et al., 1999). Our work suggests that bird's nest ferns are as attractive to skinks and geckos as they are to other animals, such as annelids, molluscs and arthropods (Ellwood et al., 2002; Ellwood and Foster, 2004).

Not only do bird's nest ferns provide a refuge in the canopy, they also act as an important food source. Although the ferns often contain aggressive, predatory arthropods such as centipedes (*Scolopendra* spp), they also support large amounts of invertebrate biomass (Ellwood & Foster, 2004). In particular, bird's nest ferns support large colonies of social insects such as ants and termites (Ellwood et al., 2002; Ellwood et al., 2016). The high rate of colonisation of those ferns observed in this study confirms the importance of bird's nest ferns as a valuable resource for arboreal skinks and geckos.

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First record of *Atractus turikensis* (Squamata: Colubridae: Dipsadinae) from the Colombian Perijá highlands

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ABSTRACT - This paper gives details of the first record of the small and poorly known dipsadine snake *Atractus turikensis* in Colombia. This includes the first specimens that do not correspond to the type series and the first vouchers of the species outside of Venezuela. We describe its morphology and some aspects of its natural history.

INTRODUCTION

The genus *Atractus* Wagler 1828 is the most speciose among the neotropical snakes, which is distributed from Panamá to Argentina (Myers, 2003). It is composed by small non-venomous snakes with terrestrial or cryptozoic habits (Silva, 2004). In Colombia, 65 species are recognised (Ecavarría-Rentería et al., 2015; Uetz et al., 2016). However, this number may be underestimated, due to new species being described every year (Passos et al., 2009b; Passos & Lynch, 2010; Khöler & Kieckbusch, 2014). *Atractus* is mostly represented by endemic or restricted distribution species, sometimes only known from their type localities. (Passos and Fernandes 2008; Passos et al., 2009).

In the Serranía del Perijá three species are recognised, *Atractus turikensis* and *A. acheronius* from the eastern flank; and *A. vertebrolineatus* on the western flank (Moreno-Arias et al., 2009; Rivas et al., 2012). However, there are three

other records of the genus in the area: *A. ventrimaculatus* (Rojas-Runjaic et al., 2007), *A. eriki* (Passos et al., 2009a; see material examined), and *A. indistinctus* (Passos et al., 2009b). This species have been discussed by Nattera et al. (2015) because of the little information given about these records in their papers, and were considered by them as "dubious". Conflict generates uncertainty regarding the taxonomic status and specific determination of any *Atractus* specimen collected in the Serranía del Perijá.

Atractus turikensis is a small and poorly known species, listed as vulnerable according to the most recent red book of the Venezuelan fauna (Rodríguez et al., 2015). It is considered endemic to Venezuela, only known by three specimens originating from the Mesa de Turik, an isolated plateau at 1800m asl, on the eastern flank of the Serranía del Perijá, Estado Zulia, Venezuela (Barros, 2000). Given the inaccessibility of the type locality and lack of specimens and field observations, all aspects of the biology of this



Figure 1. Known distribution of A. turikensis in the Serranía del Perijá. Map designed by Lorena Benítez-Cubillos.

snake are unknown (e.g. the colour definition of the original description is based on freshly preserved specimens, and not by live specimens. T.R. Barros, pers. com). Therefore, the objective of this contribution is to report the first record of *A. turikensis* for Colombia, as well as describing the colour in life and some aspects of its natural history.

MATERIALS AND METHODS

During an expedition to the highlands in western flank of the Serranía del Perijá, in September 2015, we collected two specimens of *A. turikensis* (information, see Table 1). These were deposited in the Reptile Collection of the Centro de Colecciones de la Universidad del Magdalena (CBUMAG:REP), Santa Marta, Magdalena, Colombia; under the numbers CBUMAG:REP: 00299, 00300. This locality corresponds to the quebrada El Jordán, El Contento Village,San José de Oriente,Municipio La Paz,Departamento del Cesar, Colombia. (10°17'47.3" N, 72°55'55.8" W; 2540m asl) (Fig. 1). The landscape is covered by disturbed high Andean forest with stepped successional processes, where undergrowth species are virtually nonexistent.

For species determination, we examined lepidosis and morphometric features. In addition, the head scalation patterns of our speciemens were compared with photographs of paratype specimen of *A. turikensis* MBLUZ R-302.

RESULTS AND DISCUSSION

Species determination

Although the counts of ventral scales of our specimens are greater than those of the type series, it presents other characters that resemble *A. turikensis* (Table 1, Fig. 2). Our specimens can be distinguished immediately from *A. acheronius* (characters in parentheses) by having a narrow body, < 10 mm (thick body, > 10 mm, Table 2), and seven infralabials (versus six) and three preventrals (one preventral) (see Passos et al., 2009b); likewise, they are distinguishable from *A. vertebrolineatus* and *A. indistinctus* by the absence of dorsolateral lines present in those species, having 27-28 subcaudal scales (45 subcaudal scales), and the cephalic colouration is uniform with the body (darkened in head than body) (Prado, 1940, 1941).

They can be distinguished from species with unconfirmed distribution on the Serranía del Perijá, for example A. ventrimaculatus, by having 17-17 dorsal scales (15-15 dorsal formula), having seven supralabials, third and fourth in contact with eye, (eight, fourth and fifth in contact with eye) (Esqueda & La Marca, 2005); while it differs from A. eriki by having 172-179 ventral count (162 ventrals scales), the presence of a partial line over the vertebral region (absence, pattern uniform), and by the ventral surface being golden on the sides and dark blue in the center (ventral surface cream, lightly dark brown spotted) (Esqueda et al., 2007). Finally, a species not mentioned for this region is A. pamplonensis (although there are specimens in museums that occur north of its type locality "Pamplona, Colombia", L.F. Esqueda pers. comm.), whose morphological attributes are very similar to our specimens. For example, same range of ventral and subcaudal scales, similar count of supralabial



Figure 2. Comparison of the cephalic lepidosis of *A. turikensis* paratype (MBLUZ R-302) (left) and Colombian specimen (CBUMAG:REP:00300) (right) in dorsal **(a)**, lateral **(c)**, and ventral view. Photograph by Luis Sibira (MBLUZ R-302) and Juan David Jiménez-Bolaño (CBUMAG:REP:00300).

and infralabial scales and dorsal pattern colouration, in both cases with small separated spots arranged longitudinally, giving the impression of a broken line. Contrary to the few differences between the two species is four gular scales in *A. turikensis* (one to two gular scales), four infralabial scales in contact with geneials (three infralabials in contact with geneials), the ventral region mostly darkblue and golden in margins (ventral region mostly yellowish with discontinuous spots at the edges, forming a discontinuous lateroventral stripe), and the head arched in lateral profile (not arched in the lateral profile) (Pérez-Santos & Moreno 1988; Schargel & García-Pérez, 2002; Natera et al., 2015).

Colour in life description

Atractus turikensis is a colourful species because of its iridescent scales. The dorsal surface is gold to dark gold. Likewise, some groups of dorsal scales are dark gray coloured and surrounded by golden scales. Dark gray spots are spread unevenly throughout the body, though they give the impression of forming two dorsolateral interrupted stripes. Ventral scales are dark blue in the center, turning gold around the edges . The head dorsum is dark gray with dark gold irregularly distributed scales that spread to half of supralabial scales. The other half of supralabials and infralabials are golden. The head ventral surface is golden, except for the geneial scales, which are dark gray (Fig. 3). Surely, the "light brown" and "dark brown" surfaces on the dorsal region described by Barros (2000), are golden in live specimens. Therefore, we consider the pattern of colouration of our specimens and the illustrations provided by Barros (2000) are very similar, especially in the dorsal design and

Table 1. Lepidosis of potential *Atractus* species from the Serranía del Perijá. Data provided by Prado (1940, 1941), Pérez-Santos and Moreno (1988), Barros (2002), Schargel and García-Pérez (2002), Esqueda and La Marca (2005), Esqueda et al., (2007), Passos et al., (2009b), and Natera et al., (2015). *Data of preventral scales obtained only from paratype MBLUZ R-302. Not including data from other type specimen

Species	Dorsals	Preventrals	Ventrals	Subcaudals	Supralabials (in contact with eye)	Infralabials (in contact with geneials)	Gulars
<i>A. turikensis</i> Barros, 2000 (CBUMAG)	17-17	3	172-179	27-28	7 (3/4)	7 (1/2/3/4)	4
A. turikensis (type series)	17-17	4*	158-166	20-27	7 (3/4)	7 (1/2/3)	4
<i>A. acheronius</i> Passo, Rivas & Barrio-Amorós, 2009b	17-17	1	166	23	7 (3/4)	6	4
A. pamplonensis Amaral, 1937	17-17	2-3	172-189	23-30	7 (3/4)	6-7 (1/2/3)	1-2
A. indistinctus Prado, 1940	17-17	NA	170	35	7 (3/4)	6 (1/2/3)	NA
A. vetebrolineatus Prado, 1941	17-17	NA	159	46	6 (3/4)	7 (1/2/3)	NA
<i>A. eriki</i> Esqueda, La Marca & Bazó, 2007	17-17	3	152-160	30-40	7 (3/4)	6 (1/2/3)	3
A. ventrimaculatus Boulenger, 1905	15-15	NA	143-156	16-23	8 (4/5)	7 (1/2/3/4)	4-5

Table 2. Comparisons of the morphometry of *A. turikensis* and *A. acheronius* specimens- Data provided by Barros (2000) and Passos et al., (2009b). *In Table 1 of Barros (2000), he presents the SL of female paratype as higher than HL, therefore, SL/HL ratio was erroneously calculated. **The TaL measurement was calculated as 9.7% of the SVL of the *A. acheronius* holotype.

			A. turikensis			A. acheronius
Specimen	CBUMAG:REP: 00299	CBUMAG:REP: 00300	MBLUZ R-301 (Holotype)	MBLUZ R-302 (Paratype)	MCN UNELLEZ N° 1914 (Paratype)	MHNLS 398 (Holotype)
Total length (TL)	199.12	270.85	439	420	364.03	587.992
Snout vent length (SVL)	181	244	402.61	398.06	329.97	536
Head length (HL)	9.3	10.28	10.1	9.55*	8.45	18.7
Head Width (HW)	4.23	5.58	7.62	6.68	5.84	9.6
Snout length (SL)	2.74	3.06	8.1	9.9*	5.91	5.7
Interorobital distance (IOD)	3.55	3.86	4.57	4.34	3.91	6.9
Eye diameter (ED)	1	1.13	1.44	1.34	1.27	1.9
Tail length (TaL)	18.12	26.85	36.39	21.94	34.06	51.99**
Maximum body width (MBW)	3.52	5.74	10.49	10.06	6.85	12.3
Tail base width (TBW)	1.69	3.31	5.08	5.38	4.39	NA
HW/HL (%)	45.48	54.28	75.45	69.95	69.11	51.34
SL/HL (%)	64.78	54.84	80.20	148.20*	69.94	59.38
IOD/HW (%)	83.92	69.18	59.97	64.97	66.95	71.88
TaL/LT (%)	9.10	9.91	8.29	5.22	9.36	8.84
TBW/MBW (%)	48.01	57.67	48.43	53.48	64.09	NA

chin colouration.

Nevertheless, we detected some differences between our specimens and those described by Barros (2000). The paratype specimen used in the original description of A. turikensis has a single interrupted vertebral stripe, while the two specimens collected by us have two interrupted paravertebral "stripes". We consider that A. turikensis does not present true longitudinal stripes, since the area occupied by the stripes on the scales are proportional in all their extension, even when interrupted (see photograph of A. emigdioi in the Reptile Database link for a graphic http://reptile-database.reptarium.cz/species?g description enus=Atractus&species=emigdioi). On the other hand, A. turikensis presents spots in an irregular proportion, neither completely aligned, nor consistently arranged, which may vary on each specimen.

Distribution and natural history



Figure 3. Specimen CBUMAG:REP:00300 in life. Lateral (a), ventral (b), and dorsal (c) views. Photos by Efrain Rada-Vargas.

The two specimens (both females) were captured in the morning. The specimen CBUMAG:REP: 00299 was found under a boulder, while the specimen CBUMAG:REP:00300 was under a fallen decaying trunk. Prints left by snake crawling activities on the substrate, suggest that *A. turikensis* has cryptozoic or mining habits. Another uncollected specimen was observed active during the night, suggesting nocturnal activity.

The new record extends the distribution of A. turikensis 27 km to south-west of its type locality and demonstrates that the species is present in both western and eastern versant of Perijá. Its altitudinal range is also extended from 1800 to 2540m asl, with the highest altitudinal record corresponding to the Colombian locality reported herein. Also, this new record increases the number of Atractus species known for Colombia to 66. A comprehensive search for additional specimens in the Perijá highlands (both Colombia and Venezuela), is required to determine the real distribution of this species. Although in Venezuela the species occupies an uninhabited area where no major threats are known to exist, which is the reason it is considered under least concern by the IUCN (Rivas, 2016). Atractus turikensis does not seem to be very abundant, and faces accelerated deforestation of the high Andean forests of Colombian Perijá, therefore, an assessment of population and conservation status of this snake is needed.

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New records of paedomorphic smooth newts (*Lissotriton vulgaris*) at a site in Cambridgeshire, U.K.

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The smooth newt (*Lissotriton vulgaris*) is a common I and widespread amphibian species throughout most of its range, including the UK (Beebee & Griffiths, 2000). Whilst surveying for great crested newts (Triturus cristatus) at the Orton Brick Pits SSSI/SAC (TL162941) in Cambridgeshire, England during March 2015, a small number of paedomorphic smooth newts were discovered in bottle traps or observed within the target pond by torchlight (Fig. 1). All of the individuals were greater than 7 cm in length, which would classify them as of adult size. Two of the paedomorphic smooth newts were caught from the same pond using bottle traps (on 12th March 2015) whilst the third was seen under torchlight in a neighbouring pond the following evening. The two newts caught in bottle traps were typically coloured females whereas the individual seen in the pond was darker in colour.

This is a new location for smooth newt paedomorphosis in Britain where its occurrence is believed to be relatively rare, although the phenomenon has been recorded elsewhere in the county before (Beebee & Griffiths, 2000).

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Figure 1. One of the paedomorphic smooth newts caught whilst bottle trapping for great crested newts at the Orton Brick Pits

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Limb malformation in the skipper frog (*Euphlyctis cyanophlyctis*) (Amphibia: Dicroglossidae), first evidence in Bangladesh

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n 29 August 2016, a deformed adult Skipper frog (Euphlyctis cyanophlyctis) was found in Shere-e-Bangla Agricultural University Campus (23°46'14.68"N, 90°22'41.21"E; Elev 14m; WGS 84), in Dhaka City, Bangladesh. The adult frog was found while calling near a paddy field during a night survey with almost all of its left forelimb absent, but the right forelimb was normal (Fig. 1). The following measurements were taken: SVL: 41 mm, length of the right forelimb (normal limb): 24.60 mm, length of the small fraction of the deformed left forelimb: 3.14 mm, hind limbs were found in normal condition. Figure 2 shows a radiographic image indicating bone structure of the deformed frog. An interesting aspect of the condition is that the frog had managed to survive to the adult stage, which necessitates securing prey for growth and presumably avoiding predation. A recent report (Ramalho et al., 2017) indicated blind Amazonian tree frogs, apparently congenital conditions, had also survived to the adult stage, albeit rather underweight.

This is the first evidence of forelimb malformation in amphibians in Bangladesh ever reported. *Euphlyctis cyanophlyctis* is a widely distributed species in Bangladesh and highly adapted to aquatic habitats including agricultural fields, temporary pools, etc. (Chakma, 2009; Hasan et al., 2014; Khan 2015). The paddy field in Shere-e-Bangla Agricultural University campus is used by the students for their agriculture related research purpose, such as cultivation of different paddy plants or vegetables, etc. They employ different pesticides and agro-chemicals during cultivation and hence there is the possibility that these could be involved in the malformation described here. Further investigations are in progress into this phenomenon.

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Figure 1. Forelimb malformation in adult *E. Cyanophlyctis*: Details of the dorsal side of the deformed left forelimb



Figure 2. Radiograph of the adult E. cyanophlyctis

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Notes on breeding of the barred wolf snake (*Lycodon striatus*) in Nilgiris, Tamil Nadu, India

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The barred wolf snake (Lycodon striatus) is a non-L venomous colubrid snake distributed in south Asia (India, Nepal, Pakistan, Afghanistan, Sri Lanka), as well as eastern and north-eastern Iran, western Tajikistan, southern Turkmenistan and Uzbekistan. In India, this species have been recorded from Himachal Pradesh, Kerala, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, Maharashtra and Punjab (The Reptile Database, 2017). Many details of the breeding biology of Indian snakes are poorly understood but some are known to be prolific breeders, and most reproduce seasonally (Nixon, 2015). Breeding seasonally can affect many aspects of a species biology, and in turn affect its life history and demography (Lemes-Espinal et al., 2003). This note reports on the breeding season and habitat, juvenile size and clutch size of L. striatus in the Nilgiris, Tamil Nadu, India.

On 15th November 2015, 11:45h a juvenile snake was observed at a settlement of Kurumba tribals near Mettupalayam (N 11.34270°, E 76.87007°) in Nilgiris, Tamil Nadu, India. This settlement is located along a river side habitat where the major forest type is mixed deciduous (Champion & Seth, 1968). Additional juvenile snakes were observed coming out one by one from a hole in a kitchen wall. A total of nine individuals were observed (Fig. 1). After the wall was broken we found nine egg shells indicating all snakes in the clutch had hatched successfully (Fig. 2). The clutch size, if deposited by a single female is larger than what has previously been reported. The juveniles were identified as barred wolf snakes using a field guide (Whitaker & Captain, 2004). The total length of juveniles ranged between 153 mm and 171mm, eggs between 20 mm and 25 mm.

Previous records reported the clutch size ranged between two to four eggs (Smith, 1943; Das, 2002; Whitaker & Captain, 2004). The literature indicates that this species deposits eggs in the month of July and August (Smith, 1943), April (Das, 2002) and August (Whitaker & Captain, 2004) with the incubation period usually 50-60 days depending upon geographical location and temperature (Das, 2002; Whitaker & Captain, 2004). It is possible that the eggs were laid around August to September, and hence juveniles were observed in November.



Figure 1. Nine individuals of *L. striatus* collected from a hole in a kitchen wall



Figure 2. Total of nine egg shells of L. striatus

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A very European tale – Britain still has only three snake species, but its grass snake is now assigned to another species (*Natrix helvetica*)

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Following several investigations of the phylogeography and systematics of grass snakes (Fritz et al., 2012; Kindler et al., 2013, 2014; Pokrant et al., 2016), we published a further detailed study on this topic in August (Kindler et al., 2017). Our new investigation revealed that only very limited gene flow occurs between western barred grass snakes and eastern common grass snakes. Consequently, we concluded that the barred grass snake (Fig. 1), previously a subspecies, should be elevated to a full species. August being the 'silly season' for news stories led the local media, including the highly respected BBC, to claim that Britain has now an additional snake species, i.e. four instead of three species - the northern viper (Vipera berus), the smooth snake (Coronella austriaca) as well as two species of grass snake, the common grass snake (Natrix natrix) and the newly recognised barred grass snake (Natrix helvetica).

This upheaval resulted from a complete misunderstanding of a press release by the Senckenberg Institution. The press release pointed out that Europe now has one more full snake species, which was misinterpreted as Britain gaining an additional snake species. In Brexit times, we have great sympathies with this approach. However, we have to face reality. Since the Kingdom of Hanover was lost from the United Kingdom in 1837, only one species of grass snake lives within UK borders (excluding a few introduced N. *natrix* and their offspring identified by our study). Until our paper was published (Kindler et al., 2017), the native British populations were assigned to the subspecies Natrix natrix helvetica, also widely distributed on the continent. Now, the strong evidence we presented indicates that this subspecies should be recognised as a full species, Natrix helvetica. Yet, this did not add another species to the British fauna, the British populations simply changed their identity label, as will all Britons when they leave the European Union. They will no longer be listed as EU citizens, but otherwise they will remain the same. Having now clarified this matter, we want to give a quick summary of the scientific situation.

As long ago as 1979, a thorough analysis of morphological characters demonstrated that there is a sharp break between western barred grass snakes and eastern common grass snakes (Thorpe, 1979). Geographically, this break approximates to the Rhine region in western Central Europe. Our genetic results (Kindler et al., 2013, 2017), based on analyses of mitochondrial DNA sequences and 13 microsatellite markers, largely corroborate Thorpe's



Figure 1. Young *N. helvetica* showing the distinctive lateral bars from which the species common name the 'barred grass snake' is derived (photo: © Jason Steel)

findings. However, some southern populations identified by Thorpe with barred grass snakes, for instance from northern Italy, turned out to be distinct from N. *helvetica* sensu stricto, explaining Thorpe's finding that in this region morphological divergence becomes fuzzy. These southern populations, distributed from the Padan Plain over the Apennine Peninsula, Sicily, Corsica, and Sardinia, are closely related to N. *helvetica*, but nevertheless are genetically and morphologically distinct and can be considered as subspecies of N. *helvetica* (Kindler et al., 2017).

For Central Europe, we found that the gene flow between N. helvetica and N. natrix is largely unidirectional from N. helvetica into N. natrix and restricted to a narrow belt less than 50 km wide in the Rhine region. In contrast, another contact zone in eastern Central Europe and southeastern Europe is very wide (more than 650 km). In this eastern contact zone there are two genetic lineages of N. natrix involved, and nuclear genomic admixture is more or less complete (Kindler et al., 2017). The two contact zones correspond to two very different stages of the speciation process. With respect to the western contact zone in the Rhine region, we understand our findings as hard evidence for a widely advanced divergence process of N. helvetica and N. natrix. Therefore, we proposed to recognise the two taxa as distinct species. Our view is supported by morphological differences between N. helvetica and N. natrix and the considerable divergence time of 7.3-8.2 million years between the mitochondrial lineages of the two species (Fritz et al., 2012).

Europe now has three full species of grass snake, *Natrix* astreptophora, which is the recently recognised Ibero-Maghrebian species (Pokrant et al., 2016), *N. helvetica* and *N. natrix* (Kindler et al., 2017). However, Britain still has only one native species – the barred grass snake, *N. helvetica*. The only opportunity to have more would be to merge with the European Union.

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British Herpetological Society: Ethical Policy and Guidelines

The AIMS of the BHS include 'The promotion of the study and protection of amphibians and reptiles, in particular through scientific research, captive breeding and husbandry, conservation and fieldwork, and education'. These aims are achieved in part by the publication of research on amphibians and reptiles in the Society's two scientific journals: The Herpetological Journal and The Herpetological Bulletin. It is a requirement of research published in these journals that it adheres to the Society's ethical policy.

The essential principles governing BHS policy on research on animals follow the ethical theory known as utilitarianism. In this, the main benefit of research is the increase of knowledge. Knowledge is a general benefit to people, but it can also be of practical benefit to the animals concerned in helping people to understand better how to protect them (for example, from disease) and to conserve both them and their habitats. However, research can impose costs on animals, ranging from mild (such as light stress from being closely observed), through to severe, as when an animal dies, possibly painfully, in the course of a study. Utilitarian ethics justifies research when the benefits exceed the costs. In the case of research on animals, this means that studies which impose the severest costs on animals require the greatest justification. As an example, if a study involves killing frogs, BHS would require that the knowledge gained is substantial, and not merely confirmatory; and if a large number of frogs are killed, then the knowledge gain would need to be very substantial. In addition, BHS would expect evidence that the methods used to kill the frogs is humane. BHS would be unlikely to accept a study if the frogs killed are in the higher IUCN Red Data categories.

Research on amphibians and reptiles occasionally involves potential harm to the researchers, especially when studying venomous snakes, or carrying out fieldwork in remote or hazardous locations. BHS requires a statement that any research has been preceded by a risk assessment and that the recommended procedures have been followed. Equally, there are ethical implications concerning the impact of the research on people. An assessment therefore needs to be carried out of any potential implications of the work for local people's livelihoods, religious beliefs or cultural practices. If the research subjects involve people (e.g. using a questionnaire survey to determine attitudes or knowledge), then the research must follow the principles of informed consent, ensure that the identities of participants are protected, and a statement provided concerning the ethical guidelines consulted (e.g. http://www.esrc.ac.uk/funding/guidance-forapplicants/research-ethics/).

Other organisations involved in animal research have developed substantial ethical guidelines. Rather than develop something entirely new, BHS has drawn from existing sources. The principal ones are: Guidelines for the Treatment of Animals in Behavioural Research developed by the Association for the Study of Animal Behaviour (ASAB, 2012); the Universities Federation for Animal Welfare Handbook (Hubrecht & Kirkwood, 2010), especially the chapters on terrestrial and aquatic reptiles and on amphibians; guidance on wildlife research provided by the National Centre for the Replacement, Refinement & Reduction of Animals in Research [https://www.nc3rs.org.uk/wildlife-research]) and Guidelines for Research on Live Amphibians and Reptiles developed by the American Herpetological Animal Care and Use Committee (HACC, 2004).

BHS policy has the following components:

1. Compliance with legislation: Research submitted to BHS derives from countries all over the world. Legislation governing research on amphibians and reptiles may be specific to one country, or it may be international. For example, many countries have laws regulating experimentation on animals, both in the laboratory and in the field, such as the UK's Animals (Scientific Procedures) Act. BHS expects full compliance with the legislation of the country where the research is carried out, and requires a statement from the authors confirming compliance. When a country lacks such legislation, BHS may require that the research complies with an alternative legal framework. In addition to national legislation, individual research institutions often have ethics committees that regulate animal research: BHS expects a statement of compliance with any local requirements of that kind.

An example of relevant international legislation is the Convention on Trade in Endangered Species of Fauna and Flora (CITES), to which nearly every country is a party. BHS expects full compliance with relevant international legislation and, again, a statement of confirmation.

Data from all work that involves the research, euthanasia or removal of animals from the wild must be collected in accordance with the country's national legislation, with all research and collection permits in place prior to the fieldwork or research commencing. In addition, the Nagoya Protocol on Access and Benefit Sharing (ABS) is a 2010 supplementary agreement to the 1992 Convention on Biological Diversity (CBD) that aims to ensure the fair and equitable sharing of benefits arising out of the utilisation of genetic resources, thereby contributing to the conservation and sustainable use of biodiversity. This legislation entered into force on 12 October 2014, and all genetic material collected from natural resources (i.e. living wild organisms) of party states is subject to this legislation. If material is transported out of the origin country, it is the authors' responsibility to ensure the correct collection and export permits have been lodged with the origin countries' Access and Benefit Sharing Clearing House, and the subsequent Internationally Recognised Certificate of Compliance (IRCC) issued prior to exportation of materials.

2. Laboratory practice: Few, if any, species of amphibians and reptiles can be considered as having populations adapted to laboratory life in the way that laboratory rats and mice are. Researchers therefore need to remember that they are studying wild animals kept under captive conditions, and that this could be potentially stressful to the animals. BHS therefore requires that laboratory research should be carried out under high standards of welfare and that submitted papers include a clear statement of husbandry conditions and of how welfare was achieved, either in the main text of the paper or if more appropriate, as part of supplementary material. General husbandry methods for amphibians and reptiles are provided in Hubrecht & Kirkwood (2010) and in HACC (2004), but more specialist literature may need to be consulted. Factors to consider include enclosure design; environmental conditions such as humidity, light, temperature: feeding: health, including disease prevention: interactions: environmental and behavioural social enrichment. Enrichment remains an under-reported aspect of amphibian and reptile husbandry (Burghardt, 2013; Michaels et al., 2014), and BHS welcomes the submission of new studies.

3. **Fieldwork practice**: Fieldwork on amphibians and reptiles can involve a wide range of activities, with the effects on the animals varying from negligible or mild to increasingly severe: animals may be:

- simply observed with no or minimal disturbance; animals may be measured with minimal disturbance, such as taking carapace lengths on a nesting marine turtle;
- captured for a time just long enough to take non-invasive measurements, then released;
- captured to allow invasive sampling, such as taking blood samples or skin swabs, then released;
- marked in some way, so as to allow them to be recognised again, then released;
- attached to some kind of data-recording device, then released;
- captured and taken to a laboratory for a short time, so that observations not feasible in the field can be made, then released back at the original site;
- captured and set up in an experiment which mimics aspects of the natural environment, with later release as a possible outcome. Such experiments may be termed 'semi-natural'.
- captured and killed in order to collect data not available from live animals, or in order to preserve the specimens for museum collections.

In all cases, BHS expects a clear account of the methods used, including measures taken to reduce stress or discomfort to the animals. Marking and euthanasia are discussed in the next section.

4. **Marking and euthanasia**: Although any laboratory or field procedure can cause stress in animals, among the most problematic from an ethical viewpoint are marking methods and killing techniques. Marking is much used in population and behavioural studies as a way of recognising individuals after release. Examples are the use of flipper tags to mark

marine turtles and the use of toe-clipping to mark lizards and frogs. The ethical questions are: does the creation of the mark cause pain, and if so, is it short or prolonged, and does the mark affect the behaviour and survival of the individual? Toe-clipping of frogs has long been controversial with advocates both for and against (compare Grafe et al., 2011 with Parris et al., 2010). The BHS view is that toe-clipping should only be used as a method of last resort and for important studies. For amphibians, the ready availability of cheap digital photography allied to the growing recognition of individually variable cutaneous markings should render toe-clipping obsolete for many species.

In some studies, the killing of animals is necessary in order to gather essential information, such as the documentation of a voucher specimen. Since death is a normal aspect of life, ethicists generally do not regard death as a harm in itself. However, the experience of death can be long and painful, and that is a harm. For any research that requires the death of animals, the aim must be euthanasia, i.e. death should be rapid and free of stress and pain. BHS expects a clear account of the euthanasia methods used. The number of animals killed should be kept to a minimum, since excessive killing could harm the viability of the population, and sample sizes should be justified based on existing literature and/or statistical power analyses.

5. Toxicity testing: Amphibians and reptiles are not generally used to test for the toxicity of substances such as agrichemicals when there is a concern that people may be harmed by these substances. It is therefore entirely possible that such substances may cause unintentional harm to amphibians and reptiles, and there is growing evidence of such effects (e.g. Orton & Tyler, 2015).

Experiments designed to assess the impact of potentially toxic substances on amphibians and reptiles inevitably generate ethical concerns, since there is an expectation that some animals may be harmed. Under the UK's Animals (Scientific Procedures) Act, deliberate exposure of adult amphibians and reptiles to experimental toxicity testing would require a licence, since they are covered under the heading of 'live vertebrates'. The situation is different for early developmental stages; anuran amphibians are not considered as 'live vertebrates' until the onset of active feeding, around Gosner (1960) stage 24/25. This means that toxicity testing on anuran embryonic stages is not subject to UK regulation. For amniote vertebrates, including reptiles, the equivalent critical point is mid-way through egg incubation.

For toxicity assessment papers submitted to BHS publications, the ecological relevance of the study must be clear, compliance with legislation must be explicit, and the number of individuals exposed to harm kept to a minimum and justified using existing literature and/or statistical power analyses.

6. **Conservation aspects**: Culling, disease, captive breeding and release: The motivation behind much wildlife research is conservation, i.e. the desire to maintain the diversity of wildlife and wild habitats. However, conservation procedures can come into conflict with animal welfare and therefore arouse ethical concerns (see Conservation and Animal Welfare Science Workshop (2010) for an attempt to resolve such conflicts). An obvious example is the culling of one species, often an alien invader, in order to improve the life chances of others. A herpetological example is the culling of cane toads in Australia, where their rapid spread since introduction in 1935 is regarded as a serious threat to many native species.

Another potential harm is the inadvertent spread of disease by researchers during their work aimed at conservation. The most obvious example is the spread of the chytrid fungi *Batrachochytrium dendrobatidis* and *B. salamandrivorans* via researchers' boots and sampling equipment. Where chytrid is a risk, it is good practice to clean and sterilise boots and equipment between sites. BHS expects papers describing fieldwork in areas where disease spread is a risk to include an account of the measures taken to minimise that risk.

Captive breeding and release is widely regarded as a method of last resort for saving species at severe risk of extinction (Marris, 2008), but release protocols and associated technologies are improving all the time. When considering for publication any papers reporting on release programmes, BHS expects a clear statement of the preparations made to maximise the chances that released individuals will thrive, including permissions obtained from the relevant regulatory authorities.

7. **Numbers**: In carrying out field or laboratory studies that have the potential to cause harm to individual amphibians and reptiles, it is important to consider the number of animals to be used. In many cases this should involve a statistical power analysis to ensure that the results obtained do not fail to reach statistical significance simply because of samples being too small. This is an ethical issue because such a study would have harmed animals without having any reliable result.

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