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Implications of Drought and Ranavirus on an Amphibian Community in the Great Smoky Mountains National Park

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I am submitting herewith a thesis written by Edward Davis Carter entitled "Implications of Drought and Ranavirus on an Amphibian Community in the Great Smoky Mountains National Park." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

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Implications of Drought and Ranavirus on an Amphibian Community in the Great Smoky Mountains National Park

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ABSTRACT

The Great Smoky Mountains National Park is an epicenter of amphibian biodiversity in North America. Over the last 18 years, amphibian die-off events due to the pathogen, ranavirus, have been documented at Gourley Pond in the Cades Cove region of the Park. The goal of my study was to determine if ranavirus was present and having negative impacts on the Gourley Pond amphibian community. During my study (2016 – 2017), a significant drought occurred, allowing me to investigate possible interactions between ranavirus and drought. In 2016, I documented ranavirus persisting in three post-metamorphic amphibian species (*Lithobates sylvaticus* = 8.9%; *Ambystoma maculatum* = 1.6%, and *Notophthalmus viridescens* = 1.2%); however, after extended drought, ranavirus was not detected in 2017 despite extensive sampling. The drought conditions resulted in an insufficient hydroperiod for larval development of several amphibian species, and nearly complete recruitment failure both years. I documented a 39 – 99% decrease in catch-per-unit effort for five common amphibian species between 2016 and 2017. My results provide evidence that ranavirus can persist in the post-metamorphic amphibian community; however, if the pathogen is not amplified in the aquatic environment by highly susceptible larvae, its prevalence may drop below ecologically relevant levels after one year of wetland drying. My study also found that insufficient hydroperiod at Gourley Pond could be having negative impacts on the amphibian community. If these conditions persist, the U.S. National Park Service (NPS) should consider implementing conservation strategies which extend the hydroperiod at Gourley Pond, such as installing wetland liners or diverting nearby Sea Branch to the site. At a minimum, I recommend low-intensity monitoring of the hydroperiod, amphibian community, and ranavirus prevalence to inform future management decisions. I also found public visitation to Gourley Pond is high (ca. 10 trail passes per day), suggesting that humans could play a role in translocating ranavirus from the site if the pathogen re-emerges. If ranavirus

is detected in the future at Gourley Pond, the NPS should consider informative signage about the pathogen and restricting access to the site.

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1. Introduction

Amphibian population declines have been documented around the world (Daszak et al. 1999, Houlahan et al. 2000, Blaustein et al. 2011, Alroy 2015). Considering the rate of amphibian declines and extinctions from 1971 – 2000, it is estimated that about 7% of anuran (frog) species could disappear during the 21st century (Alroy 2015). When compared to birds and mammals, amphibian species are at greater risk, with 32.5% being globally threatened compared to 12 and 23% of birds and mammals, respectively (Stuart et al. 2004). Current rates of extinction for amphibians are conservatively estimated to be between 100 – 211 times greater than background extinction rates based on analysis of fossil records (Barnosky et al. 2011, Ceballos et al. 2015). The overwhelming evidence of amphibian declines has motivated scientists and natural resource practitioners to identify causes of declines, and to develop conservation strategies that ameliorate those declines (Collins and Crump 2009).

Six major hypotheses have been presented as driving factors in global amphibian declines (Collins and Storfer 2003): invasive species introduction, over-exploitation, land-use changes, increased application of contaminants, global climate change and emerging infectious diseases. Each of these causes for declines are in some way linked to anthropogenic activities and, in many cases, are the result of synergistic interactions among them. Emerging infectious diseases are unique from the other factors, because they have caused amphibian declines in pristine areas seemingly absent from human influence (Daszak et al. 1999).

The two most common pathogens associated with amphibian mortality events are the fungus *Batrachochytrium dendrobatidis* (*Bd*) and viruses in the genus *Ranavirus* (Daszak et al. 1999). *Bd* has been linked to the decline of >200 amphibian species worldwide (Lips 2016). The pathogen is believed to be from Asia (O'Hanlon et al. 2018), and likely was disseminated

globally through unclean international trade of amphibians (Schoelgel et al. 2009, Kolby et al. 2014). Similar to *Bd*, ranaviruses are found on every continent that amphibians exist (Duffus et al. 2015). Several species of *Ranavirus* can infect amphibians, reptiles and fish, although most cases of declines have been associated with frogs and salamanders (Duffus et al. 2015). Price et al. (2014) provided evidence of population declines for several amphibian species in northern Spain due to ranavirus. Outbreaks can progress quickly lasting less than two weeks (Brunner et al. 2015), resulting in thousands of dead animals (Wheelwright et al. 2014). Earl and Gray (2014) demonstrated through simulations that recurring ranavirus-induced die-offs could lead to population extirpation of very susceptible host species in <6 years. In general, most die-offs have occurred with the larval cohort (likely due to reduced immune function, Grayfer et al. 2015), resulting in population decline due to reduced recruitment (Earl and Gray 2014).

Within the Great Smoky Mountains National Park (GSMNP) of eastern North America, larval amphibian die-offs have been attributed to *Frog Virus 3* (FV3)-like ranaviruses at Gourley Pond (GP) on at least five separate occasions: 1999, 2000, 2001, 2009, and 2012 (Green et al. 2002; Todd-Thompson 2010; P. Super, U.S. National Park Service, person. commun.). Moribund and dead larval marbled salamanders (*Ambystoma opacum*), spotted salamanders (*A. maculatum*), wood frogs (*Lithobates sylvaticus*), spring peepers (*Pseudacris crucifer*), and upland chorus frogs (*P. feriarum*) were observed during these outbreaks (Green et al. 2002, Todd-Thompson 2010). Importantly, the mortality events observed from 1999 – 2001 were during an amphibian monitoring and inventory program conducted by U.S. Geological Survey personnel (Dodd 2003), and GP was not monitored again until 2009 (Todd-Thompson 2010). Inconsistent monitoring at GP has resulted in uncertainty about whether mortality events due to

ranavirus have gone undetected, and whether die-offs are having negative impacts on the local amphibian community.

Inasmuch as GP is an ephemeral wetland with the hydroperiod lasting generally <6 months (Dodd 2003, Todd-Thompson 2010), the reoccurring nature of ranavirus outbreaks suggests the pathogen is reintroduced by subclinically infected hosts or on fomites attached to recreationists or non-host wildlife (e.g., Brunner et al. 2004). The environmental persistence of ranavirus is likely <1 week (Johnson and Brunner 2014, Munro et al. 2016), hence annual drying of GP likely inactivates free-floating virions (Brunner et al. 2007). Major questions are: (1) are amphibian or reptilian hosts, humans, or non-host wildlife responsible for reintroduction, and (2) which of these groups represent the greatest threat to translocating ranavirus to other GSMNP sites? It is possible that GP represents a site of ranavirus amplification where high concentrations of infectious virions are produced by very susceptible hosts or due to environmental conditions then disseminated by carrier species across the landscape.

Great Smoky Mountains National Park is the second most visited national park in the United States and it is an epicenter of biodiversity, with over 5,500 native species present (Becky and Keith 2007). It is a global diversity hotspot for amphibians that provides habitat for 31 caudate and 13 anuran species representing about 18% of the total amphibian diversity of the United States and about 55% of amphibian species found in Tennessee (Dodd 2004, Wiens 2007). Protecting this diversity from threats such as emerging infectious diseases is a priority of the U.S. National Park Service (NPS). Management options for disease intervention include strategies such as changing conditions in the environment, host community factors, and modifying human disturbance (Langwig et al. 2015, Heard et al. 2018). In order to understand the most appropriate strategies to implement, host-pathogen interactions at a site need to be

investigated (Gray et al. 2017). For example, if reservoir species that carry subclinical infections can be identified, their densities could be selectively decreased through capture and relocation or culling in order to reduce pathogen reintroduction (Heard et al. 2018). It also is unlikely that ranaviruses can persist for several years in hosts without eventual inactivation by the immune system (Grayfer et al. 2015); hence, keeping a site dry through human manipulation of the hydrology to prevent amphibian breeding (when ranavirus amplification often occurs, Brunner et al. 2015) for a few years could eliminate the pathogen from a population, especially if it is isolated from other ranavirus-positive sites. If site visitation by the public is high, access could be reduced to minimize introduction from other sites as well as human disturbance, which could act as a stressor on the host immune system and increase the prevalence of ranavirus (St-Amour et al. 2008). The key to any proactive disease intervention study is to identify influential pathways for pathogen introduction and persistence, and devise management strategies to interrupt them (Gray et al. 2017).

The **objectives** of my thesis research were to: (1) determine whether evidence existed for decreases in population size at GP, (2) identify possible sources of ranavirus introduction into GP as well as factors that could result in translocation of ranavirus from GP, (3) identify environmental and host factors responsible for ranavirus emergence and persistence at GP, and (4) provide strategies to the NPS on how to limit the effects of ranavirus on amphibians in the GSMNP. During my study, there was a substantial drought in eastern Tennessee, hence I added an objective to investigate how weather variables and wetland hydroperiod affected the GP amphibian community. I also included a small pond located <150 m from GP with a longer hydroperiod, hereafter referred to as Little Gourley Pond (LGP), that could possibly function as a source of individuals for re-colonization of GP or as a source for ranavirus introduction at GP.

2. Methods

Study Area and Sampling Frequency

I conducted my research under GSMNP permit # SCI-1256 and University of Tennessee, Knoxville Institutional Animal Care and Use Committee protocol #2426. My field sites were GP (N 35°59'34.67", W 83°78'88.44") and LGP (N 35°59'27.13", W 83°78'75.49"; Figure 1). Both wetlands are ephemeral and typically fill with water during winter and dry early to late summer. The U.S. Department of Agriculture soil classification for GP and LGP is Northcove-Maymead-Nowhere complex, which consists of cobbly sandy loam in the upper soil horizon (https://websoilsurvey.sc.egov.usda.gov/). The porosity of this soil type likely contributes to the ephemeral nature of the wetlands. Gourley Pond is 0.3 ha with a maximum depth of 1.5 m at full pool. Although LGP is smaller (0.036 ha, 1.3 m deep), it tends to hold water longer, perhaps due to differences in the substrate or its greater canopy closure. Given the proximity of GP and LGP to each other, it is likely these sites represent one amphibian population. A small stream (Sea Branch) with intermittent water is located 50 and 86 m to the southeast of GP and LGP, respectively, and could serve as a dispersal corridor for amphibians. The closest permanent water wetlands to GP and LGP are the impoundments for the Cades Cove campground wastewater located 1.2 km to the northeast, which is beyond the dispersal limit of most amphibian species (Semlitsch and Bodie 2003, Rittenhouse and Semlitsch 2007). Breeding site fidelity has also been observed in several studies. Berven and Grudzien (1990) found that all of the adult wood frogs marked in their study returned to the site they originally bred and 82% of metamorphs returned to their natal ponds despite available ponds in close proximity. Thus, GP and LGP likely represent an isolated amphibian community, especially for pond-dwelling species such as anurans (e.g., wood frogs) and mole salamanders (Ambystomatidae). A well-established trail

leads to GP from the Cades Cove loop (<150 m) facilitating public access, and a less noticeable trail runs between GP and LGP.

I conducted this study over two amphibian breeding seasons in 2016 and 2017. I began sampling during February each year when explosive breeding species (e.g., wood frog; spotted salamander, *Ambystoma maculatum*) were known to use the wetlands (Dodd 2003, Todd-Thompson 2010), and ended sampling after each site was dry for two consecutive weeks. I sampled GP and LGP from 2 February to 28 April 2016. Sampling in 2017 began on 3 February and ended 20 June. During these periods, I sampled larval and post-metamorphic amphibians once weekly. In addition, I performed sampling each fall (21 October 2016, 11 and 16 October 2017) to capture post-metamorphic marbled salamanders (*A. opacum*), which are the only fall-breeding species at GP and LGP.

Field Sampling Procedures

I used the following techniques to estimate occurrence and relative abundance of amphibians and reptiles, and determine if there was evidence of population fluctuations during the years I sampled: pitfall sampling, funnel traps, cover boards, enclosure sampling, and area searches (Gray et al. 2013). I installed drift fences and pitfalls that encircled approximately 75% of each wetland (Figure 2). I divided each wetland into the four cardinal quadrants, and placed drift fence (i.e., 0.75-m tall silt fencing) above the high-water elevation along the wetland perimeter in each quadrant. At the end of each cardinal drift fence segment, a 10-m section of drift fence was installed perpendicular to the main cardinal drift fence to help direct animals toward the pitfalls. Pitfalls were 19-L buckets that were buried so their tops were flush with the ground. Two pitfalls were placed opposite of each other at the intersection of the cardinal and perpendicular fences. Additional pitfalls were placed along each cardinal fence alternating on

each side of it every 5 m for a total of 57 pitfalls for GP and 32 pitfalls for LGP. To reduce predation of captured amphibians by mesocarnivores, I installed predator exclusion devices like those used by Campbell (2013), and encircled the GP sampling area with electric fence wire. I reduced small mammal bycatch by tying jute string to each predator exclusion device (Karraker 2001). Moist leaf litter was placed in the bottom of each pitfall as well as the snake funnel traps (discussed below) to reduce the likelihood of captured amphibians desiccating.

To capture reptile species, I placed a box-type funnel trap designed similar to Sutton et al. (2010) at the midpoint of each cardinal drift fence segment at GP only (Figure 2). To aid in the capture of more terrestrial amphibian species and snakes, I placed cover objects 50 m from the center of each cardinal drift fence segment. Cover objects were 120 x 75 cm and were constructed from untreated plywood or corrugated tin (Scheffers et al. 2009, Wilson JD and JW 2010). Each quadrant at GP had four cover objects (n = 16 total) while LGP had two per quadrant (n = 8 total); half of the cover objects in each quadrant were made of each material. I performed pitfall sampling, opened funnel traps, and checked cover objects once per week, and targeted sampling near rain events within weeks to maximize capture probability of amphibians. I opened pitfalls and funnel traps in the afternoon and checked them as soon as possible the next morning for captured animals (i.e., generally <12 hours capture duration, Gray et al. 2013).

On the day that traps were opened, I also performed larval sampling as described by Werner et al. (2007). I used a 120-L garbage can with the bottom cut out as a portable larval enclosure. During each larval sampling event, I randomly generated two azimuths in each cardinal quadrant and a random distance from shore to place the larval enclosure in GP and LGP. I netted in the enclosure until ten consecutive attempts recovered no additional larvae. Each captured larva was removed from the net (dimensions = 22 x 15 cm), and isolated in a 50-mL

plastic cup containing water from the wetland until processing. The total number of captured larvae and total number of netting attempts were recorded to estimate catch-per-unit effort (CPUE) for each sampling event. After sampling larvae, I searched in each quadrant for egg masses. I also estimated the total number of egg masses detected for each amphibian species.

I collected tissue samples for ranavirus testing from up to 30 individuals of each species per week, except for *A. opacum*, which I collected up to 50 samples during the fall sampling events. I collected toe clips and tail clips from post-metamorphic and larval amphibians, respectively, using a sterile scalpel blade with only one use per animal (Miller et al. 2015). I collected ca. 5-mm tail-clip samples from chelonians or squamates and used a small amount styptic powder (Kwik Stop®) to stop any associated bleeding. The 30 samples collected from each species per week were evenly distributed among the quadrants at GP and LGP when possible. I placed each tissue sample in 90% EtOH for storage until DNA extraction was performed. Morphometric measurements were recorded for each post-metamorphic animal captured including, snout vent length (SVL) and mass. All post-metamorphic amphibians were marked with unique toe clip combinations following Woodbury et al. (1956) so recaptures could be identified. Chelonians were marked by filing carapace scutes (Cagle 1939).

I processed each animal inside of disposable plastic bags and changed nitrile gloves between collecting tissues samples to reduce the likelihood of cross-contaminating samples (Gray et al. 2017, 2018). I disinfected all equipment that was re-used with 1% chlorhexidine (Nolvasan®, Zoetis US, Parsippany, New Jersey) for at least 5-min contact duration (Bryan et al. 2009). I returned processed animals to their capture location. Post-metamorphic amphibians caught in pitfalls were released on the opposite side of the fence from where they were captured to allow movement in the direction they were traveling prior to capture (Gray et al. 2013).

To test for the presence of ranavirus DNA, I extracted genomic DNA from all tissue samples using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). After extraction, total gDNA content of each sample was measured using a Bio-TEK Synergy HT spectrophotometer. The qPCR reactions followed protocols described in Hoverman et al. (2010) and Picco et al. (2007), and consisted of 1 uL of 10uM primers (rtMCP-F [5'-ACA CCA CCG CCC AAA AGT AC-3'] and rtMCP-R [5'CCG TTC ATG ATG CGG ATA ATG-3']), 1 ul of 5uM probe (rtMCP-probe [5'CCT CAT CGT TCT GGC Cat CAA CCA-3']), 7.5 uL of TaqMan® Universal PCR Master Mix, and a standardized amount of gDNA plus the appropriate amount of DNA-grade nuclease free water to total 4.5 ul. Each sample was run in duplicate in a 96-well plate containing positive and negative ranavirus controls as well as a reaction mixture containing only DNA-grade water (i.e., no gDNA). I ran each reaction on an Applied biosystems Quantstudio 6 Flex qPCR instrument and applied a known standard curve to each reaction using the instrument software to estimate the number of ranaviral copies/uL. The standard curve was created by amplifying 10-fold serial dilutions of a synthetic DNA fragment (gBlock Gene Fragments, Integrated DNA Technologies), with a concentration range of $5x10^9$ to 5 ranavirus copies/uL. The synthetic DNA sequence corresponds to the major capsid protein (MCP), which is conserved among ranaviral species (Jancovich et al. 2015). Based on this standard curve, I used a threshold value of 40 cycles as the cutoff to declare the presence of ranavirus DNA (i.e., PCR-positive). If one of the duplicate samples was positive and the other negative, I re-ran the sample in duplicate, and declared the sample as positive if three out of the four total replicates were positive. Each year, I tested 500 tissue samples from the most frequently captured species for the presence of ranavirus DNA. I reported results as ranavirus infection prevalence and load (Gray et al. 2015).

Although I make inferences on infection, qPCR only detects ranavirus DNA – it does not verify that the pathogen is viable (Miller et al. 2015).

I estimated pond visitation by humans and wildlife using a trail counter and wild game cameras. The GSMNP personnel installed a trail counter along the main trail to GP, which collected data on number of trail passes each hour during each field season. I also installed nine game cameras around the perimeter of GP to document wildlife using the pond and public visitation. Two game cameras per quadrant were installed at GP and one camera was placed along the GP trail.

To determine variables potentially influencing amphibian movements and trail usage, I used data recovered from the U.S. NPS meteorological station GRSM-CC located in Cades Cove (ca. 1.3 km from GP). Available parameters included: scalar wind speed (m/s), vector wind speed (m/s), vector wind direction (degrees), ambient temperature (°C), station temperature (°C), rainfall (mm), relative humidity (%), and solar radiation (W/m^2). I also estimated the size of each wetland weekly by measuring the width in each cardinal direction (m) and maximum depth (m).

Data Analyses

All data analyses were performed using the statistical analysis software R (Rstudio 2017)**.** Given that my research was conducted on one amphibian community in the GSMNP, it represents a case study with no replication (Hurlbert 1984, Yin 2017). Thus, my inferences herein are constrained to this community only and are directed at decision making for NPS (Yin 2017). For the analyses, I designated weekly sampling events as the case-study replicates, and the primary main-effect variables of interest were year (2016, 2017), site (GP, LGP), and month. I standardized all capture data by first calculating CPUE. I used CPUE instead of mark-recapture population estimators, because the recapture rate was low (Williams et al. 2002). For post-

metamorphic captures, CPUE was calculated as the number of animals captured divided by the total number of pitfalls opened per sampling event at GP (n=57) and LGP (n=32). I calculated CPUE for larval captures as the total number of larvae captured within the enclosure divided by the number of netting attempts required to capture no additional animals after 10 consecutive attempts. I averaged CPUE among enclosures for an estimate of larval CPUE per day. I tested for differences ($\alpha = 0.05$) in larval and post-metamorphic CPUE across the levels of the main-effect variables using a nonparametric Kruskal-Wallis test (Conover 1980). For variables with more than two main-effect levels, I tested for pairwise differences in CPUE using Wilcoxon tests if the Kruskal-Wallis test was significant (Conover 1980). To identify weather variables that were significantly related to CPUE, I performed separate simple linear regressions between each variable and CPUE at $\alpha = 0.10$. I used a greater significance level because these analyses were exploratory. Those variables identified as significant ($P<0.10$) were used as candidate variables in a global model. I then used the 'dredge' function in the "MUMln" R package to create a full list of all possible predictor variable combinations, estimate the relative importance of each variable, and ultimately select the best models. I used Akaike Information Criterion adjusted for small sample size (AIC_c) to select best performing candidate models (Burnham and Anderson 2001). Specifically, those models with $\Delta AIC_c < 3$ were deemed best performing (Burnham and Anderson 2002). Although AIC model selection inherently guards against multicollinearity (Burnham and Anderson 1998), I also calculated variance inflation factors (VIF) for the variables in the final models to verify no linear dependencies (Fox and Weisberg 2011). I reported parameter estimates (β), associated standard errors (SE), standardized parameter estimates ($z = \beta / SE$), relative model weight (ω), *P*-values (*P*), and a ranking of the importance of each predictor variable as the proportion of top models including it.

I was interested in determining if there was evidence for amphibian movements between GP and LGP, because if so, ranavirus and population dynamics could be interdependent, and influence conservation decisions by NPS. For these analyses, I assigned each pitfall a directional degree and classified animals as immigrating or emigrating based upon which side of the drift fence they were captured. I used the 'roa.spacing' function from the R package "Circ.Stats" to determine whether immigration and emigration from either site was non-uniform (Jammalamadak and SenGupta 2001).

Because changes in sex ratios can provide an indication of population viability (Berven Keith 1990), I compared sex ratios between years using chi-square tests of homogeneity for each amphibian species that could be reliably sexed. Fisher Exact Tests were used when chi-square assumptions were violated (i.e., expected values <5, McHugh 2013). Given that body mass is correlated with reproductive fitness in amphibians (Wells 2007), I estimated a body mass index (BMI) by running a regression of SVL and mass measurements for each species, and used the standardized residuals as a measure of body condition (Sutton et al. 2015). Positive residuals were representative of higher than average BMI, while negative values indicated lower than average BMI. I tested for differences of BMI among years, ponds and sexes using two-sample *T*-tests (Kim 2015). I estimated ranavirus prevalence and the Clopper-Pearson 95% confidence intervals using the 'propCI' function from the "prevalence" package in R, and tested for differences among species, years and months using Fisher Exact Tests (McHugh 2013).

I compared trail usage by year, month, and weekday using nonparametric Kruskal-Wallis tests. Pairwise Wilcoxon tests were performed for significant Kruskal-Wallis tests that included more than two main-effects. Similar to the method described for creating the CPUE models, I

created linear models for identifying what weather variables were most important in predicting trail usage.

3. Results

Post-metamorphic Community

I captured 2,381 post-metamorphic amphibians comprising 11 genera and 18 species (Table 1). I also captured three squamate and one chelonian species (Table 2). The most commonly captured anuran and salamander species in 2016 were wood frogs (11.8%) and spotted salamanders (65.1%), respectively (Table 1). In 2017, American toads (9.3%) and spotted salamanders (36.7%) were the most commonly captured. Eastern newts (21.7%) also represented a sizable percentage of captures in 2017, while wood frogs were captured infrequently (0.9%, Table 1). Across species, catch-per-unit effort (CPUE) was 6X greater in 2016 ($\bar{x}$ = 1.33, SE = 0.725) than in 2017 ($\bar{x}$ = 0.221, SE= 0.05, P = 0.04; Table 3). The CPUE declined from 2016 to 2017 for the following species: wood frogs (98.5%), spotted salamanders (90.5%), eastern newts (71.7%), marbled salamanders (50.1%), and American toads (39.2%; Table 3). There were no significant differences in CPUE detected between GP ($\bar{x}$ = 0.85, SE=0.41) and LGP ($\bar{x}$ = 0.36, SE = 0.11) or among months both years (Table 4). For the species that could be sexed reliably (i.e., eastern newts, marbled salamanders, spotted salamanders, American toads, and spring peepers), spring peeper captures were male-biased in 2016 (odds ratio = 0.12, 95% CI = 0.002-1.16, P = 0.05), and eastern newts were male biased in 2016 and female biased in 2017 (odds ratio = 0.59, 95% CI = 0.33 – 1.02, P = 0.05; Table 5).

Based on separate generalized linear regressions, five variables predicted significant variation in CPUE and were included in the global model (Table 6). Using the 'dredge' function from the program R, I identified three candidate models with $\Delta AIC_c < 3$. The model with the greatest support ($\omega_i = 0.58$) for explaining CPUE included date, the amount of precipitation during the previous 24 hours, and average solar radiation in the previous 12 hours (Table 7).

Across both years, sampling date (β = - 0.007 ± 0.003) and solar radiation (β = - 0.1 ± 0.04) were negatively related while precipitation (β = 2.07 ± 0.41) was positively related with CPUE (Figure 3). Variance inflation factors (VIF) for the predictor variables included in the top three models indicated no linear dependencies (VIF < 1.6).

Based on the frequency of animal captures and pitfall location position, I detected non-uniform directionality in immigration and emigration at GP and LGP (Table 8). Both immigrating and emigrating captures were greatest in the southeast quadrant of GP and northwest quadrant of LGP (Figure 4). Of the 102 recaptured individuals (n = 1,539 marked), 8.8% were found moving between GP and LGP. Only spotted salamanders (n = 91) and eastern newts (n = 11) were recaptured. Median duration between first capture and recapture for eastern newts was greater than spotted salamander (Table 9). No individuals captured in 2016 were recaptured in 2017.

Body mass index (BMI) for marbled salamanders and spring peepers increased between 2016 and 2017 and decreased for American toads (Table 10). The BMI for marbled salamanders was greater (P = 0.02) at GP ($\bar{x}$ = 0.20, SE=0.13) than LGP ($\bar{x}$ = -0.27, SE = 0.15), and it was greater (P = 0.04) for females ($\bar{x}$ = 0.157, SE = 0.116) than males ($\bar{x}$ = -0.14, SE = 0.09) for spotted salamanders (Table 10).

Of the 500 tissue samples tested each year for ranavirus DNA, the greatest infection prevalence occurred in February 2016 ($\hat{p}$ = 5%), and it was the greatest in post-metamorphic wood frogs ($\hat{p}$ = 8.9%; Table 11). I also detected ranavirus in post-metamorphic spotted salamanders ($\hat{p}$ = 1.6%) and eastern newts ($\hat{p}$ = 1.2%). No ranavirus PCR-positive samples were detected in post-metamorphic amphibians in 2017. I did not detect ranavirus or observe a ranavirus-induced mortality event in amphibian larvae either year. No reptile samples (n = 3

Coluber constrictor, n = 3 *Storeria dekayi*, n= 2 *Diadophis punctatus*, n=1 *Terrapene carolina*) were PCR-positive.

Larval Community

Despite capturing 18 amphibian species as adults, I captured only four amphibian species as larvae (Table 1). Recruitment was low each year due to insufficient hydroperiod duration. During my study, GP had surface water present from only 29 February to 24 March 2016 – it did not flood in 2017. No larvae were captured or observed at GP; however, I counted 34 spotted salamander, five wood frog, and two spring peeper egg masses on 14 March 2016 – 10 days before it dried completely. Although LGP filled both years, there was substantial egg and larval mortality due to its short hydroperiod. In 2016, LGP had surface water prior to the start of sampling on 2 February. I counted 75 and 374 wood frog and spotted salamander egg masses, respectively, between 14 – 24 March; however, many of these eggs desiccated prior to hatching. LGP was completely dry by 21 April 2016. Although eastern Tennessee was not in drought at that point, the National Oceanic and Atmospheric Administration classified July – September 2016 as moderate drought conditions under the Palmer Hydrological Drought Index (PHDI). The dry conditions continued throughout the fall and winter of 2016 and into 2017, including extreme drought PHDI conditions for November 2016 and severe drought classifications for February and March 2017. These dry conditions ultimately led to GP remaining dry and LGP filling up later than normal on 13 April in 2017. Prior to LGP filling with water, a large raft of wood frog egg masses measuring approximately 1.5 m in diameter was deposited in the dry wetland. Increased precipitation in March allowed LGP to fill between 6 and 13 April 2017. Once LGP filled, *Pseudacris* spp larvae (either spring peepers or upland chorus frogs), and marbled salamander larvae were observed.

Larval density at LGP did not differ between years or among cardinal quadrants (Table 12). In 2016, larval density was marginally greater in April ($\bar{x}$ = 2.19, SE = 1.10) compared to March ($\bar{x}$ = 0.328, SE=0.14; P = 0 06). In 2017, larval density was negatively correlated with weekly surface area of LGP (r = –0.90, P = 0.02). Composition of the larval community at LGP was different between years. In 2016, marbled salamander ($\bar{x}$ = 1.47, SE = 0.59) larvae were more abundant than wood frogs ($\bar{x}$ = 0.19, SE = 0.07; P = 0.02). In 2017, spring peeper and upland chorus frog larvae were grouped together as *Pseudacris* spp. due to an inability to differentiate them, and they dominated the larval community ($\bar{x}$ = 5.65, SE = 2.09). Only three marbled salamander larvae were caught in 2017.

Regarding possible recruitment from LGP, the only metamorphs captured in 2016 were 10 marbled salamanders. In 2017, I captured 30 *Pseudacris* metamorphs. No larval or metamorph samples were PCR-positive for ranavirus DNA in 2016 (n = 106) or 2017 (n = 119).

Wildlife and Public Access

The public used the trail that leads to GP frequently, with an average of 9.76 trail passes per day (SE = 0.57) across both years (2016: February – May, 2017: March – October). Daily trail passes were greater in 2016 ($\bar{x}$ = 12.2, SE = 1.21) compared to 2017 ($\bar{x}$ = = 8.78, SE = 0.618, P = 0.006; Figure 5). Number of daily trail passes varied among months (P < 0.001). The greatest number of daily trail passes occurred in March ($\bar{x}$ = 12.4, SE = 1.78), while the least occurred in July ($\bar{x}$ = 5.97, SE = 1.05; Table 13, Figure 5). The trail was used more frequently on Saturday ($\bar{x}$ = 11.6, SE = 1.44) and Sunday ($\bar{x}$ = 11.0, SE = 1.49) than on Thursday ($\bar{x}$ = 7.66, SE = 1.36; P = 0.02 and 0.05, respectively).

The best model for predicting trail count passes included days since January 1st, precipitation, and solar radiation (ω_i = 0.18, Figure 6). Seven additional models were within two

ΔAIC_c values of the top model (Table 14). Daily precipitation was the most important and was included in all the top models ($\beta = -4.6 \pm 0.16$). Day, daily solar radiation, daytime temperature and scalar wind speed were included in six, five, four and three of the top models, respectively (Table 14). Days that were wetter and warmer had less trail counts, while an increase in solar radiation and scalar windspeed had the opposite effect.

Wild game cameras detected a total of 30 images. White-tailed Deer were the most commonly observed wildlife (n = 19) followed by humans (n = 8) and black bears (n = 3). Four out of eight observations of humans took place while GP was filled in 2016. The three black bear observations and 11 deer observations were from the wild game camera located along the main trail to GP.

4. Discussion

Discussion of Results

I documented significant fluctuations in the GP and LGP amphibian community between 2016 and 2017 for five amphibian species. The greatest decrease in post-metamorphic CPUE was detected in wood frog and spotted salamander populations (98.5% and 90.5% decrease, respectively). These amphibians are explosive, early-breeding species, that typically complete breeding in <2 weeks during January or February in Tennessee (Niemiller and Reynolds 2011). The best-performing AIC models that I developed indicated that increasing solar radiation and decreasing precipitation were negatively and positively related with post-metamorphic CPUE, respectively. Eastern Tennessee experienced substantial drought during my study, which fueled the infamous Great Smoky Mountains wildfires in November 2016 (Case and Zavodsky 2018). Moderate to severe drought conditions as classified by the Palmer Hydrological Drought Index (PHDI) existed from July 2016 through March 2017 (NOAA). Consequently, GP was flooded for only 24 days in 2016 and zero days in 2017. I captured no amphibian larvae in GP either year, hence failed recruitment may have contributed to the decrease in post-metamorphic CPUE between years at that wetland. The LGP was flooded for at least 79 days in 2016. Despite counting >400 egg masses in LGP during March 2016 and capturing some larvae in April, marbled salamanders were the only species that successfully metamorphosed before LGP dried on 24 April 2016. In 2017, LGP did not flood until 13 April, and I did not detect any wood frog or spotted salamander eggs or capture larvae of either species. The only larvae captured in 2017 were spring peeper, upland chorus frog, and marbled salamander. Thus, reduced recruitment of wood frogs and spotted salamanders at LGP was likely impacted by limited hydroperiod in 2016 and the pond filling after the normal breeding season for these species in 2017. In fact, I

observed wood frog egg masses laid in LGP on dry soil in 2017 prior to this site filling. Collectively, my data suggest that reduced recruitment mediated by drought was a driving factor affecting amphibian community fluctuations at the GP and LGP wetlands during my study.

Several studies have reported that amphibian species that breed in ephemeral wetlands can experience population fluctuations if the hydroperiod is shortened due to drought conditions or long-term climate shifts. Catastrophic recruitment failure (CRF) caused by abbreviated hydroperiod increases the odds of local amphibian populations going extinct (i.e., extirpation, Taylor et al. 2006). The probability of population extirpation is linked to the frequency of CRF, the survival of post-metamorphic adults, and the life span of each species (Taylor et al. 2006). Wood frogs become reproductively mature 1 – 2 years after metamorphosis, have life expectancies ranging from 3 – 4 years, and hence may breed only 1 – 2 years (Berven 1982), which is typical for many explosive breeding, r-selected species (Wells 2007). Thus, explosive breeding species that use ephemeral wetlands may be impacted most by factors that cause CRF, such as drought (Taylor et al. 2006). Amphibian populations can persist despite CRF if the population is part of a larger metapopulation that is connected via dispersal and results in sufficient immigration to counteract years with an insufficient hydroperiod for recruitment (i.e., rescue effect, Daszk et al. 2005). Unfortunately, immigration of pond-breeding amphibian species at GP and LGP is unlikely due to their distance from other wetlands within Cades Cove. Typical home ranges and regular dispersal distances for several of the pond breeding amphibians at GP do not span the distance from GP to other wetlands or are unknown (Table 15). Infrequent longer dispersal events may occur however. Although the abbreviated hydroperiods at GP and LGP might be the result of abnormal drought years, other natural areas, such as, Yellowstone National Park have attributed more frequent drier and warmer conditions to climate change

(McMenamin et al. 2008). Climate change was considered the main cause of a 4-fold increase in the number of dry ponds, a decrease in the proportion of ponds supporting amphibians, and an overall decrease in amphibian species richness within Yellowstone National Park (McMenamin et al. 2008). Greenberg et al. (2017) modeled the hydrological consequences of climate change and demonstrated that predicted shifts in temperature and precipitation patterns could result in multiple successive years of insufficient hydroperiod for larval development of five amphibian species inhabiting ephemeral wetlands in Florida. Sufficient hydroperiods for breeding and larval development have been reported for GP (Dodd 2003, Todd-Thompson 2010); however, the frequency of these events is unknown. As such, I recommend future long-term monitoring of the hydroperiods at GP and LGP to determine if the abbreviated hydroperiods of 2016 and 2017 were abnormalities, or if these years are representative of a long-term drying trend at these sites. Management strategies that could be used to increase surface water at GP and LGP include diverting water from nearby Sea Branch via water control structures. Also, due to the porous soil at these sites, rubber liners could be installed belowground in portions of the GP and LGP wetland basins to increase water retention (Biebighauser 2011). My results also indicated that GP and LGP are linked through amphibian dispersal, hence management activities could focus on one wetland, with rescue-effect benefits likely to the other site when it is naturally flooded.

Because of the abbreviated hydroperiods in 2016 and 2017, I did not document a ranavirus outbreak in the larval community at GP and LGP. Typically, ranavirus prevalence remains low in larval amphibian populations and increases through spring as water temperatures warm and amphibian development reaches metamorphosis (Brunner et al. 2015). The only larvae that I captured were at LGP, and none tested PCR-positive for ranavirus. For ephemeral wetlands, it is unlikely that ranavirus persists in the soil because drying conditions can inactivate

the pathogen (Brunner et al. 2007). Thus, it has been hypothesized that sub-clinically infected adults returning to sites for breeding shed the pathogen and result in transmission to more susceptible larvae (Brunner et al. 2004).

In 2016, I detected ranavirus DNA in post-metamorphic tissue samples for three amphibian species: wood frogs (8.9%), spotted salamanders (1.6%), and eastern newts (2.3%). Compared to larval amphibians, surveillance for ranavirus in post-metamorphic amphibians is limited (Brunner et al. 2015). Across 28 study sites, Crespi et al. (2015) estimated ranavirus prevalence in post-metamorphic wood frogs ranging from 0 – 71% ($\bar{x}$ =28.28% ± 5.99). Another study estimated ranavirus prevalence was 3% (95% CI = 0.86-9.55%) in post-metamorphic green frogs (*Lithobates clamitans*) across five sites in Prince Edward Island, Canada (Forzan and Wood 2013). In Costa Rica, ranavirus prevalence among 21 species was 16.6% (Whitfield et al. 2013). Sutton et al. (2015) reported that ranavirus prevalence was 18% among 14 plethodontid salamanders at three lotic sites in the GSMNP, and that prevalence varied significantly among years. Thus, the ranavirus prevalence levels that I documented were within typical ranges reported elsewhere (Brunner et al. 2015), and provide evidence that the pathogen could be maintained at GP and LGP as sub-clinical infections in post-metamorphic amphibians.

Brunner et al. (2004) reported that ranavirus prevalence of 6.7% (95% CI = 0.8-22%) in tiger salamanders returning to a pond was sufficient to initiate subsequent outbreaks in the larval cohort. Overall ranavirus prevalence in the post-metamorphic amphibian community in my study was 1.1% across all samples from 2016 and 2017. Despite evidence that ranavirus reintroduction occurs, it is unknown what combination of factors is required for ranavirus to be reintroduced into a system and result in an outbreak (Brunner et al. 2015, Brunner and Yarber 2018). Ranavirus can be transmitted very easily through direct contact (Brunner et al. 2007) or exposure

to contaminated water or soil (Harp and Petranka 2006, Brenes et al. 2014). However, transmission is dependent on the infection load in the host, where the probability of transmission increases as the infection becomes systemic and clinical disease develops (M. Gray and D. Miller, University of Tennessee; A. Peace, Texas Tech University, unpubl. data). In addition, transmission in water is dose-dependent (Warne et al. 2011). Hall et al. (2016) found that ranavirus loads in the water at wetlands lagged infection prevalence in wood frog larvae, presumably because it takes time for shed virus to accumulate. It is likely that some of the post-metamorphic amphibians that tested PCR-positive in my study shed ranavirus into GP and LGP in 2016. In the case of GP, this wetland went dry before eggs hatched, essentially eliminating the opportunity for transmission, because ranavirus does not appear to be able to penetrate egg masses (Haislip et al. 2011). For LGP, I hypothesize that either insufficient virus was shed into the pond to result in transmission, or water conditions at the pond resulted in virus inactivation prior to transmission. Various microbes can reduce the persistence of ranavirus (Johnson and Brunner 2014), such that ranavirus in pond water likely is inactivated in <1 week (Munro et al. 2015). If that was the case, free floating virions that were shed could have been inactivated before the eggs hatched. It has been hypothesized that the introduction of ranavirus may be more important later in larval development when host densities are high and immune function drops near metamorphosis (Brunner et al. 2015). In either case, short hydroperiods at GP and LGP likely interrupted host-ranavirus transmission cycle, which is a novel finding.

I found that wood frogs had the greatest prevalence of ranavirus at GP and LGP, which may be due to their increased susceptibility as larvae compared to spotted salamanders or eastern newts (Hoverman et al. 2011), assuming species susceptibility to ranavirus as an adult is correlated with other developmental stages. Earl et al. (2016) reported high susceptibility of

Mississippi gopher frogs across all life stages. The susceptibility of adult wood frogs is unknown; however, Haislip et al. (2011) reported high susceptibility of wood frog hatchlings, larvae and metamorphs. Interestingly, infection prevalence in eastern newts was 2%. Unlike wood frogs and spotted salamanders which leave the pond after breeding and depositing eggs, adult eastern newts will inhabit a wetland for as long as water is present (Petranka 1998). Their extended duration in the wetland could allow for more frequent contact with larvae as well as transmission during later developmental stages. This species also has excellent dispersal capability (Gill 1978, Roe and Grayson 2008), hence could represent a source of overland dispersal of the virus.

I did not detect ranavirus DNA in any samples in 2017. It is possible that ranaviruses might be able to evade the amphibian immune system in an inactive state in host macrophages similar to other viruses (Grayfer et al. 2012), although the duration of within-host persistence is unknown. For my study, I hypothesize that either subclinically infected individuals eventually cleared the pathogen or died due to natural causes or ranaviral disease. The immunological stress associated with drought conditions during my study could have facilitated viral recrudescence and the development of clinical disease in subclinically infected individuals (Rollins-Smith 2017), although no clinically infected individuals were found during 2016. The reduced hydroperiod also could have played a role given that ranavirus in most amphibian systems is amplified during the larval stage (Brunner et al. 2015), resulting in increased opportunities for transmission to adults at breeding sites. Thus, years of failed or minimal larval development might disrupt the ranavirus-amphibian host cycle. The results of my study suggest that complete failed recruitment of one year might be sufficient to interrupt the ranavirus-host cycle at a community-level infection prevalence of 2.2% or below for isolated wetland systems like GP and

LGP. Thus, a logical disease intervention strategy for isolated wetlands with ranavirus die-offs might be draining the wetland (e.g., through pumping) for one year to prevent amphibian breeding. Clearly, the benefits and consequences of such a management strategy would need to be carefully considered, because as my study also demonstrated, removal of an entire larval recruitment class could negatively impact a local population.

I did not detect differences in sex ratios between years for any of the species except spring peepers, which were male biased in 2016 and female biased in 2017. Breven (1990) reported male-biased sex ratios in wood frog and suggested it was likely due to the faster maturation rate of male frogs compared to females. Analysis of BMI between years and sexes for each species provided mixed results. The increase in BMI between 2016 and 2017 observed in spring peepers along with the female bias detected in 2017 for this species suggests that females had higher BMI than males, however my analysis did not confirm this. However, I did detect differences in BMI between male and female spotted salamanders. Differences in mass and SVL measurements are often observed between males and female spotted salamanders (Hills 1977, Davis and Maerz 2007, Morgan et al. 2014). The only species I observed a decrease in BMI between 2016 and 2017 was the American toad. Other studies have observed that warmer and dryer years can negatively affect body condition of amphibians (Reading and Clarke 1994, Reading 2006). St-Amour et al. (2010) also observed that ranavirus prevalence in positively correlated with body condition.

The trail count results indicate that GP is heavily visited. Average trail passes per day was about 10 across all months and years and about 12 passes per day from March – April. Due to the temporal pattern of historic ranavirus outbreaks at GP (Dodd 2003 and Todd-Thompson 2010), pond visitation occurring in May represents the greatest risk. Ranavirus virions can

survive in mud and pond water (Robert et al. 2011, Brenes et al. 2014, Hall et al. 2016), and thus can attach to footwear or recreational gear and be translocated to other locations in GSMNP. Given the high visitation of GP by the public compared to non-amphibian wildlife, humans likely represent the greatest risk factor for ranavirus translocation from GP. If ranavirus has not been eliminated from GP and LGP due to the 2016 drought, I recommend public access be limited from April – June, when amplification of ranavirus at these sites is most likely to occur (Todd-Thompson 2010). Trail signage could be used to inform the public of the re-occurring mortality events at GP and describe ways in which the public could observe the pond without posing a threat of translocating ranavirus to other areas of GSMNP. The NPS also might want to consider installing footwear and equipment disinfecting stations at strategic public-access sites to reduce the likelihood of ranavirus (and other pathogen) translocation among watersheds in the GSMNP. Future sampling at GP and LGP for ranavirus infection should be done opportunistically each year to determine if ranavirus remains absent. I recommend collecting at least 50 tissue samples from larvae per year during May to increase the probability of detection (Gray et al. 2015).

Management Recommendations and Future Directions

My study provided evidence of significant decreases in catch-per-unit effort of amphibians at GP and LGP between 2016 and 2017 that were most likely mediated by abbreviated hydroperiods caused by drought. Although several years of catastrophic recruitment failure will result in population decline and eventually local extirpation, Pechmann et al. (1991) reported that amphibian populations can recover from low relative abundance, especially explosive breeding species. Unfortunately, my two-year data set is too limited to make conclusions on population declines, emphasizing the need for future population monitoring.

Monitoring could consist of less intensive sampling techniques than what I performed, such as breeding call surveys and occasional dip netting for larvae (Gray et al. 2013). As discussed earlier, other National Parks are experiencing drought conditions more frequently, which is negatively impacting amphibian populations (McMenamin et al. 2008). If droughts are becoming more frequent in the southern Appalachian Mountains, NPS should consider management strategies (e.g., water diversion from Sea Branch, installation of wetland liners) that increase the hydroperiod duration at GP and LGP in order to preserve the biodiversity at this site. Although long-term data on the hydroperiod do not exist, photos that I took in 2017 show invasion of hardwood trees in GP compared to a photo taken in 2009 (Figure 7), suggesting this site is becoming drier. I recommend that NPS begin collecting monthly data on hydroperiod duration at GP and LGP to inform future management decisions.

A very interesting and unexpected finding of my study was that the drought of 2016 – 2017 may have eliminated ranavirus from GP and LGP. Ranavirus was detected in the post-metamorphic amphibian community in 2016 but not in 2017. The surveillance effort that I performed for ranavirus in 2017 was intense enough to detect the virus at a prevalence of less than 2% in an amphibian population of over 100,000 individuals (Gray et al. 2015). In general, this is an important and novel finding for natural resource biologists that are attempting to manage ranavirus outbreaks, because it suggests that dewatering a site for one year (as occurred during the drought) might interrupt the ranavirus-host cycle. Even though ranavirus prevalence decreased to non-detectable levels in 2017, it is possible that the pathogen could be reintroduced by long-dispersing species that are known to harbor subclinical infections, such as the eastern newt. Sea Branch is a logical dispersal corridor for Cades Cove amphibians. As such, I recommend that 30 – 50 amphibian larvae be tested for ranavirus infection in GP and LGP each

year. Sampling should occur in May or June when previous ranavirus outbreaks have occurred at the site.

Clearly, there is a trade-off between management actions to promote increased water presence at GP to thwart negative impacts of drought on the amphibian community and potentially increased opportunity for ranavirus transmission due to water presence. Given that drought resulted in two years of essentially no recruitment during my study, I hypothesize this factor is a greater threat to the amphibian community at GP. In the only other longitudinal study performed at GP, Todd-Thompson (2010) found that some amphibian species survived a large-scale ranavirus outbreak. Thus, if conservation of biodiversity at GP is a concern, I recommend that actions be taken first to counteract the insufficient hydroperiod if this condition persists. Hydroperiod and low-intensity breeding and larval population monitoring can help guide this decision. If habitat management actions are performed at GP to increase the hydroperiod (e.g., installation of wetland liners, Biebighauser 2011), ranavirus prevalence should be monitored to ensure outbreaks do not occur. In the event an outbreak occurs, my study suggests that dewatering (e.g., pumping) a site for one year should eliminate ranavirus from the system.

My study revealed substantial public visitation to GP and LGP. Hence, if ranavirus re-emerges in the GP amphibian community, I recommend that informational signage on the pathogen be erected and that access into the standing water is discouraged or restricted unless decontamination of footwear occurs prior to departure. The most likely months that visitors would encounter contaminated substrate or water containing shed ranavirus virions at GP is May and June (Todd-Thompson 2010), hence reduced access could be limited to those months.

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APPENDICES

APPENDIX A: TABLES

Table 1. Total (n) and percent (%) capture of post-metamorphic amphibian species at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Order	Family	Genus	Species	Year	n	Year %[1]	Total %[2]
Anuran	Bufonidae	Bufo	*A. americanus*	16	52	2.7	2.2
Anuran	Hylidae	Pseudacris	*P. crucifer*	16	18	0.9	0.8
Anuran	Hylidae	Pseudacris	*P. feriarum*	16	18	0.9	0.8
Anuran	Microhylidae	Gastrophryne	*G. carolinensis*	16	7	0.4	0.3
Anuran	Ranidae	Lithobates	*L. clamitans*	16	8	0.4	0.3
Anuran	Ranidae	Lithobates	*L. palustris*	16	7	0.4	0.3
Anuran	Ranidae	Lithobates	*L. sylvaticus*	16	225	11.8	9.6
Urodela	Ambystomatidae	Ambystoma	*A. maculatum*	16	1243	65.1	52.9
Urodela	Ambystomatidae	Ambystoma	*A. opacum*	16	59	3.1	2.5
Urodela	Plethodontidae	Plethodon	*P. glutinosis*	16	2	0.1	0.1
Urodela	Plethodontidae	Plethodon	*P. serratus*	16	6	0.3	0.3
Urodela	Plethodontidae	Pseudotriton	*P. ruber*	16	17	0.9	0.7
Urodela	Salamandridae	Notophthalmus	*N. viridescens*	16	247	12.9	10.5
Anuran	Hylidae	Pseudacris	*P. crucifer*	17	24	5.4	1.0
Anuran	Hylidae	Pseudacris	*P. feriarum*	17	3	0.7	0.1
Anuran	Microhylidae	Gastrophryne	*G. carolinensis*	17	7	1.6	0.3
Anuran	Ranidae	Lithobates	*L. clamitans*	17	4	0.9	0.2
Anuran	Ranidae	Lithobates	*L. palustris*	17	3	0.7	0.1
Anuran	Ranidae	Lithobates	*L. sylvaticus*	17	4	0.9	0.2
Urodela	Ambystomatidae	Ambystoma	*A. maculatum*	17	162	36.7	6.9
Urodela	Ambystomatidae	Ambystoma	*A. opacum*	17	38	8.6	1.6
Urodela	Plethodontidae	Desmognathus	*D. monticola*	17	5	1.1	0.2
Urodela	Plethodontidae	Desmognathus	*D. quadramaculatus*	17	2	0.5	0.1
Urodela	Plethodontidae	Eurycea	*E. longicauda*	17	3	0.7	0.1
Urodela	Plethodontidae	Eurycea	*E. wilderae*	17	2	0.5	0.1
Urodela	Plethodontidae	Gyrinophilus	*G. porphyriticus*	17	4	0.9	0.2
Urodela	Plethodontidae	Plethodon	*P. glutinosis*	17	7	1.6	0.3
Urodela	Plethodontidae	Plethodon	*P. serratus*	17	26	5.9	1.1
Urodela	Plethodontidae	Pseudotriton	*P. ruber*	17	11	2.5	0.5
Urodela	Salamandridae	Notophthalmus	*N. viridescens*	17	96	21.7	4.1

[1] *The percentage of the total number of captures each species represented from the total number of amphibian captured during that year.*

[2] *The percentage of the total number of captures each species represented from the total number of amphibians captured during both years combined.*

Table 2. Total (n) and (%) of reptile species captured at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Order	Family	Genus	Species	Year	n	Year %[1]	Total %[2]
Squamata	Colubridae	*Coluber*	*constrictor*	16	1	33.3	11.1
Squamata	Dipsadidae	*Diadophis*	*punctatus*	16	1	33.3	11.1
Testudines	Emydidae	*Terrapene*	*carolina*	16	1	33.3	11.1
Squamata	Colubridae	*Coluber*	*constrictor*	17	2	33.3	22.2
Squamata	Colubridae	*Storeria*	*dekayi*	17	3	50.0	33.3
Squamata	Dipsadidae	*Diadophis*	*punctatus*	17	1	16.7	11.1

[1] *The percentage of the total number of captures each species represented from the total number of reptiles captured during that year.*

[2] *The percentage of the total number of captures each species represented from the total number of reptiles captured during both years combined.*

Table 3. Catch per unit effort mean ($\bar{x}$), standard deviation (SD), and standard error (SE) for the five most commonly captured species at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Common Name	Year	$\bar{x}$	SD	SE
Spotted salamander	2016	0.873	2.390	0.599
Spotted salamander	2017	0.083	0.157	0.033
Marbled salamander	2016	0.041	0.137	0.034
Marbled salamander	2017	0.019	0.089	0.019
American toad	2016	0.034	0.047	0.012
American toad	2017	0.021	0.043	0.009
Eastern newt	2016	0.173	0.171	0.043
Eastern newt	2017	0.049	0.061	0.013
Wood frog	2016	0.158	0.441	0.110
Wood frog	2017	0.002	0.007	0.002

Table 4. Catch-per-unit effort (CPUE) comparisons of post-metamorphic amphibians among years, ponds and months using Wilcoxon[a] and Kruskal-Wallis[b] tests at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Test	**W or $X^{2\,(1)}$**	**df**	***P***
Total CPUE~ Year[a]	247.0	NA	0.037
Pond CPUE~Pond[a]	912.0	NA	0.282
Total CPUE~Month[b]	12.7	7	0.079

[1]The test statistics W and X^2 scores reported for Wilcoxon tests and Kruskal-Wallis test, respectively. No degrees of freedom (df) are reported for Wilcoxon tests.

Table 5. Differences in sex ratios (M:F) between years (2016 and 2017) using Fisher Exact Tests for species that were captured and could be sexed reliably at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park.

Species	**2016 (M:F)**	**2017 (M:F)**	**odds ratio**	**95% CI**	***P***
Spring Peeper	8:1	11:12	0.12	0.002–1.16	0.05
Eastern Newt	96:103	30:55	0.59	0.33–1.02	0.05
Spotted Salamander	361:282	81:72	0.89	0.61–1.27	0.53
Marbled Salamander	31:17	21:16	0.72	0.27–1.90	0.51
American Toad	29:10	10:3	1.15	0.22– 7.77	1.00

Table 6. Simple linear regressions of various weather variables collected at the U.S. National Park Service Cades Cove weather station and post-metamorphic catch-per-unit effort at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Predictor	***P*[1]**
Average precipitation during previous 24 hr	<0.001
Average ambient temperature during previous 12 hr	0.06
Average station temperature during previous 12 hr	0.086
Palmer Hydrological Drought Index	0.092
Average solar radiation previous 12 hr	0.096
Average relative humidity previous 12 hr	0.226
Average Scalar wind speed previous 12 hr	0.277
Average vector wind speed direction previous 12 hr	0.299
Average vector wind speed previous 12 hr	0.445
Days since January 1st	0.492
Relative humidity during previous 24 hr	0.583
Average precipitation during previous 12 hr	0.741

[1] *Predictors are arranged by significance, rows highlighted in gray are those which were included in the global generalized linear model created to predict CPUE.*

Table 7. Variables included in the final model of predicting catch-per-unit effort (CPUE) of post-metamorphic amphibians at Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Variable	Num.mod[1]	Importance[2]	β[3]	SE	z[4]	*P*
Average precipitation (mm) over the previous 24 hr	4	1.00	2.0664	0.498	4.813	<0.001
Average solar radiation (W/m^2) over the previous 12hr	4	1.00	-0.0070	0.003	-2.132	0.033
Number of days since January 1st	4	1.00	-0.1060	0.041	-2.493	0.013
Palmer Hydrological Drought Index	2	0.26	0.0621	0.098	0.614	0.539
Average ambient temperature (C°) over the previous 12 hr	2	0.20	-0.0204	0.506	0.389	0.697

[1]*The total number of models each predictor variable was included in out of top four models.*

[2]*The percent of models within the model subset that contain that variable.*

[3]*The parameter estimate (β) associated with the variable listed in the same row.*

[4]*The standardized parameter estimate (z = β / SE).*

Table 8. The results of each Rao spacing test of uniformity are reported including the test-statistic, critical value and result for immigration and emigration directions of post-metamorphic amphibians at Gourley Pond (GP) and Little Gourley pond (LGP), Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Test	**Test-statistic (U)**	**Critical Value**	**Result[1]**
GP Immigration	350.0	136.94	Reject HΘ
GP Emigration	356.0	136.94	Reject HΘ
LGP Immigration	334.0	142.35	Reject HΘ
LGP Emigration	334.2	142.35	Reject HΘ

[1]*A rejected HΘ indicates that immigration and emigration were not uniformly distributed around each pond at* α*=0.05.*

Table 9. Total number of spotted salamander and eastern newt recaptures (n), mean time between initial capture and recapture ($\bar{x}$), median time between captures (M), standard deviation (SD), standard error (SE) and p-value (*P*) of recaptured post-metamorphic amphibians from Gourley and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Species	**n**	**$\bar{x}$**	**M**	**SD**	**SE**	**W[1]**	***P***
Spotted salamanders	91	12.9	1	42.8	4.48	226.5	<0.001
Eastern newt	11	30.3	25	29.3	8.84		

[1]*Wilcoxon test statistic of the comparison of each population mean time between recaptures for spotted salamanders and eastern newts.*

Table 10. Differences in estimated body mass index for each post-metamorphic amphibian species captured with >10 captures per year at Gourley pond and Little Gourley pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Test	**t**	**df**	***P***	$\bar{x}_1$ [1]	$\bar{x}_2$ [2]
Spotted salamander BMI ~ Year	1.728	197.45	0.086	0.128	-0.115
Spotted salamander BMI ~ Pond	0.737	73.79	0.942	0.004	-0.010
Spotted salamander BMI ~Sex	2.031	171.86	0.044	0.157	-0.138
Marbled salamander BMI ~ Year	-4.634	63.19	<0.001	-0.368	0.560
Marbled salamander BMI ~ Pond	2.312	88.16	0.023	0.198	-0.270
Marbled salamander BMI ~ Sex	-0.025	64.07	0.980	-0.003	0.002
Eastern newt BMI ~ Year	0.136	222.11	0.891	0.006	-0.020
Eastern newt BMI ~ Pond	-0.637	165.52	0.525	-0.024	0.058
Eastern newt BMI ~ Sex	1.692	213.03	0.092	0.087	-0.149
American toad BMI ~ Year	2.618	77.92	0.011	0.265	-0.311
American toad BMI ~ Pond	0.460	65.05	0.654	0.022	-0.082
American toad BMI ~ Sex	-0.633	26.34	0.532	-0.140	0.049
Spring peeper BMI ~ Year	-4.020	22.94	<0.001	-0.647	0.503
Spring peeper BMI ~ Pond	-1.038	1.36	0.449	-0.024	0.426
Spring peeper BMI ~ Sex	1.742	28.69	0.092	0.330	-0.224
Red salamander BMI ~ Year	-1.417	17.16	0.174	-0.237	0.352
Red salamander BMI ~ Pond	-0.705	1.04	0.606	-0.072	0.942

Test variables are as follows: Year=year measurements were taken (2016 or 2017), Pond=pond animal was captured (Gourley Pond or Little Gourley Pond), Sex= male or female.

[1] *$\bar{x}_1$ represents the calculated mean of BMI for 2016, Gourley pond and female for tests on year, pond and sex respectively.*

[2] *$\bar{x}_2$ represents the calculated mean of BMI for 2017, Little Gourley Pond and males for tests on year, pond and sex respectively.*

Table 11. Ranavirus prevalence for each amphibian species tested in 2016 and 2017 from samples collected at Gourley and Little Gourley ponds, Cades Cove, Great Smoky Mountains National Park.

Species	Year	Prevalence	95% CI[1]
Wood Frog (*Lithobates sylvaticus*)	2016	0.089	0.03–0.20
Spotted Salamander (*Ambystoma maculatum*)	2016	0.016	0.0002–0.04
Marbled Salamander (*Ambystoma opacum*)	2016	0.000	0–0.07
Eastern Newt (*Notophthalmus viridescens*)	2016	0.012	0. 001–0.04
Overall Prevalence 2016	**2016**	**0.023**	**0.01–0.04**
Wood Frog (*Lithobates sylvaticus*)	2017	0.000	0–0.6
Spotted Salamander (*Ambystoma maculatum*)	2017	0.000	0–0.04
Marbled Salamander (*Ambystoma opacum*)	2017	0.000	0–0.09
Eastern Newt (*Notophthalmus viridescens*)	2017	0.000	0–0.04
American Toad (*Anaxyrus americanus*)	2017	0.000	0–0.09
Seal Salamander (*Desmognathus monticola*)	2017	0.000	0–0.6
Black belly Salamander (*Desmognathus quadramaculatus*)	2017	0.000	0–0.8
Long-tailed Salamander (*Eurycea longicauda*)	2017	0.000	0–0.7
E. Narrow-mouthed Toad (*Gastrophryne carolinensis*)	2017	0.000	0–0.41
Spring Salamander (*Gyrinophilus porphyriticus*)	2017	0.000	0–0.7
Green Frog (*Lithobates clamitans*)	2017	0.000	0–0.7
Pickerel Frog (*Lithobates palustris*)	2017	0.000	0–0.7
Slimy Salamander (*Plethodon glutinosus*)	2017	0.000	0–0.34
Red-backed Salamander (*Plethodon cineria*)	2017	0.000	0–0.14
Spring Peeper (*Pseudacris crucifer*)	2017	0.000	0–0.15
Upland Chorus Frog (*Pseudacris feriarum*)	2017	0.000	0–0.6
Red Salamander (*Pseudotriton ruber*)	2017	0.000	0–0.15
Overall Prevalence 2017	**2017**	**0.000**	**0–0.008**
Overall Prevalence 2016--2017	**2016-17**	**0.010**	**0.006–0.02**

Results are organized by year and then by greatest prevalence for each species. Overall prevalence in 2016, 2017 and for both years are displayed as well.

[1]95% confidence interval estimated using the Clopper-Pearson method.

Table 12. Wilcoxon and Kruskal-Wallis tests comparing differences in larvae/m^2 among year, month, quadrant, and species at Little Gourley pond, Cade Cove, Great Smoky Mountains National Park, 2016 and 2017.

Test	**df**	**W or X^2**	***P***
Larvae/m^2~Year	1	13.50	0.196
Larvae/m^2~Month 2016	2	3.65	0.162
Larvae/m^2~Month 2017	2	3.15	0.201
Larvae/m^2~Quadrant 2016	3	1.03	0.794
Larvae/m^2~Quadrant 2017	3	2.64	0.450
Larvae/m^2~Species 2016	1	5.23	0.022

[a]*Wilcoxon tests comparing larval densities with Wilcoxon W test statistic reported.*

[b]*Kruskal-Wallis tests comparing larval densities with X^2 test statistic reported.*

Table 13. Summary data for trail passes per day along the main trail to Gourley Pond, Cades Cove, Great Smoky Mountains National Park are shown for each month in 2016 and 2017, including the number of days observations were made for each month (n), mean number of trail passes per day (μ), standard deviation (SD) and standard error (SE).

Month	n	μ	SD	SE
April	60	12.00	9.83	1.27
August	31	6.48	9.31	1.67
July	31	5.97	5.83	1.05
June	30	7.60	5.52	1.01
March	34	12.40	10.40	1.78
May	43	11.20	9.59	1.46
October	3	7.33	8.74	5.04
September	30	10.00	9.61	1.76

Table 14. Predictor variables included in the final model of trail passes per day along the main trail to Gourley Pond, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017.

Variable	**Num.mod**[1]	**Importance**[2]	**β**[3]	**SE**	**z**[4]	***P***
Days since January 1st	6	0.74	-0.0023	0.0011	-2.000	0.0455
Average daytime precipitation (mm)	8	1.00	-0.4585	0.1579	-2.892	0.0038
Average daytime solar radiation (W/s)	5	0.69	0.0009	0.0005	1.790	0.0735
Average daytime ambient temperature (C°)	4	0.51	-0.0241	0.0150	-1.607	0.1080
Average daytime scalar windspeed (m/s)	3	0.24	0.0691	0.0861	0.799	0.4244

[1]*The total number of models each predictor variable was included in out of top four models.*

[2]*The percent of models within the model subset that contain that variable.*

[3]*The parameter estimate (β) associated with the variable listed in the same row.*

[4]*The standardized parameter estimate (z = β / SE).*

Table 15. Natural history characteristics of 10 pond breeding amphibian species captured at Gourley Pond, Cades Cove, Great Smoky Mountains National Park in 2016 and 2017; data for each species was compiled from multiple literature sources (Wells 2002, Lannoo 2005, Hoverman et al. 2011).

Species	Breeding Season	Larval Development[1] (days)	Longevity[2] (yrs)	Clutch Size[3] (# of eggs)	Maximum Dispersal[4] (m)	Average Range[5] (m^2)
Lithobates sylvaticus	Winter	65-130	5	300-4000	2,530	13.3
Lithobates clamitans	Summer	90-243	5	1,000-7,000	4,800	20-200
Lithobates palustris	Spring	60-90	Unknown	3,000	Unknown	Unknown
Anaxyrus americanus	Spring	57.5	5	2,000-20,000	4,023	809
Pseudacris crucifer	Winter	90-100	2	700-1,000	Unknown	Unknown
Pseudacris feriarum	Winter	55		1,000	Unknown	Unknown
Gastrophryne carolinensis	Summer	20-70	6	850-1,600	914	42-914
Ambystoma maculatum	Winter	203.5	32	500-1000	567	0-249
Ambystoma opacum	Fall	150	10	30-200	600	1-225
Notophthalmus viridescens	Summer	90	15	200-375	1,000	267-353

[1]*Larval development is the duration measured in days from hatching to completing metamorphosis.*

[2]*Longevity is the maximum number of years individuals for each species have been found living.*

[3]*Clutch size is the range of eggs typically laid by one female of each species in one egg laying period.*

[4]*Maximum dispersal represents the maximum movement or dispersal distance in meters found in the literature.*

[5]*Average range is the average home range measured in squared meters of each amphibian species found in the literature.*

APPENDIX B: FIGURES

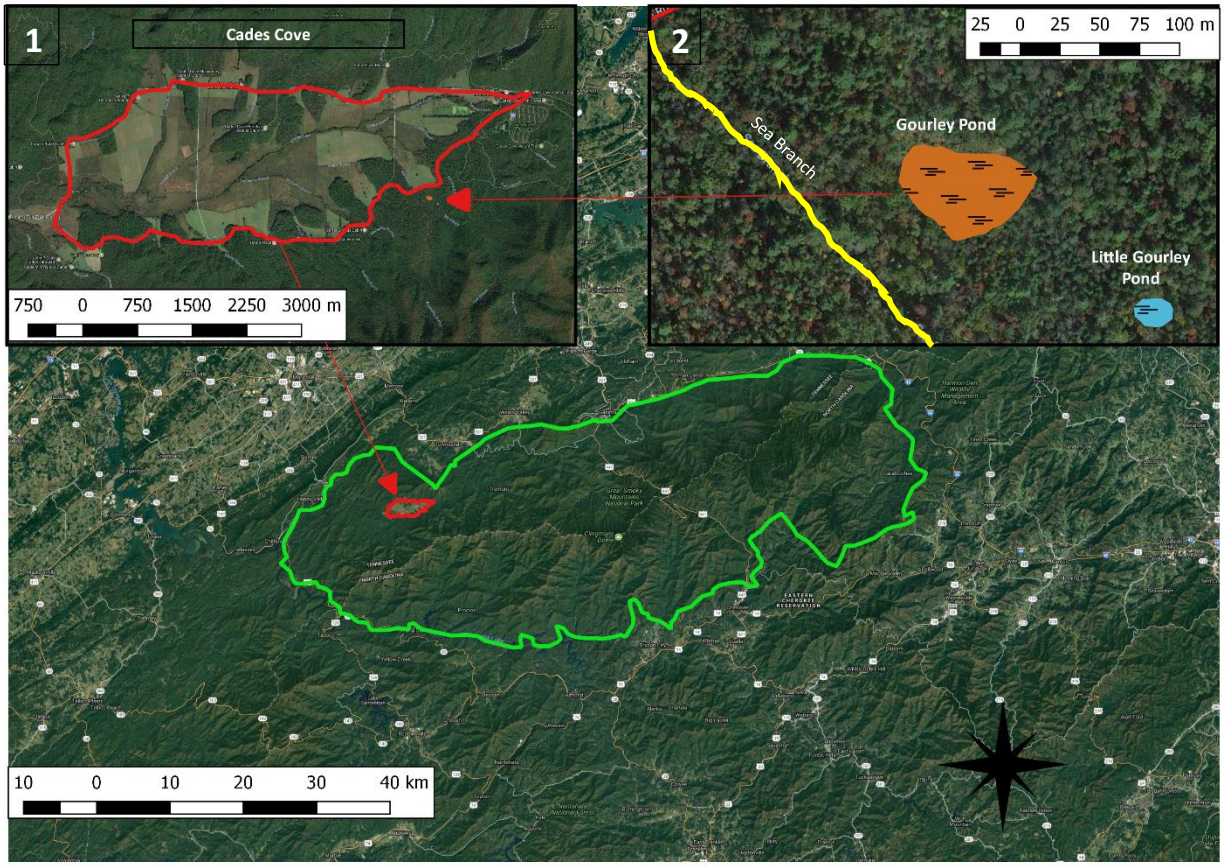

Figure 1. Satellite image of Great Smoky Mountains National Park (green outline) with inset 1 showing Cades Cove (red outline) and inset 2 showing Gourley Pond, Little Gourley Pond and Sea Branch colored orange, blue, and yellow, respectively.

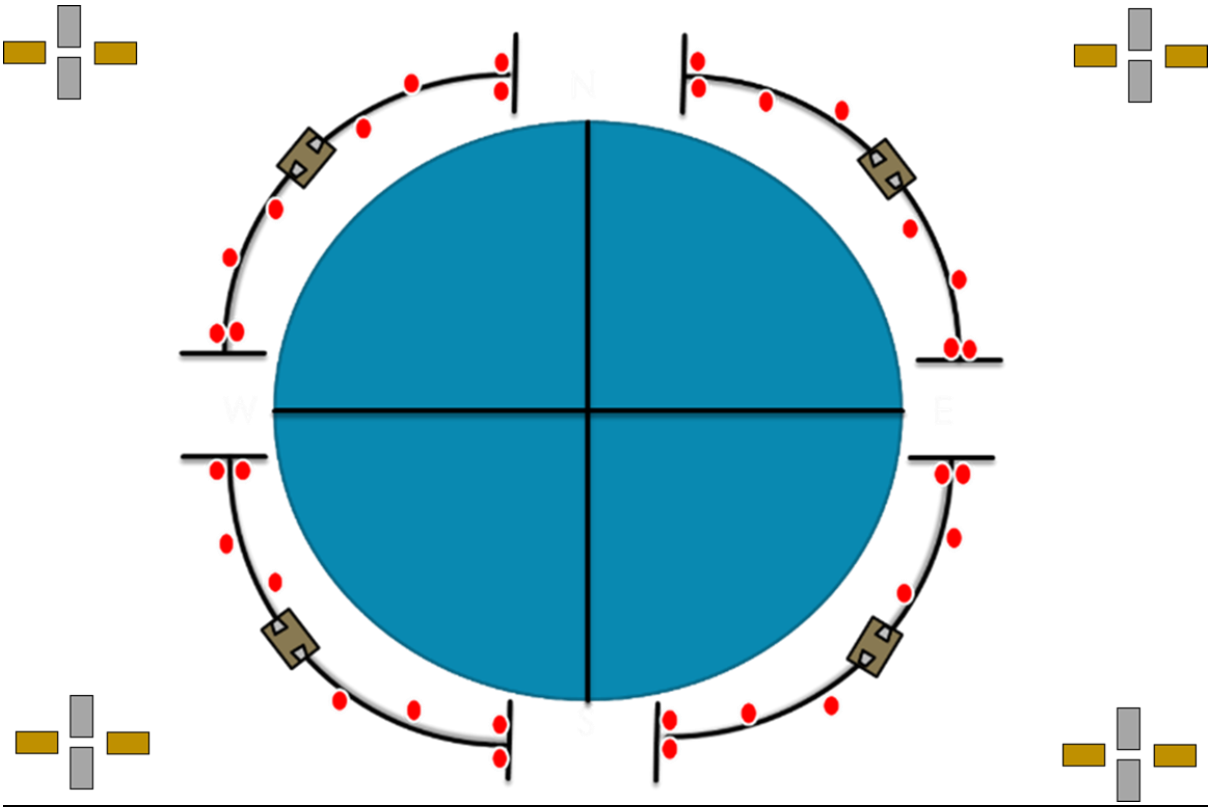

Figure 2. Post-metamorphic amphibian sampling design at Gourley and Little Gourley ponds, Cades Cove, Great Smoky Mountains National Park. Each wetland (blue circle) had approximately 75% of its perimeter encircled with drift fence (black lines) and pitfall traps (red circles). Pitfall traps alternated sides of the fence every 5 m and each drift fence terminus had two pitfalls on either side of the fence. At GP, each drift fence segment had a snake funnel trap (brown rectangle) placed at its midpoint. Artificial cover objects (brown = plywood and gray = tin rectangles) were placed 50 m from each snake funnel trap.

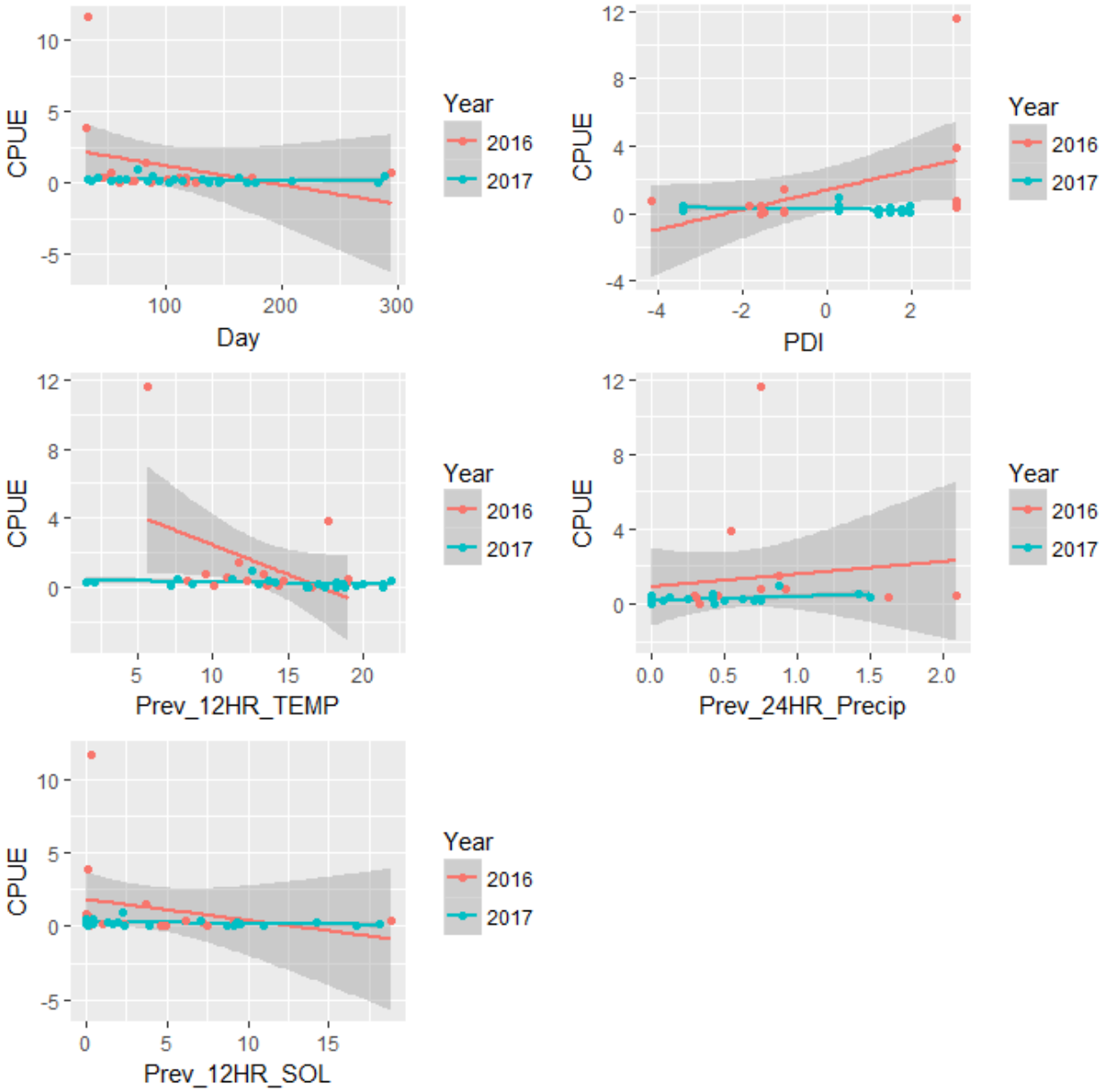

Figure 3. Scatterplots with fitted lines showing the relationship between each of the weather variables included in the final model (x axis) and catch-per-unit effort (CPUE, y axis) of post-metamorphic amphibians at Gourley and Little Gourley ponds, Cades Cove, Great Smoky Mountains National Park, 2016 (red) and 2017 (blue). Weather variables were Day = days since January 1 for each year, PHDI = Palmer Hydrological Index, Pre_12HR_Temp = average temperature (C°) during the previous 12 hr, Pre_24HR_Precip =average precipitation (mm) during the previous 24 hr, and Prev_12HR_SOL= average solar radiation (W/m^2) during the previous 12 hr.

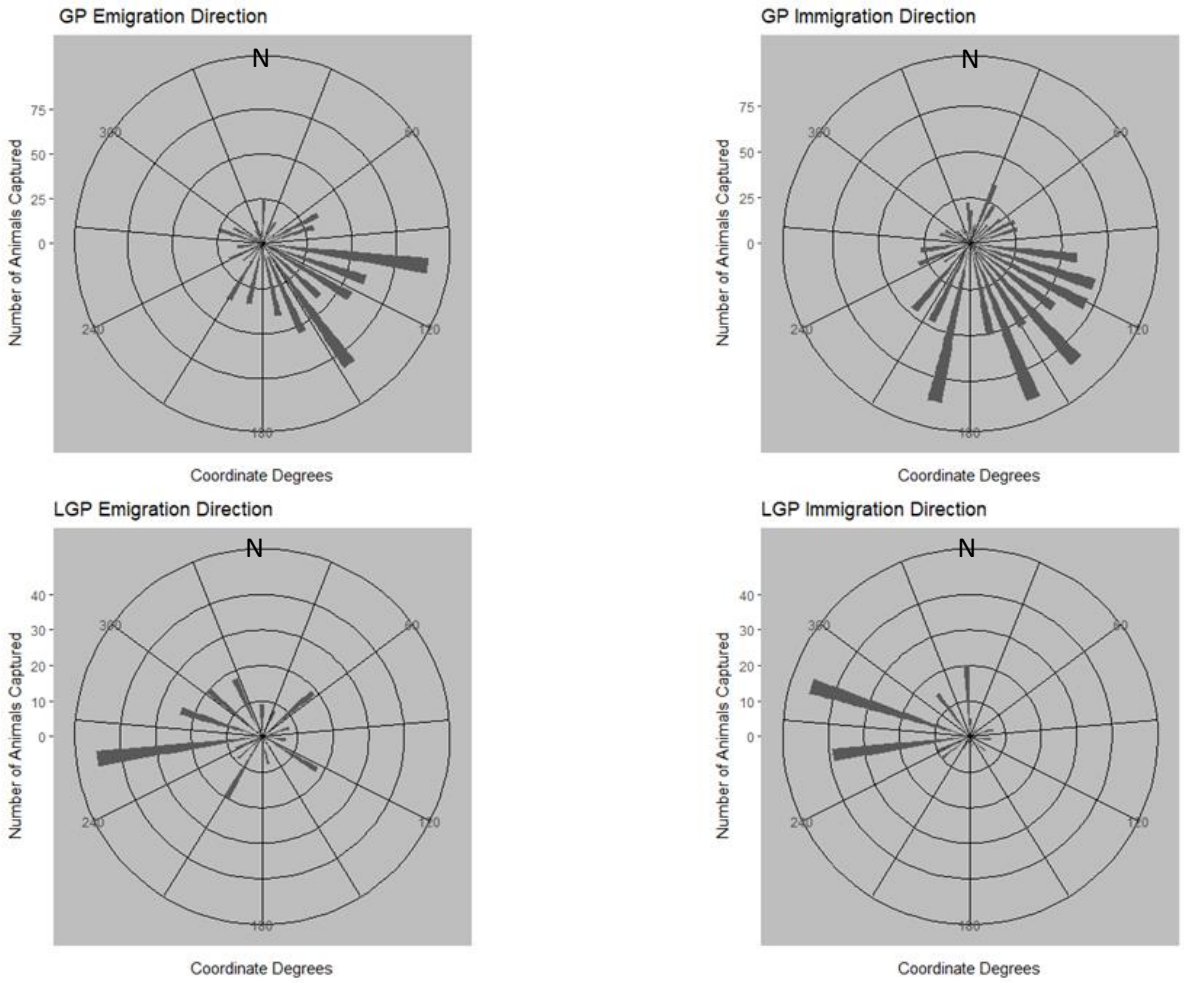

Figure 4. Plots of immigration and emigration for post-metamorphic amphibian captures at Gourley (GP) and Little Gourley (LGP) ponds, Cades Cove, Great Smoky Mountains National Park, 2016 and 2017 (data combined across years). Greater frequencies of captures in each direction correspond to larger bars extending from the center of each plot. North (N) is located at 0,360 degrees.

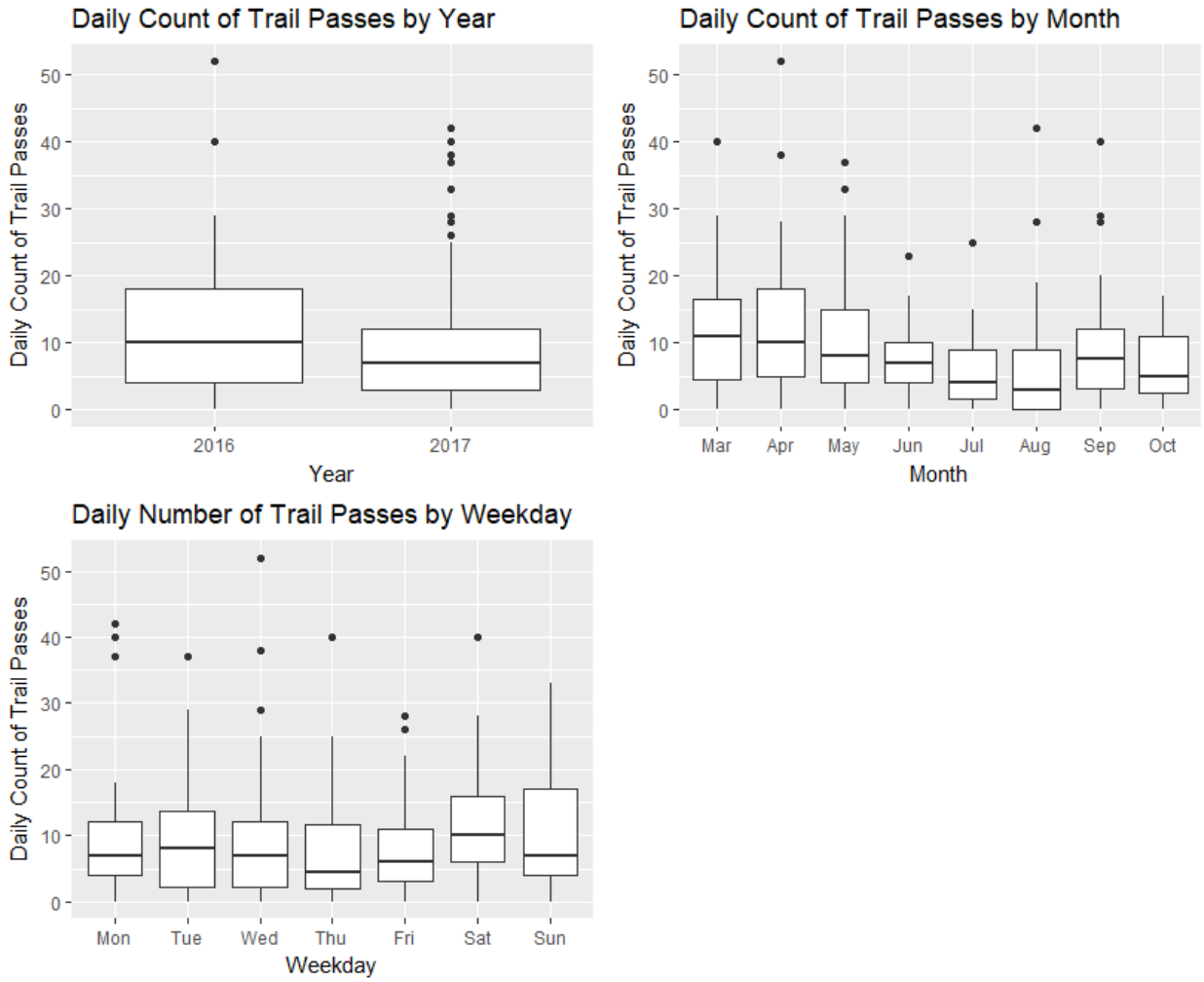

Figure 5. Daily passes estimated between years and among weekdays and months by the U.S. National Park Service trail counter along the trail leading from Cades Cove Loop to Gourley Pond, Great Smoky Mountains National Park. The bottom line extending from each graph represents quartile 1- 1.5*interquartile range (IQR), while the line extending from the upper portion of each box represents quartile 3+1.5* IQR. The lower and upper portion of each box represent the first and third quartile. The midline of each boxplot represents the median. Black points extending beyond the boxplot represent outliers.

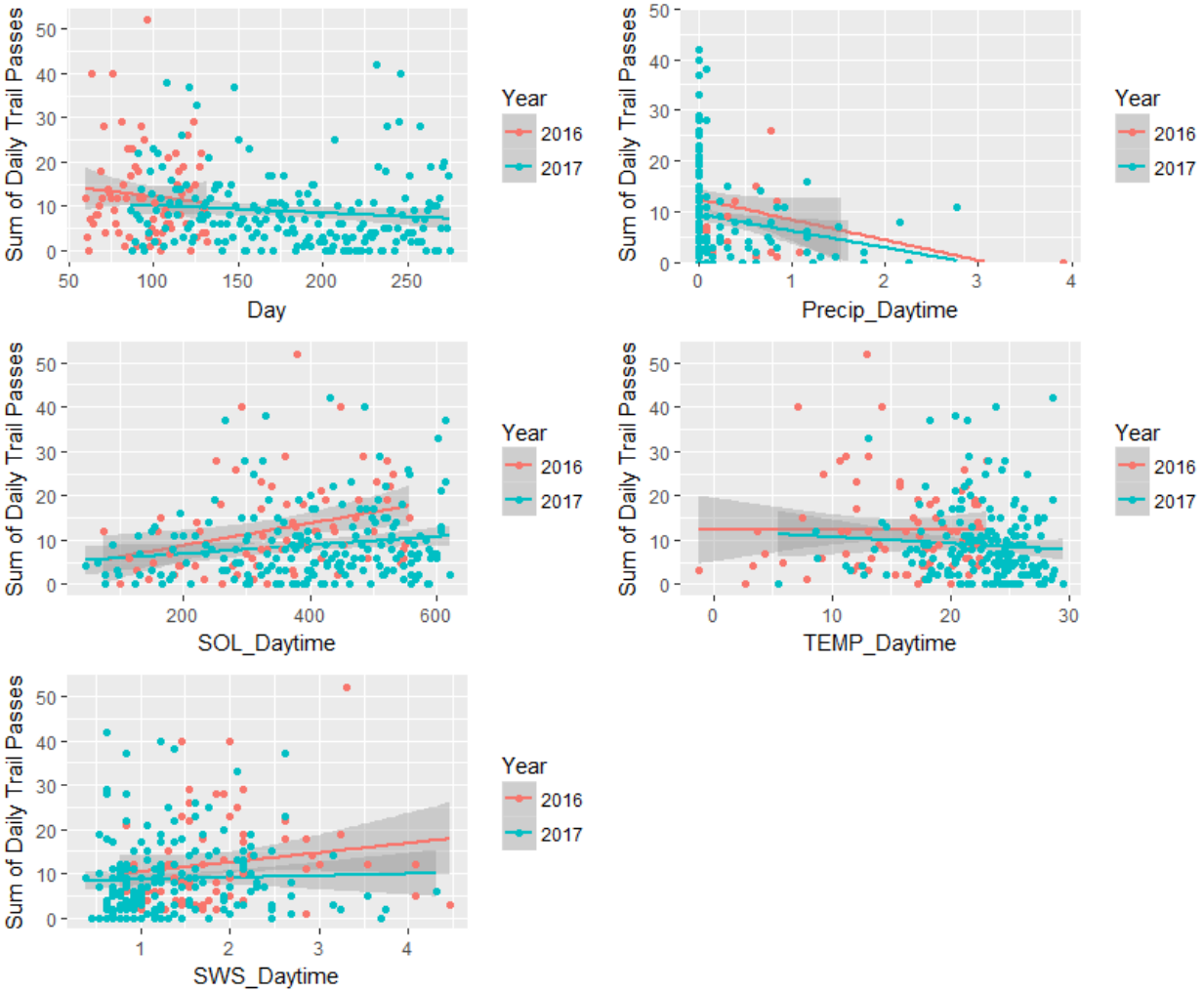

Figure 6. Scatterplots with fitted lines showing the relationship between each of the weather variables included in the final model (x axis) and trail passes per day (y axis) of post-metamorphic amphibians at Gourley and Little Gourley ponds, Cades Cove, Great Smoky Mountains National Park, 2016 (red) and 2017 (blue). Weather variables were Day = days since January 1 for each year, Precip_Daytime= average total daytime precipitation (mm), SOL_Daytime = average daytime solar radiation (W/m^2), TEMP_Daytime= average daytime temperature (C°), and SWS_Daytime= average daytime scalar wind speed (m/s).

Figure 7. Photos taken in 2009 (top) and 2017 (bottom) of Gourley Pond, Great Smoky Mountains National Park. Note the invasion of trees occurring in the bottom photo likely due to more frequent dry conditions.

VITA

Davis Carter is originally from Charlotte, North Carolina. He earned his Bachelor of Science in biology in Hampden-Sydney, Virginia at Hampden Sydney College. While attending Hampden-Sydney College, Davis conducted two undergraduate research projects which involved ranavirus and herpetofauna. After graduating in 2015, he began working in the UT Center for Wildlife Health as a research technician under the supervision of Dr. Matthew Gray and Dr. Debra Miller. In 2016, he started his M.S. on ranaviruses under the guidance of Dr. Gray and Dr. Miller.