

Using Glow Sticks to Increase Funnel Trap Capture Rates for Adult Vernal Pool Amphibians

Amphibians are declining around the globe, and causes include climate change, disease, over-exploitation, and habitat loss (Kiesecker et al. 2001; Stuart et al. 2004). In the United States, vernal pools are ephemeral wetlands that are critical habitat to dozens of North American amphibian species (Petranka 1998; Lannoo 2005). Given that they dry on a regular basis, they provide prime breeding grounds free of fish predation (Karraker and Gibbs 2009). However, vernal pools and their surrounding habitat are particularly vulnerable to habitat loss because they are not protected by federal law (Semlitsch and Bodie 1998; Gibbons 2003). Even if the wetlands themselves are preserved, many species migrate to these pools from great distances, making them sensitive to land-use change in surrounding upland areas (Gibbs and Shriver 2005; Harper et al. 2008). Salamander and anuran populations have also declined in areas like U.S. national parks despite having both wetland and upland habitat protected (Adams et al. 2013). With such complex drivers of population decline, many species that require vernal pool habitat are often designated as endangered, threatened, or species of conservation concern in the states they occur (e.g., 50% of *Ambystoma* in the northeastern U.S.; Mitchell et al. 2006). Determining and monitoring population status has become increasingly important, and many resources have been devoted to improving amphibian monitoring: e.g., the United States Geological Survey Amphibian Research and Monitoring Initiative (armi.usgs.gov), products from Partners

in Amphibian and Reptile Conservation (Graeter et al. 2013). However, monitoring changes in species' populations is dependent on effective sampling techniques; thus, improving sampling techniques is a critical component to monitoring and conserving species.

Many pond-breeding amphibians are highly cryptic for most the year. Monitoring efforts have consequently focused on the breeding season when migrating adults and egg masses are conspicuous at vernal pool breeding grounds (Miller and Grant 2015; Davis et al. 2017). Despite the availability of adults for sampling, egg masses are usually surveyed as an index of adult breeding females instead (Crouch and Paton 2000; Miller and Grant 2015; Amburgey et al. 2017). To capture adults, established survey techniques fall broadly into two categories: active sampling where surveyors capture animals and passive sampling where animals encounter traps. The most frequently used methods include drift fences with pitfall traps, aquatic funnel traps, visual encounter surveys (VES), and dip-net surveys (Heyer et al. 1994; Hutchens and DePerno 2009; Willson and Gibbons 2009). Each method varies considerably in the amount of effort and material required. Drift fences are passive arrays usually deployed by completely encircling vernal pools with fencing and pitfall traps to ensure a near-census of migrating breeding adults (Dodd 1991; Crouch and Paton 2000; Gibbons et al. 2006; Grayson et al. 2011). The amount of resources needed to install, maintain, and monitor drift fences is high, so drift fence studies are generally restricted to one or a few vernal pools (Dodd 1991; Gibbons et al. 2006; Grayson et al. 2011). Active sampling methods, such as VES and dip-net surveys, are versatile and require less effort and material to conduct surveys; however, outcomes are often biased by observer skill, and they frequently damage essential habitat (Heyer et al. 1994; Grant et al. 2005; Sutherland 2006; Bennett et al. 2012).

Unlike active surveys where encountering and capturing an available animal is mainly dependent on the quality of surveyor,

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FIG. 1. A) The aquatic funnel trap with float (labeled with trap name). The right trap must be adjusted to eliminate gap between trap halves. B) Deployed aquatic funnel trap baited with glow stick treatment. C) Internal view of aquatic funnel trap with 2.54 cm trap opening by which animals entered. Shown with adult *Ambystoma jeffersonianum*.

passive surveys are dependent on traps being available, on animals encountering them, and on animals being caught and retained (Luhring et al. 2016). As amphibians move terrestrially to wetland breeding sites, drift fence surveys ensure high encounter rates by restricting access to the pool via the trapping array. An alternative passive method is to capture amphibians in the aquatic environment itself. For vernal pools, aquatic funnel traps (Fig. 1A) have commonly been used to study larval amphibians or adult newts (Heyer et al. 1994; Buech and Egeland 2002; Wilson and Pearman 2010; Bennett et al. 2012). Funnel traps offer advantages for monitoring efforts because they allow for rapid deployment and retrieval—compared to drift fences—and eliminate biases and damaging methods common to active sampling methods. In this study, our goal is to improve the efficacy of aquatic funnel traps for capturing adult breeding amphibians. In particular, aquatic funnel traps have much lower encounter rates than drift fences by nature of their design. Using lures, we hope to increase encounter rates, and thus capture rates for adult amphibians moving in and around vernal pools during breeding season. Past studies found that baiting traps with glow sticks increased the capture rates of larval amphibians 2 to 8 times compared to funnel traps with no lure (Grayson and Roe 2007; Bennett et al. 2012). No studies have tested the effectiveness of glow stick lures on the capture rates of adult, vernal pool-breeding amphibians. We specifically focus on the adult life stage because life history suggests adults play the most critical role in population persistence (Stearns 1992; Petranks 1998). Capturing adults also make techniques like mark recapture feasible, providing important estimates of demographic parameters like abundance and survival to improve conservation decisions (Williams et al. 2002; Nichols

2014). Captures in aquatic funnel traps have also been shown to linearly scale with adult amphibian population density, suggesting captures are a potential index of adult population size (Wilson and Pearman 2010).

Here we experimentally test the efficacy of glow stick lures in increasing captures of Jefferson's Salamander (*Ambystoma jeffersonianum*), the Spotted Salamander (*Ambystoma maculatum*), the Eastern Red-spotted Newt (*Notophthalmus viridescens*), and the Wood Frog (*Lithobates sylvaticus*). We predict that the glow sticks will increase captures by providing a visual stimulus that draws adult amphibians to the traps. By increasing the efficacy of aquatic funnel traps with a simple glow-stick lure, aquatic funnel traps can be a more powerful tool for monitoring efforts.

METHODS

Study Site.—We monitored pools in State Game Lands 176 in Centre County, Pennsylvania, USA (40.778715°N, 78.006278°W), which are managed by the Pennsylvania Game Commission. The study area is mixed deciduous forest that contains a dense network of vernal pools. Within this network, we surveyed twelve pools that were part of a long-term monitoring study. These pools were spatially organized into three clusters with each cluster containing four pools. The pools vary in size (mean perimeter length: 66 m \pm 25 m SD, range: 40–120 m) but have similar habitat characteristics: no aquatic vegetation, leaf-litter bottoms, mixed deciduous upland habitat. Each pool dries mid to late summer in most years. Surveys for this experiment were conducted from 31 March 2015 to 9 April 2015, and traps were continuously deployed during surveys.

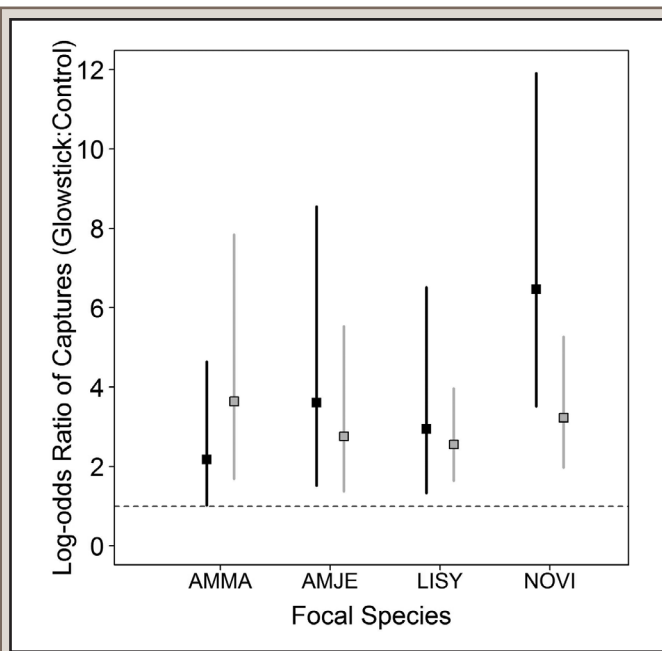


FIG. 2. Log-odds ratio of captures (treatment vs. control) for the focal species. AMMA = Spotted Salamander (*Ambystoma maculatum*), AMJE = Jefferson's Salamander (*Ambystoma jeffersonianum*), LISY = Wood Frog (*Lithobates sylvaticus*), and NOVI = Eastern Red-spotted Newt (*Notophthalmus viridescens*). Females are indicated in black and males in grey. A value of 1:1 indicates no effect (dashed line). Means are represented by squares, and segments are 95% confidence intervals.

Amphibian Sampling.—Unaltered aquatic funnel traps (Frabill®, Plano Molding Company, Illinois, USA) were used to survey for salamanders. The traps are torpedo shaped and are constructed of black vinyl coated steel with 6.35-mm mesh (Fig 1A). They measure 420 cm long with a 19 cm diameter. They have two 2.54-cm cone-shaped openings on either side allowing animals to enter with limited chances of exiting (Fig 1C). A waterproof foam float (FOAMULAR®, Owens Corning, Toledo, Ohio, USA) placed inside the trap raised the unit partially above water to prevent drowning of air-breathing animals. A total of seventy-nine traps were deployed each night, and traps were positioned every 10 m along the pool perimeter, resulting in four to twelve traps per pool depending on size. Traps were placed along the pool's edge in deep enough water that everything but the float was submerged (typically less than 1 m from pond's edge; Fig. 1B). Openings were oriented perpendicular to the pond's edge and were attached to the bank with rope so they would not drift. Every night, half of the traps in each pool were systematically assigned to the treatment or control, with treatment alternating between traps. The treatment was a 15.24 cm activated green glow stick placed inside the funnel trap (The Glow Company Ltd., Doncaster, United Kingdom). Because movements from upland habitats mainly occur at night (Petranka 1998), glow sticks were activated between 1600 h and 1800 h, and all glow sticks emitted light throughout the night until researchers checked them the next day. To reduce individual trap bias, treatment assignments were alternated each trap night, meaning each night there were either $N = 39$ or $N = 40$ replicates of glow stick treatment. Each trap received approximately the same number of trap nights with glow stick

or control (either 4 for 5 nights). Traps were checked daily, and we recorded species, sex, and trap location of each capture.

Analysis.—We analyzed our captures (count data) using a generalized linear mixed model with a negative binomial distribution and log link function using the “glmmADMB” package (Fournier et al. 2012) in the statistical software R (R Core Team 2014):

$$\text{Captures} \sim \text{Negative Binomial}(\mu_i, k_i)$$

$$\log(\mu_i) = \beta_0 + \beta_1 \text{site}_i + \beta_2 \text{treatment}_i + \beta_3 \text{migration night}_i + \beta_4 (\text{treatment}_i * \text{migration night}_i) + \gamma \text{trap}_i$$

$$\text{where } \gamma \sim \text{normal}(0, \sigma^2)$$

where β represents fixed effect regression coefficients estimated by the model and γ is a random intercept for individual traps. The fixed effects included the site (specific pool), “migration night”, and treatment. Because capture rates are greatly influenced by the number of amphibians that chose to migrate on a given night, we created a “migration night” indicator variable (big migration night = 1; non-migration night = 0). Because of this, we expected a migration night by treatment interaction—the treatment is more effective on nights when more amphibians have moved to the pools. No migration night effect was predicted for *N. viridescens* given adults are permanent residents of pools. We used a random effect for individual trap to account for repeated measures and other trap-specific variability. After initial analysis, fixed effects were removed if there were insufficient data to estimate them. The full set of effects was included for both sexes of *A. maculatum* and male *L. sylvaticus*. Only a treatment effect was included for female *L. sylvaticus*, and some sites were unable to be estimated for *A. jeffersonianum* and *N. viridescens* (Table 1).

RESULTS

Over the course of nine trapping nights, we captured 4935 amphibians (Table 2). Regression coefficients for treatment were significant for all species (Table 3). Migration night was a significant predictor of captures except for *N. viridescens*. No species or sexes had a significant treatment by migration night interaction. We converted our regression coefficient estimates to log-odds ratios which show that glow sticks increased the estimated mean number of captures of *A. maculatum* by 2.18–3.64 times, *A. jeffersonianum* by 2.76–3.61 times, *L. sylvaticus* by 2.55–2.94 times, and *N. viridescens* by 3.22–6.47 times compared to control traps (Fig. 2). Captures also varied significantly among pools (results not reported). The random effect for trap absorbed little variability, indicating traps performed similarly.

DISCUSSION

Monitoring species' populations require effective sampling techniques that provide informative data without commanding too many resources (e.g., effort, materials). Monitoring vernal pools is particularly important, as amphibian communities in these systems are likely experiencing regional decline (Mitchell et al. 2006; Adams et al. 2013). These systems are also vulnerable to predicted increases in precipitation variability

TABLE 1. Summary of fixed effects included for each analysis (“Y” = yes included, “N” = excluded). Parameters were excluded if not enough data were available to reliably estimate them.

Species	Site	Migration night	Treatment	Interaction
<i>A. maculatum</i> ♀	Y	Y	Y	Y
<i>A. maculatum</i> ♂	Y	Y	Y	Y
<i>A. jeffersonianum</i> ♀	N	Y	Y	Y
<i>A. jeffersonianum</i> ♂	N	Y	Y	Y
<i>L. sylvaticus</i> ♀	N	N	Y	N
<i>L. sylvaticus</i> ♂	Y	Y	Y	Y
<i>N. viridescens</i> ♀	N	Y	Y	Y
<i>N. viridescens</i> ♂	N	Y	Y	Y

TABLE 2. Pooled captures of females (♀) and males (♂) of our focal species across 12 vernal pools from 31 March to 9 April 2015.

Species	Captures
<i>A. maculatum</i> ♀	291
<i>A. maculatum</i> ♂	1944
<i>A. jeffersonianum</i> ♀	121
<i>A. jeffersonianum</i> ♂	423
<i>L. sylvaticus</i> ♀	48
<i>L. sylvaticus</i> ♂	1818
<i>N. viridescens</i> ♀	148
<i>N. viridescens</i> ♂	142

TABLE 3. Results from generalized linear mixed models. Mean model regression coefficients are reported with standard error (\pm) and 95% confidence interval in parentheses. The treatment effect is the impact of including an activated glow stick in a trap. A p-value < 0.05 is designated by an asterisk (*). Migration night is an indicator of nights when large groups of animals migrate to the pools. Interaction is the interaction term between treatment and migration night.

Species	Treatment	Migration night	Interaction
<i>A. maculatum</i> ♀	0.778 \pm 0.387 (0.020, 1.54) *	1.78 \pm 0.369 (1.06, 2.51) *	0.560 \pm 0.465 (-0.350, 1.47)
<i>A. maculatum</i> ♂	1.29 \pm 0.392 (0.522, 2.06) *	3.47 \pm 0.374 (2.73, 4.20) *	0.608 \pm 0.461 (-0.295, 1.51)
<i>A. jeffersonianum</i> ♀	1.28 \pm 0.440 (0.419, 2.15) *	2.59 \pm 0.521 (-1.57, 3.61) *	-0.885 \pm 0.656 (-2.17, 0.400)
<i>A. jeffersonianum</i> ♂	1.02 \pm 0.355 (0.320, 1.71) *	2.70 \pm 0.440 (1.84, 3.56) *	0.192 \pm 0.579 (-0.942, 1.33)
<i>L. sylvaticus</i> ♀	1.079 \pm 0.405 (0.285, 1.87) *	NA	NA
<i>L. sylvaticus</i> ♂	0.938 \pm 0.225 (0.498, 1.38) *	1.67 \pm 0.226 (1.22, 2.11) *	0.015 \pm 0.293 (-0.560, 0.590)
<i>N. viridescens</i> ♀	1.87 \pm 0.311 (1.26, 2.48) *	1.07 \pm 0.560 (-0.024, 2.17)	0.340 \pm 0.635 (-0.903, 1.58)
<i>N. viridescens</i> ♂	1.17 \pm 0.250 (0.681, 1.66) *	0.115 \pm 0.598 (-1.06, 1.29)	0.930 \pm 0.697 (-0.436, 2.30)

under climate change, meaning monitoring data will become only more important for informing conservation decisions (Brooks 2004; Hayhoe et al. 2007; Anderson et al. 2015; Davis et al. 2017). While most use of aquatic funnel traps has focused on the larval stage of the amphibian (Heyer et al. 1994; Buech and Egeland 2003), we demonstrate that aquatic funnel traps are a helpful addition in the monitoring toolkit for adult, breeding amphibians. We show that the use of commercially available glow sticks significantly improve capture rates for four vernal pool species. Mean captures of *A. maculatum*, *A. jeffersonianum*, *N. viridescens*, and *L. sylvaticus* increased 2–6 times in glow stick traps over control funnel traps. Captures were highest on migration nights, but our results did not show a migration night by treatment interaction, meaning glow sticks had consistently higher capture rates throughout the breeding season. This

study adds to the growing body of literature that demonstrates the utility of using glow sticks for increasing amphibian capture rates, and the results of our study expand the utility of glow sticks to adult *Ambystoma* spp. and *L. sylvaticus* (Grayson and Roe 2007; Bennett et al. 2012).

The exact mechanism for adult amphibians to be attracted to the glow sticks is unclear. For larval amphibians and adult *N. viridescens*, light attracts prey, making traps a food incentive (Bennett et al. 2012). When breeding at the vernal pools, *Ambystoma* spp. and *L. sylvaticus* are thought not to forage (Petranka 1998; Lannoo 2005). This suggests that the glow sticks may merely provide a visual stimulus or may help salamanders see movement of other individuals—either way, likely increasing the encounter rate of amphibians with traps. The glow sticks also increased captures of female amphibians,

which may improve encounter, capture, and retention rates of male amphibians during the breeding season (Wilson and Pearman 2010). Because migration might affect captures, it is likely movement into and out of ponds leads to the highest encounter and capture rates. If glow sticks are effective because they act as a visual cue, their brightness, wavelength, and environmental factors such as moonlight or turbidity may limit their effectiveness (Grayson and Roe 2007; Chen et al. 2008; Bennett et al. 2012).

There were clear differences between the number of female and male amphibians captured in our aquatic funnel traps (Table 2). Petranks (1998) reports observed sex ratios (male:female) for *A. maculatum* (1.5:1–3.5:1), *A. jeffersonianum* (1.5:1–3:1), and *N. viridescens* (0.7:1–2.6:1) which are lower than our observed *Ambystoma* spp. sex-ratios but similar to our *N. viridescens* sex-ratio: 6.7:1, 3.5:1, and 0.96:1, respectively. Sex-ratios for *L. sylvaticus* range from 1:1 to 12.3:1 (Berven 1990). Our observed sex-ratio was 37.9:1. Without further data, it is challenging to know if these values, for all species, represent the true population structure or if they reflect sex-biased catchability and retention. Males of migrating species arrive earlier than females (unpublished data) and are likely to have higher encounter rates if they wait along the perimeter for arriving females (where traps are located). Egg masses are oviposited near the periphery of these ponds (Petranks 1998; C. L. Davis, pers. comm.), so females should also have high encounter rates with the traps. Despite having potentially equal encounter rates, male courtship behavior (seeking females, repeated contact and gestures once found) and male-male competition may translate into males having higher capture rates. To breed successfully in a male-biased population, males may have more incentive to explore and enter traps. Females may not have to move far for males to begin courting them. Regardless, inclusion of glow sticks significantly increased captures of both sexes.

Although this study shows that glow sticks significantly increase the capture rates of breeding amphibians in vernal pools, the benefit comes at an increased cost of \$0.52 USD per trap night, in addition to increased waste in form of spent glow sticks. These higher costs may be avoided if glow sticks are optimally deployed only on migration nights (versus the whole breeding season)—maximizing the number of captures and minimizing costs. Ensuring traps are fully functional also improves retention rates. In one instance, two *A. maculatum* were stuck between two trap halves and the trap had to be adjusted with pliers (e.g., Fig. 1A right). The 6.55-mm mesh size on the traps was also large enough that some *N. viridescens* would get stuck trying to escape. While glow sticks improve encounter and capture rates, appropriate trap sizes and trap condition may improve these and retention rates (Luhning et al. 2016). Trap entrances were large enough that gravid *Ambystoma* spp. and *Lithobates sylvaticus* would not be deterred from entering the trap.

Future research into glow stick lures should focus on determining which aspect of the glow stick leads to increased capture rates: visual cues, food cues, or potentially chemical cues. Salamander eyes are sensitive to green and blue light, but other species may be receptive to different wavelengths of glow sticks (Chen et al. 2008). It would also be beneficial to understand which aspects of passive sampling are impacted by glow sticks. The visual cue may improve encounter rates, but altering other trap features (e.g., size) may better help capture and retention rates of amphibians (Luhning et al. 2016). By continuing to improve the efficacy of aquatic funnel traps, monitoring efforts

can have a versatile tool for gathering high quality data on adult, vernal pool amphibians without the intensive efforts of drift fence surveys or the biases of active sampling techniques.

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