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Long term amphibian monitoring at wetlands lacks power to detect population trends

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ABSTRACT

Amphibians are declining worldwide due to habitat destruction, disease, and environmental stressors. Extremely variable breeding populations and a paucity of long-term monitoring data limits rigorous testing of amphibian population trends, or bias associated with sampling regimes. We used 24 years of continuous trapping data to compare annual probability of presence, and population trends and statistical power for six species among seven wetlands using five sampling scenarios (SS) based on the interval and span of years analyzed. Richness within a year and wetland ranged 29–89% of total species captured there (all years), and 27–82% of total species captured during the study (all years, pooled wetlands). SS had little effect on probability of presence for most common species but did for less common species. Population trends were inconsistently significant or nonsignificant among wetlands within SSs, and among SSs within the same wetlands. The direction (+/−) of trends among wetlands and scenarios for a species generally agreed, but not always. Low statistical power for virtually all population trend estimates, including the All-years SS indicated results were inconclusive. Juvenile recruitment was correlated with adult populations in some subsequent years for four of the six species. We illustrate how probability of presence and population trend estimates can differ among similar wetlands within a landscape, and according to the span, or subset of years sampled. Our results indicate that amphibian monitoring at wetlands cannot conclusively gauge population trends for breeding populations that fluctuate widely among wetlands and from year to year.

1. Introduction

Population declines, range reductions, and even extinction of some amphibian species are occurring worldwide due to habitat destruction, disease, and other environmental stressors including climate change, pollution, invasion by non-native species, and increased ultraviolet radiation (Adams et al., 2013; Blaustein et al., 1994, 2011; Grant et al., 2016; Houlahan et al., 2000; Stuart et al., 2004). High variation in amphibian breeding among years reduces the statistical power to conclusively detect the presence or absence of population trends, and the variables that determine them (Marsh, 2001; Reed and Blaustein, 1995). Rigorous testing of population trends, or the sampling timespan and frequency needed to detect them, has been limited by a paucity of long-term continuous monitoring programs. Nearly 30 years after scientists first reported amphibian population declines, long-term studies remain exceedingly rare; few extend beyond one turnover of the sampled population, and most focus only on select species (e.g., Blaustein et al., 1994), making it difficult to gauge whether long-term population trends differ among co-occurring amphibian species. The few existing long-term data sets typically are intensive sampling at single sites (e.g., Rainbow Bay at Savannah River Site; Pechmann et al., 1991; Semlitsch et al., 1996), or non-concurrent or intermittent "snapshot" sampling at multiple sites (e.g., Marsh and Trenham, 2001; Muths et al., 2005; Skelly et al., 2003; Werner et al., 2007) that limit the spatial or temporal scale of inference.

Two general approaches to monitoring amphibian populations are common. Spatial patterns of persistence (presence or absence) are used to assess distributional changes of target species over time, as an indicator of population stability, decline, or extinction and recolonization by re-sampling the same sites at regular intervals (e.g., Adams et al., 2013; Grant et al., 2016; Marsh and Trenham, 2001; Muths et al., 2005; Skelly et al., 2003; Werner et al., 2007). Alternatively, population

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trends can be assessed using counts, or relative abundance data from the same site(s) over multiple years (Daszak et al., 2005; Pechmann and Wilbur, 1994). Standard amphibian monitoring techniques at wetlands include acoustic, aquatic (box trap or dip net), and terrestrial drift fence sampling (Dodd Jr, 2010); all can provide important information but are limited by method-specific bias in detecting presence or relative abundance of amphibians (Dodd Jr, 2010; Greenberg et al., 2017a). Long-term continuous, year-round mark-recapture trapping at wetlands with drift fences and pitfall traps is likely the most effective method for assessing persistence and population trends of many amphibian species (Blaustein et al., 1994) but is rarely used as a monitoring method because it is labor-intensive and more costly than other sampling methods (Dodd Jr, 2010).

Estimates of amphibian population trends, distributional change, or local extinctions and recolonizations at breeding wetlands are extremely sensitive to the timing and frequency (Skelly et al., 2003) of both within- and among-year sampling, for several reasons. Amphibian breeding populations and juvenile recruitment are often highly variable among years (Pechmann et al., 1989, 1991; Pechmann and Wilbur, 1994; Semlitsch et al., 1996; Trenham et al., 2003) and wetlands (Greenberg et al., 2017b; Werner et al., 2007). Detection and adult abundance estimates of any given species may reflect weather patterns, hydroregime (the timing, duration, and depth of water in wetlands) (Greenberg et al., 2017a, 2017b; Saenz et al., 2006; Semlitsch et al., 1996), life history factors, or past juvenile recruitment success (e.g., Berven, 1990). Intermittent sampling increases the likelihood of “missing” detections or underestimating abundance due to narrow breeding seasons, infrequent or unpredictable breeding behavior (Skelly et al., 2003), and rapid turnover of amphibian eggs and larvae within years and wetlands due to high mortality rates, followed by deposition of new eggs and larval cohorts (Greenberg et al., 2017a). Similarly, species detections and population estimates based on sampling at multi-year intervals may be heavily influenced by which years or wetlands are sampled. Only intensive, concurrent and continuous sampling of multiple sites and species over multiple years can provide insight into amphibian community dynamics at a broad temporal and spatial scale, and potentially distinguish between natural fluctuations and true population declines or extinctions (Blaustein et al., 1994, 2010; Pechmann et al., 1991).

We used 24 years (March 1994–December 2017) of continuous, concurrent trapping data at seven isolated wetlands to assess the value of long-term data and spatial replication in assessing probabilities of amphibian species' presence, and adult breeding population trends of six anuran species. We also examine how estimates of species presence and breeding population trends might differ among individual wetlands, pooled wetlands, or based on the interval or span of years sampled (sampling scenario). We asked the following questions: (1) How does species richness and annual probability of each species' presence differ among individual and pooled wetlands, and how are probability of presence estimates affected by sampling scenario? (2) Do adult population trend estimates differ among individual wetlands, pooled wetlands, or sampling scenario? (3) Is the statistical power adequate to detect adult population trends, and does it differ among wetlands and sampling scenarios? (4) Does juvenile recruitment affect adult breeding populations in subsequent years? For the purposes of this paper, we were not interested in the environmental factors (e.g., hydroregime, wetland characteristics, or weather) contributing to species presence or abundance in any specific year, or in developing the best models for each species (see Greenberg et al., 2017b), but rather to compare overall patterns of persistence and population trend estimates among wetlands and sampling scenarios.

2. Methods

2.1. Study area

Our seven study wetlands were a representative selection of small (0.1–0.35 ha), isolated, groundwater-driven sinkhole wetlands, embedded within xeric longleaf pine-wiregrass uplands of the Floridan Aquifer System region, Ocala National Forest, Marion and Putnam Counties, Florida (Greenberg et al., 2015). Within our study area, average weekly temperatures (February 1997–December 2017) ranged from a minimum of $-1.0\,^{\circ}\mathrm{C}$ in January, to a maximum of $41.1\,^{\circ}\mathrm{C}$ in July. Average annual precipitation (1995–2017) was 139.2 cm, with more than half occurring during late spring and summer). Heavy precipitation providing groundwater recharge was associated with thunderstorms and tropical systems in summer and fall, and wet autumn, winter, or spring frontal systems (Winsberg, 1990). Wetland depths were generally highest in winter and lowest in summer due to rainfall patterns and groundwater recharge, as well as low evapotranspiration in winter (Greenberg et al., 2017b; Knowles Jr et al., 2002). Hydroregimes differed among wetlands, with each drying several times for varying lengths of time, with maximum depths of > 240 cm for some, within the 24-year study period (Greenberg et al., 2017b).

2.2. Amphibian sampling

We installed drift fences 7.6-m long and spaced 7.6-m apart around the perimeter of each ($n = 7$) wetland near the high-water line, such that 50% of each wetland was fenced, with fences and spaces equally distributed and encircling wetlands. Pitfall traps (19-l buckets) were positioned inside and outside of each end of each fence (four per fence), and a double- or single-ended funnel trap (one of each per fence) was positioned at the midpoint of each fence on opposite sides, to detect directional movement by amphibians to and from wetlands. Additionally, we placed a pvc pipe (5-cm diameter; approximately 1.4-m height) between each drift fence to attract treefrogs. We placed a sponge in each pitfall trap and moistened as needed during trap checks to reduce the likelihood of desiccation. All traps were checked approximately three times weekly from 31 January 1994 through December 2017. We identified, sexed (when possible), and measured snout-vent length (SVL) and weight of captured animals. We assigned age-class (adult or recent metamorph/first-year juvenile) based on a specified SVL cutoff for each focal species used in analyses (Table 1). All individuals were marked by wetland number and year of capture by toe clipping; exceptions were new metamorphs of some species that were too small to toe-clip. Because all wetlands were sampled continuously and in proportion to basin size we did not further adjust for trapnights.

2.3. Statistical analyses

2.3.1. Sampling scenarios

We used our 24-year monitoring data from seven wetlands to compare annual probability of presence and population trends under five sampling scenarios differentiated by sampling interval and span of years sampled: (1) all years: 1994–2017 (All-yr); (2) alternate years: 1994–2016 (2-yr); (3) 5-year interval:1994–2014 (5-yr); (4) all years between a randomly selected start and stop year: 1996–2016 (RSS), and; (5) a randomly selected span of 10 consecutive years: 2007–2016 (R10).

2.3.2. Species richness and probability of presence

We used annual amphibian species richness for individual ponds and pooled across wetlands. Annual richness was defined as the number of all amphibian species captured at least once in the given year. The probability of presence for each species was calculated as the proportion of sample years that the species was captured at least once. We chose to represent the entire amphibian community in our species

Table 1
Peak periods of adult and juvenile captures of six focal anuran species, and wetland-years discarded from data analysis[a] when trap effectiveness was compromised due to flooding for prolonged periods during their respective peak capture periods, Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017).

Species	Adult Peak Captures	Juvenile Peak Captures	Adults: years discarded	Juveniles: years discarded
Anaxyrus quercicus (J if < 20 mm SVL)	Jun Wk 1 – Aug Wk 1	Jun Wk 3 – Sep Wk 3	2005 – All Wetlands; Pooled	2003 Wetland 7; Pooled 2005 – All Wetlands; Pooled 2008 – Wetlands 1, 2; Pooled
Anaxyrus terrestris (J if < 25 mm SVL	Mar Wk 4 – Sep Wk 4	Apr Wk 3 – Aug Wk 1	1998 – Wetlands 1,2; Pooled 2003 – Wetland 7; Pooled 2005 – All Wetlands; Pooled	1998 – Wetlands 1,2; Pooled 2005 – All wetlands; Pooled
Gastrophryne carolinensis (J if < 23 mm SVL)	Jun Wk 1 – Aug Wk 4	Aug Wk 2 – Oct Wk 2	2003 – Wetland 7; Pooled 2005 – All Wetlands; Pooled	2003 – Wetland 7; Pooled 2004 – All Wetlands; Pooled 2005 All Wetlands; Pooled 2008 Wetlands 1, 2; Pooled 2009 – Wetland 1; Pooled 2017 Wetlands 1, 2; Pooled
Rana capito (J if < 50 mm SVL)	All year	May Wk 2 – Jul Wk 4	1998 – Wetlands 1, 2, Pooled 2003 – Wetland 7, Pooled 2004 – Wetlands 1,2,3,6,7,8; Pooled 2005 – All Wetlands; Pooled 2006 – Wetlands 1, 2; Pooled 2017 – Wetland 1; Pooled	2005 – All Wetlands; Pooled
Rana sphenocephalus (J if < 52 mm SVL)	All year	Apr Wk 4 – Sep Wk 1	1998 – Wetlands 1, 2, Pooled 2003 – Wetland 7, Pooled 2004 – Wetlands 1,2,3,6,7,8; Pooled 2005 – All Wetlands; Pooled 2006 – Wetlands 1, 2; Pooled 2017 – Wetland 1; Pooled	2005 – All Wetlands; Pooled
Scaphiopus holbrookii (J if < 18 mm SVL)	After heavy rainfall	2–3 wks after breeding		1998 – Wetlands 3, 6; Pooled 2004 – Wetland 5; Pooled

[a] Wetland-years listed for adults were discarded from adult population trend regression analyses; wetland-years listed for adults, juveniles, or both were discarded from adult-juvenile correlations.

richness and probability of presence calculations, and thus included two terrestrial-breeding species, *Eleutherodactylus planirostris* and *Plethodon grobmani*. We discarded richness data for 2005 because traps at most wetlands were compromised for much of the year due to prolonged flooding. All other years ($n = 23$) were included in analyses despite some wetlands flooding during some years, which generally occurred in late fall and winter when overall capture rates were low and unlikely to affect detecting species' presence (see Greenberg et al., 2017b). We used one-way ANOVA (PROC MIXED; SAS 9.3) in a randomized block design with wetland as the random block effect (replicate) to compare probability of presence estimates among the five sampling scenarios.

2.3.3. Population trends and power analyses

We selected six commonly captured wetland-breeding "focal" anuran species for population trend and power analyses including *Anaxyrus quercicus*, *A. terrestris*, *Gastrophryne carolinensis*, *Rana capito*, *R. sphenocephalus*, and *Scaphiopus holbrookii*. Models were fitted to estimate adult (males and females) population trends for each focal species at each wetland and for pooled wetlands, for each of the five sampling scenarios (5 scenarios × (6 species × 7 wetlands + 6 additional models for pooled wetland) = 240 total models). We used simple linear regression to model each species' adult population as a function of year (year = 0 was 1994 and year = 23 was 2017). If an individual was recaptured in a given year, it was only counted once; recaptures from prior years (approximately 2 to 9% of adult individuals per species) were included in totals. Since the dependent variable (population of each species) was an integer ranging possibly from 0 to very large values, normality was not assumed on theoretical grounds. In addition, the variance/mean ratio on a wetland basis averaged 39.8 and ranged from 1.2 to 211.6, indicating a clumped distribution like the negative binomial. Thus, initially we tried fitting negative binomial models with the log link function and annual autocorrelation parameters. However, autocorrelation was abandoned due to model fitting problems arising from very variable annual data, little evidence of autocorrelation, deleted years for certain species and ponds, and the large number of models that had to be fitted. In addition to the parameter estimates, we obtained 95% confidence intervals (CI) to assess whether population trend estimates (slope parameter) differed (approximate non-overlapping CI test) among sampling scenarios for each wetland and pooled wetlands, for each species. We discarded data from some years for one or more wetlands, if most traps were flooded for prolonged periods during peak adult capture periods, which differed among species (Table 1). For pooled wetlands regressions of focal species, we omitted any year that did not include all seven wetlands, to ensure that models were based on the same number of wetlands.

We conducted power analyses for each focal species at each wetland and pooled wetlands, to estimate the probability of finding significant population trends (reject null hypothesis that slope is equal to zero) at a negative 0.05 effect size (5% annual population decrease) for each sampling scenario. A 0.05 effect size is assumed to be biologically meaningful, and power ≥ 0.80 is generally assumed to be adequate in detecting population trends (Hayes and Steidl, 1997). We also generated power curves for each focal species using pooled wetlands under the five sampling scenarios to illustrate how statistical power changes with effect sizes of 0.00 to negative 0.20 and differs among sampling scenarios. A simulation approach was used for all power analyses by generating 1000 sample scenarios from the specific negative binomial population obtained from each of the population models. The power was then computed as the proportion of the simulated scenarios that rejected the null hypothesis. Theoretically the power should increase as the effect size increases. Occasionally this did not occur which was due to the stochastic nature of the 1000 power simulations which provide approximate but not exact power at each size effect which would require an infinite number of simulations. In addition, sometimes one or more of the 1000 simulations from the negative binomial regression did not provide parameter estimates for a variety of reasons (for example, all zero data for a population regression) resulting in fewer than 1000 simulations and also a possible bias.

We used Kendall's tau rank correlations to test the relationship between juvenile recruitment and adult populations (captures) during any of the subsequent eight years, for each of the six focal species. Each adult-juvenile pair from each of the 7 wetlands and 24 years was considered to be an independent data point, for a total of 168 possible pairs per species. However, due to the lag juvenile pairs which resulted in some missing values, this was reduced accordingly. We discarded data from wetland-year combinations when capture data were likely compromised due to flooding during peak capture for periods for adults, juveniles, or both (Table 1). Kendall's was selected over Pearson tests because the response variables (adult and juvenile captures) did not meet assumptions of normality. For all population analyses, we omitted same-year recaptures, but included recaptures from prior years, as we were interested in the number of individuals captured each year.

3. Results

3.1. Species richness and probability of presence

We captured a total of 22 amphibian species from the seven study wetlands during our 24-year study period (1994–2017). Average annual species richness was 16.0 for pooled wetlands, and ranged from 10.3 to 12.8 among individual wetlands (Fig. 1). Species richness within individual wetlands ranged from 6 to 18 among years, and 14 to 19 for pooled wetlands (Fig. 1). Species richness within any given year and wetland ranged from 29% to 89% of total species captured over all 24 years at that wetland, and from 27% to 82% of total species captured at all seven (pooled) wetlands over the entire 24-year study period. Species richness of pooled wetlands in any given year ranged from 64% to 86% of total richness during the entire study period.

Mean annual probability of presence varied considerably among species, with commonly captured species having higher probabilities than rarely captured species (Fig. 2). Probability of presence differed among sampling scenarios for some, but not all species (Table 2). The probability of *Acris gryllus*, *Eleutherodactylus planirostris*, *Notophthalmus perstriatus*, *Pseudacris ocularis*, *Rana catesbeianus*, and *R. gryllio* presence was significantly lower under the R10 than all other scenarios. The probability of *Eurycea quadridigitata* presence was greater under the 2-yr than R10 scenario, and greater under the 5-yr than RSS or R10 scenarios. The probability of *Pseudacris crucifer* presence was greater under the All-yr and RSS scenarios than the 2-yr, 5-yr, or R10 scenarios. The probability of *R. sphenocephalus* presence was greater under All-yr, 2-yr, and 5-yr scenarios than R10, and greater under the 2-yr and 5-yr than the RSS scenarios. In contrast, the probability of *H. squirella* presence increased under the R10 compared to All-yr, 5-yr, and RSS scenarios. Several other species (*Osteopilus septentrionalis*, *Plethodon grobmani*, *R. capito*, *R. heckscheri*, and *Scaphiopus holbrookii*) also showed marginally significant differences in probability of presence among sampling scenarios (Table 2).

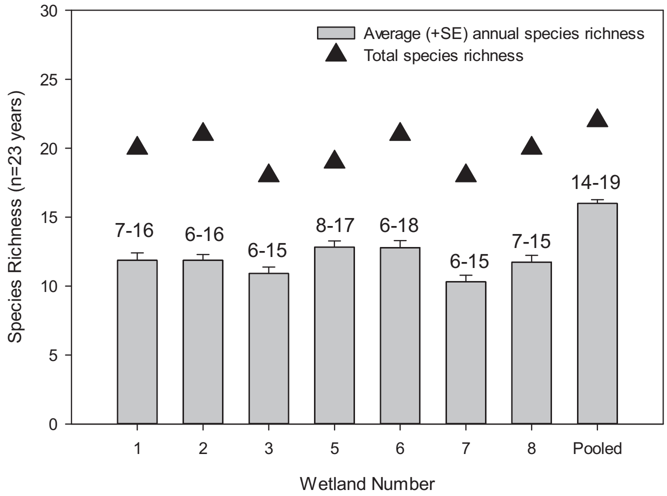

Fig. 1. Average (+SE) annual, range (numbers), and total amphibian species richness at each of seven wetlands and pooled wetlands, based on continuous, concurrent sampling with drift fences and pitfall traps in the Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017).

3.2. Population trends and statistical power

Breeding adult populations and juvenile recruitment of all six focal species was highly variable among years and wetlands (Fig. 3). All six focal species exhibited wild annual oscillations, with adults of some species ranging from near zero to several hundred, and juveniles ranging from zero to several thousand (e.g., *S. holbrookii*) at any given wetland. Often peaks and dips followed each other with little recognizable population trend. This lead to adult population trend estimates differing among wetlands, and among sampling scenarios within individual and pooled wetlands (Table 3).

Adult *A. quercicus* (Fig. 3) population trends were negative at wetland 3 and pooled wetlands under the All-yr, 2-yr, and RSS scenarios, and at wetlands 1, 2, and pooled wetlands under the 5-yr scenario; trends at other wetlands, pooled wetlands, and scenarios were non-significant. Overlapping CI's among all significant wetland-scenario combinations indicated that they did not statistically differ (Table 3). Statistical power (negative 0.05 effect size) ranged from 0.030 to 0.461 among all individual wetlands and scenarios except at wetland 1 under the 5-yr scenario, where power was 1.000 (Table 3). Statistical power for pooled wetlands ranged from 0.092 to 0.555 among scenarios (Table 3; Fig. 4). Power curves for pooled wetlands were adequate for detecting a negative 0.10 change for all scenarios except R10. Power generally increased with the number of years included in the different sampling scenarios, with the exception of the R10 scenario ($n = 10$) which remained much lower than the 5-yr scenario ($n = 5$) for all tested effect sizes. Kendall's tau rank correlations indicated no significant relationship between juvenile recruitment and adult *A. quercicus* populations during any of the subsequent eight years.

Adult *A. terrestris* (Fig. 3) population trends were positive at wetland 7 under the All-yr scenario, at wetlands 2, 5, 6, and pooled wetlands under the 2-yr scenario, and at wetland 5 under the 5-yr scenario; trends at other wetlands, pooled wetlands, and scenarios were non-significant (Table 3). Overlapping CI's among all significant wetland-scenario combinations indicated that they did not statistically differ (Table 3). Statistical power (negative 0.05 effect size) ranged from 0.022 to 0.827 among wetlands and scenarios, and was generally higher for individual wetlands under the All-yr, 2-yr, and RSS scenarios than the 5-yr or R10 scenarios (Table 3). Statistical power for pooled wetlands ranged from 0.198 to 0.986 among scenarios, and was also higher for All-yr, 2-yr, and RSS scenarios (≥0.791) than the 5-yr and R10 scenarios (≤0.362). Power curves for pooled wetlands were adequate for detecting a negative 0.05 change for all scenarios except 5-yr and R10. Power generally increased with the number of years included in the different sampling scenarios, with the exception of the R10 scenario ($n = 10$) which was lower than the 5-yr scenario ($n = 5$) for all tested effect sizes. Kendall's tau rank correlations indicated no relationship between juvenile recruitment and adult *A. terrestris* populations during any of the subsequent eight years (Table 4).

Adult *Gastrophryne carolinensis* (Fig. 3) population trends were significant only under the R10 scenario, at all individual wetlands except 7, and for pooled wetlands; all significant trends were positive, and overlapping CI's indicated that they did not differ from one another (Table 3). Statistical power ranged from 0.019 to 0.588 among wetlands and scenarios and was generally higher under the All-yr, 2-yr, and RSS scenarios than the 5-yr or R10 scenarios at all wetlands (Table 3). Power models for six of the seven study wetlands did not converge under scenario R10, the only scenario with significant population

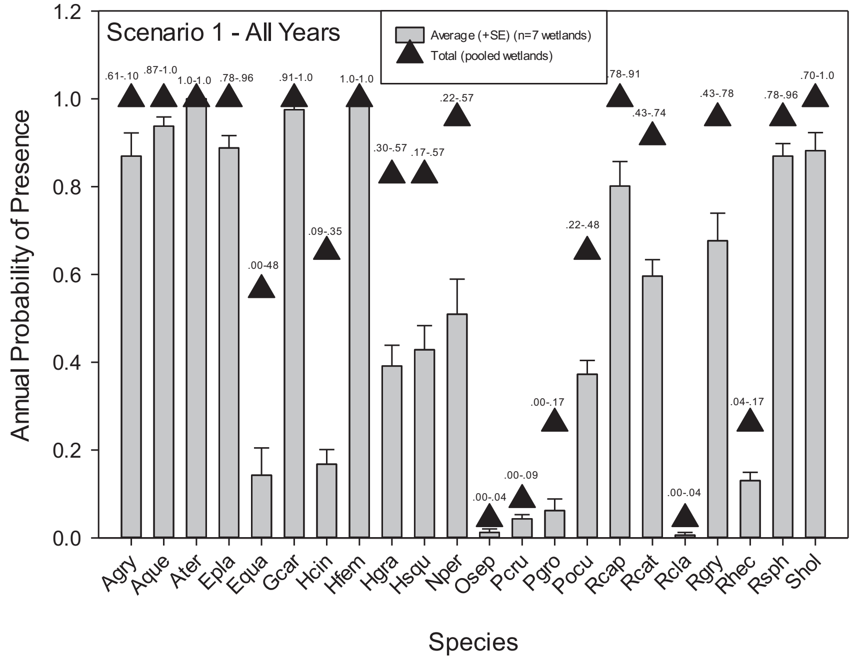

Fig. 2. Average (+SE; $n = 7$ wetlands), range (numbers), and total (pooled wetlands) annual probability of presence for each of 22 amphibian species captured at seven ephemeral wetlands with drift fences and pitfall traps, Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017, except 2005). See Table 2 for scientific names of species.

trends. Statistical power (0.05 effect size) for pooled wetlands ranged from 0.016–0.586 among scenarios and was highest for the All-yr and RSS scenarios. Power curves for pooled wetlands were adequate for detecting a 0.10 change for all scenarios except 5-yr and R10.The pooled R10 scenario power curve could not be completed over the range of effect sizes above negative 0.06 due to the extremely low intercept parameter estimate of 0.6678 coupled with larger negative effect sizes, which resulted in many simulated samples consisting of only zero values. This terminated so many PROC GLIMMIX fits that it was not feasible to obtain such power estimates. Power curves generally increased with the number of years included in the different sampling scenarios. Kendall's tau rank correlations indicated a significant, positive relationship between juvenile recruitment and adult *G. carolinensis* populations during each of the subsequent 4 years (Table 4).

Adult *R. capito* (Fig. 3) population trends were negative at wetlands 2, 8, and pooled wetlands under the All-yr and 2-yr scenario, and at wetland 8 under the RSS scenario; trends at other wetlands, pooled wetlands, and scenarios were nonsignificant. Overlapping CI's among

Table 2

Least squares means (± SE) and results of one-way ANOVA comparing the annual probability of presence for all captured amphibian species among five sampling scenarios: (1) all years (All-yr); (2) alternate-years, beginning with 1994 (2-yr); (3) 5-year interval, starting with 1994 (5-yr); (4) all years, with a randomly selected start- and stop year (1996–2016; RSS), and; (5) a randomly selected span of 10 years (2007–2016; R10), based on continuous (1994–2017)[a], concurrent drift fence trapping at seven isolated, ephemeral wetlands, Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017). Different letters within a row indicate significant differences among sampling scenarios.

Species	Sampling scenario					*p*-Value
	All-yr	2-yr	5-yr	RSS	R10	
Acris gryllus	0.870 ± 0.063A	0.869 ± 0.063A	0.914 ± 0.063A	0.850 ± 0.063A	0.743 ± 0.063B	0.0008
Anaxyrus quercicus	0.938 ± 0.028	0.929 ± 0.028	0.914 ± 0.028	0.929 ± 0.028	0.929 ± 0.028	0.9378
A. terrestris	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	–[b]
Eleutherodactylus planirostris	0.888 ± 0.041A	0.905 ± 0.041A	0.943 ± 0.041A	0.871 ± 0.041A	0.743 ± 0.041B	< 0.0001
Eurycea quadridigitata	0.143 ± 0.072AB	0.167 ± 0.072 AC	0.257 ± 0.072A	0.093 ± 0.072BC	0.029 ± 0.072B	0.0007
Gastrophryne carolinensis	0.975 ± 0.021	0.952 ± 0.021	0.971 ± 0.021	0.971 ± 0.021	0.971 ± 0.021	0.7997
Hyla cinerea	0.168 ± 0.049	0.226 ± 0.049	0.114 ± 0.049	0.193 ± 0.049	0.186 ± 0.049	0.1916
H. femoralis	0.994 ± 0.014	0.988 ± 0.014	0.971 ± 0.014	1.000 ± 0.014	1.000 ± 0.014	0.4269
H. gratiosa	0.391 ± 0.049	0.393 ± 0.049	0.343 ± 0.049	0.400 ± 0.049	0.357 ± 0.049	0.6233
H. squirella	0.429 ± 0.068A	0.440 ± 0.068AB	0.37 ± 0.068A	0.393 ± 0.068A	0.571 ± 0.068B	0.0032
Notophthalmus perstriatus	0.509 ± 0.090A	0.512 ± 0.090A	0.486 ± 0.090A	0.507 ± 0.090A	0.343 ± 0.090B	0.0025
Osteopilus septentrionalis	0.012 ± 0.012	0.024 ± 0.012	0.000 ± 0.012	0.014 ± 0.012	0.029 ± 0.012	0.0781
Plethodon grobmani	0.062 ± 0.024	0.071 ± 0.024	0.029 ± 0.024	0.050 ± 0.024	0.000 ± 0.024	0.0577
Pseudacris crucifer	0.044 ± 0.005A	0.000 ± 0.005B	0.000 ± 0.005B	0.043 ± 0.005A	0.000 ± 0.005B	< 0.0001
P. ocularis	0.373 ± 0.054A	0.393 ± 0.054A	0.486 ± 0.054A	0.336 ± 0.054A	0.129 ± 0.054B	0.0004
Rana capito	0.801 ± 0.061	0.810 ± 0.061	0.829 ± 0.061	0.800 ± 0.061	0.729 ± 0.061	0.0623
R. catesbeianus	0.596 ± 0.056A	0.607 ± 0.056A	0.600 ± 0.056A	0.593 ± 0.056A	0.386 ± 0.056B	< 0.0001
R. clamitans	0.006 ± 0.009	0.012 ± 0.009	0.000 ± 0.009	0.007 ± 0.009	0.014 ± 0.009	0.4269
R. gryllio	0.677 ± 0.062A	0.655 ± 0.062A	0.657 ± 0.062A	0.679 ± 0.062A	0.529 ± 0.062B	0.0001
R. heckscheri	0.130 ± 0.027	0.107 ± 0.027	0.114 ± 0.027	0.107 ± 0.027	0.057 ± 0.027	0.0649
R. sphenocephalus	0.870 ± 0.032AB	0.940 ± 0.032A	0.943 ± 0.032A	0.850 ± 0.032BC	0.786 ± 0.032C	< 0.0001
Scaphiopus holbrookii	0.882 ± 0.051	0.893 ± 0.051	0.800 ± 0.051	0.864 ± 0.051	0.914 ± 0.051	0.0539

[a] 2005 omitted from analyses for all species due to prolonged flooding at most wetlands.

[b] Probability of presence was 1.0 for all sampling scenarios, so no statistical test could be performed.

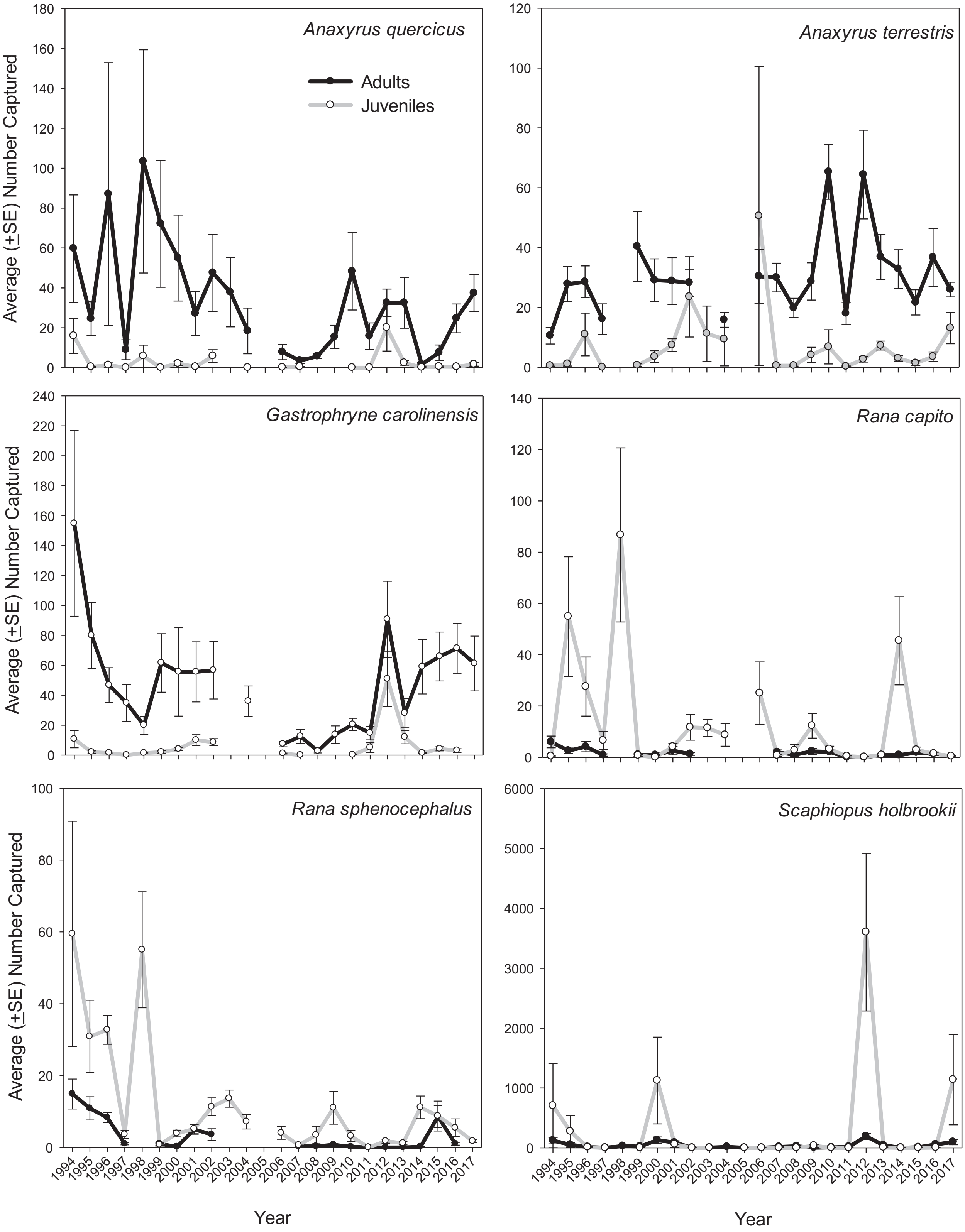

Fig. 3. Average (+SE) annual number of individual adult and juvenile *Anaxyrus quercicus*, *A. terrestris*, *Gastrophryne carolinensis*, *Rana capito*, *R. sphenocephalus*, and *Scaphiopus holbrookii* captured ephemeral (n = 7) wetlands based on continuous, concurrent sampling with drift fences and pitfall traps in the Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017). Averages here include only years when data for all seven wetlands were included, which differed among species and age-classes (see Table 1).

all significant wetland-scenario combinations indicated that they did not statistically differ (Table 3). Statistical power (negative 0.05 effect size) ranged from 0.000 to 0.677 among individual wetlands and scenarios (Table 3). Several power models did not converge, likely due to multiple years when few or no *R. capito* adults were captured. Statistical power for pooled wetlands ranged from 0.099 to 0.629 among

Table 3
Estimated slope and 95% confidence intervals (first line) when significant, number of years used (*n*) and statistical power for a negative 0.05 effect size (second line) of significant[a] ($p \le 0.05$) adult population trends of six focal anuran species, at each of seven wetlands (1, 2, 3, 5, 6, 7, 8) and pooled wetlands over a 24-year period (1994–2017)[b], using five sampling scenarios: (1) all years (1994–2017; All-yr); (2) alternate-years starting 1994 (2-yr); (3) 5-year interval starting 1994 (5-yr); (4) all years, with a randomly selected start- and stop year (1996–2016; RSS), and; (5) a randomly selected span of 10 years (2007–2016; R10), Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017).

Wetland No.	Sampling scenario				
	All-yr	2-yr	5-yr	RSS	R10
Anaxyrus quercicus					
1	NS	NS	−0.337 (−0.426, −0.247)	NS	NS
	n = 23; power = 0.259	*n* = 12; power = 0.150	*n* = 5; power = 1.000	*n* = 20; power = 0.184	*n* = 10; power model NC
2	NS	NS	−0.275 (−0.463, −0.087)	NS	NS
	n = 23; power = 0.228	*n* = 12; power = 0.139	*n* = 5; power = 0.231	*n* = 20; power = 0.160	*n* = 10; power model NC
3	−0.100 (−0.171, −0.028)	−0.111 (−0.213, −0.008)	NS	−0.132 (−0.217, −0.047)	NS
	n = 23; power = 0.277	*n* = 12; power = 0.195	*n* = 5; power = 0.134	*n* = 20; power = 0.230	*n* = 10; power = 0.087
5	NS	NS	NS	NS	NS
	n = 23; power = 0.242	*n* = 12; power = 0.162	*n* = 5; power = 0.156	*n* = 20; power = 0.166	*n* = 10; power = 0.043
6	NS	NS	NS	NS	NS
	n = 23; power = 0.461	*n* = 12; power = 0.253	*n* = 5; power = 0.140	*n* = 20; power = 0.337	*n* = 10; power = 0.079
7	NS	NS	NS	NS	NS
	n = 23; power = 0.197	*n* = 12; power = 0.122	*n* = 5; Power model NC	*n* = 20; power = 0.143	*n* = 10; power = 0.083
8	NS	NS	NS	NS	NS
	n = 23; power = 0.313	*n* = 12; power = 0.127	*n* = 5; power = 0.090	*n* = 20; power = 0.271	*n* = 10; power = 0.030
Pooled	−0.052 (−0.097, −0.008)	−0.076 (−0.143, −0.008)	−0.164 (−0.284, −0.044)	−0.074 (−0.130, −0.019)	NS
	n = 23; power = 0.555	*n* = 12; power = 0.338	*n* = 5; power = 0.313	*n* = 20; power = 0.419	*n* = 10; power = 0.092
Anaxyrus terrestris					
1	NS	NS	NS	NS	NS
	n = 22; power = 0.684	*n* = 11; power = 0.375	*n* = 5; power = 0.244	*n* = 19; power = 0.501	*n* = 10; power = 0.094
2	NS	+0.074 (+0.014, 0.134)	NS	NS	NS
	n = 22; power = 0.693	*n* = 11; power = 0.409	*n* = 5; power = 0.152	*n* = 19; power = 0.545	*n* = 10; power = 0.107
3	NS	NS	NS	NS	NS
	n = 23; power = 0.815	*n* = 12; power = 0.426	*n* = 5; power = 0.167	*n* = 20; power = 0.640	*n* = 10; power = 0.183
5	NS	+0.049 (+0.002, 0.096)	+0.090 (+0.001, 0.179)	NS	NS
	n = 23; power = 0.693	*n* = 12; power = 0.451	*n* = 5; power = 0.080	*n* = 20;power = 0.521	*n* = 10; power = 0.070
6	NS	+0.065 (+0.009, 0.121)	NS	NS	NS
	n = 23; power = 0.785	*n* = 12; power = 0.404	*n* = 5; power = 0.136	*n* = 20; power = 0.660	*n* = 10; power = 0.151
7	+0.058 (+0.012, 0.105)	NS	NS	NS	NS
	n = 22; power = 0.584	*n* = 12; power = 0.417	*n* = 5; power = 0.022	*n* = 19; power = 0.502	*n* = 10; power = 0.173
8	NS	NS	NS	NS	NS
	n = 23; power = 0.827	*n* = 12; power = 0.756	*n* = 5; power = 0.168	*n* = 20; power = 0.720	*n* = 10; power = 0.127
Pooled	NS	+0.047 (+0.006, 0.087)	NS	NS	NS
	n = 21; power = 0.986	*n* = 11; power = 0.791	*n* = 5; power = 0.362	*n* = 18; power = 0.953	*n* = 1; power = 0.198
Gastrophryne carolinensis					
1	NS	NS	NS	NS	+0.284 (0.063, 0.505)
	n = 23; power = 0.418	*n* = 12; power = 0.251	*n* = 5; power = 0.111	*n* = 20; power = 0.326	*n* = 10; power model NC
2	NS	NS	NS	NS	+0.311 (0.094, 0.528)
	n = 23; power = 0.456	*n* = 12; power = 0.195	*n* = 5; power = 0.411	*n* = 20; power = 0.301	*n* = 10; Power model NC
3	NS	NS	NS	NS	+0.324 (0.045, 0.603)
	n = 23; power = 0.376	*n* = 12; power = 0.210	*n* = 5; power = 0.588	*n* = 20; power = 0.286	*n* = 10; power model NC
5	NS	NS	NS	NS	+0.284 (0.104, 0.461)
	n = 23; power = 0.587	*n* = 12; power = 0.291	*n* = 5; power = 0.217	*n* = 20; power = 0.457	*n* = 10; power model NC
6	NS	NS	NS	NS	+0.231 (0.029, 0.434)
	n = 23; power = 0.512	*n* = 12; power = 0.290	*n* = 5; power = 0.123	*n* = 20; power = 0.356	*n* = 10; power model NC
7	NS	NS	NS	NS	NS
	n = 22; power = 0.458	*n* = 12; power = 0.239	*n* = 5; power = 0.066	*n* = 19; power = 0.292	*n* = 10; power = 0.029
8	NS	NS	NS	NS	+0.234 (0.118, 0.349)
	n = 23; power = 0.336	*n* = 12; power = 0.174	*n* = 5; power = 0.095	*n* = 20; power = 0.219	*n* = 10; Power model NC
Pooled	NS	NS	NS	NS	+0.267 (0.111, 0.423)
	n = 22; power = 0.586	*n* = 12; power = 0.288	*n* = 5; power = 0.241	*n* = 19; power = 0.453	*n* = 10; power = 0.016
Rana capito					
1	NS	NS	NS	NS	NS
	n = 19; power = 0.259	*n* = 9; power = 0.173	*n* = 4; power = 0.164	*n* = 17; power = 0.171	*n* = 10; power model NC
2	−0.174 (−0.271, −0.077)	−0.272 (−0.444, −0.100)	NS	NM	NS
	n = 20; power = 0.479	*n* = 9; power = 0.677	*n* = 4; power = 0.096	power model NC	*n* = 10; power model NC
3	NS	NS	NS	NS	NS
	n = 22; power model NC	*n* = 11; Power model NC	*n* = 4; Power model NC	*n* = 19; Power model NC	*n* = 10; power = 0.000
5	NS	NS	NS	NS	NS
	n = 23; power = 0.233	*n* = 12; power = 0.166	*n* = 5; power = 0.038	*n* = 20; power = 0.044	*n* = 10; Power model NC
6	NS	NS	NS	NS	NS
	n = 22; power = 0.327	*n* = 11; power = 0.185	*n* = 4; Power model NC	*n* = 19; power = 0.193	*n* = 10; power = 0.110
7	NS	NS	NS	NS	NS
	n = 21; power = 0.072	*n* = 11; power model NC	*n* = 4; Power model NC	*n* = 18; power = 0.054	*n* = 10; power = 0.101
8	−0.147 (−0.235, −0.060)	−0.148 (−0.256, −0.041)	NS	−0.158 (−0.264, −0.052)	NM
	n = 22; power = 0.333	*n* = 11; power = 0.256	*n* = 4; Power model NC	*n* = 19; power = 0.255	power model NC

(*continued on next page*)

Table 3 (*continued*)

Wetland No.	Sampling scenario				
	All-yr	2-yr	5-yr	RSS	R10
Pooled	−0.055 (−0.102, −0.007) *n* = 18; power = 0.629	−0.077 (−0.143, −0.011) *n* = 9; power = 0.367	NS *n* = 4; power = 0.247	NS *n* = 16; power = 0.424	NS *n* = 10; power = 0.099
Rana sphenocephalus					
1	−0.106 (−0.192, −0.020) *n* = 19; power = 0.225	−0.230 (−0.380, −0.080) *n* = 9; power = 0.244	NS *n* = 4; power = 0.409	NS *n* = 17; power = 0.135	NS *n* = 10; power model NC
2	−0.105 (−0.198, −0.013) *n* = 20; power = 0.189	−0.250 (−0.458, −0.043) *n* = 9; power = 0.438	NS *n* = 4; power = 0.253	NS *n* = 17; power = 0.091	NS *n* = 10; power model NC
3	−0.195 (−0.291, −0.099) *n* = 22; power = 0.535	−0.256 (−0.379, −0.133) *n* = 11; power = 0.761	NS *n* = 4; power = 0.063	−0.188 (−0.317, −0.059) *n* = 19; power = 0.265	NS *n* = 10; power model NC
5	NS *n* = 23; power = 0.172	−0.169 (−0.264, −0.074) *n* = 12; power = 0.361	NS *n* = 5; power = 0.129	NS *n* = 20; power = 0.141	NS *n* = 10; power model NC
6	NS *n* = 22; power = 0.175	−0.129 (−0.226, −0.032) *n* = 11; power = 0.234	NM power model NC	NS *n* = 19; power = 0.140	+0.744 (0.072, 1.416) *n* = 10; Power model NC
7	NS *n* = 21; power = 0.125	NS *n* = 11; power = 0.098	NS *n* = 4; power model NC	NS *n* = 18; power = 0.088	NS *n* = 10; Power model NC
8	−0.129 (−0.208, −0.049) *n* = 22; power = 0.351	NS *n* = 11; power = 0.191	NS *n* = 4; Power model NC	−0.088 (−0.175, −0.001) *n* = 19; power = 0.260	NS *n* = 10; Power model NC
Pooled	NS *n* = 18; power = 0.203	−0.160 (−0.266, −0.055) *n* = 9; power = 0.206	NS *n* = 4; power = 0.199	NS *n* = 16; power = 0.154	NS *n* = 10; power model NC
Scaphiopus holbrookii					
1	NS *n* = 24; power = 0.124	NS *n* = 12; power = 0.146	−0.446 (−0.718, −0.175) *n* = 5; power = 0.410	NS *n* = 21; power = 0.109	NS *n* = 10; Power model NC
2	NS *n* = 24; power = 0.146	NS *n* = 12; power = 0.117	−0.268 (−0.491, −0.045) *n* = 5; power = 0.420	NS *n* = 21; power = 0.109	NS *n* = 10; Power model NC
3	NS *n* = 24; power = 0.204	NS *n* = 12; power = 0.151	NS *n* = 5; power = 0.084	NS *n* = 21; power = 0.157	NS *n* = 10; power = 0.019
5	NS *n* = 24; power = 0.235	NS *n* = 12; power = 0.137	NS *n* = 5; power = 0.120	NS *n* = 21; power = 0.174	NS *n* = 10; power = 0.066
6	NS *n* = 24; power = 0.214	NS *n* = 12; power = 0.139	−0.273 (−0.396, −0.151) *n* = 5; power = 0.325	NS *n* = 21; power = 0.162	NS *n* = 10; power = 0.067
7	NS *n* = 24; power = 0.239	NS *n* = 12; power = 0.195	NS *n* = 5; Power model NC	NS *n* = 21; power = 0.199	NS *n* = 10; power = 0.102
8	NS *n* = 24; power = 0.172	NS *n* = 12; power = 0.112	NS *n* = 5; power = 0.093	+0.129 (+0.012, 0.245) *n* = 21; power = 0.103	NS *n* = 10; Power model NC
Pooled	NS *n* = 24; power = 0.233	NS *n* = 12; power = 0.176	−0.219 (−0.321, −0.118) *n* = 5; power = 0.348	NS *n* = 21; power = 0.184	NS *n* = 10; power = 0.080

[a] NS denotes non-significant; NC denotes non-convergence, indicating that the power calculation could not be completed due to model fitting problems related to the simulated data that lacked sufficient variability for numerous replications; NM denotes no model, as data were to sparse to fit a mode.

[b] See Table 1 for years omitted from regression analyses for a given wetland or pooled wetlands, for each species (adults only).

scenarios and was highest for the All-yr scenario. Power curves for pooled wetlands were adequate for detecting a 0.10 change for all scenarios except 5-yr and R10. Power generally increased with the number of years included in the different sampling scenarios, with the exception of the R10 scenario which remained much lower than the 5-yr scenario ($n = 4$) for all tested effect sizes. Kendall's tau rank correlations indicated a significant, positive correlation between juvenile recruitment and adult *R. capito* populations 5 and 6 years later, but no relationship for other tested years (Table 4).

Adult *R. sphenocephalus* (Fig. 3) population trends were negative at wetlands 1, 2, 3, and 8 under the All-yr scenario, at wetlands 1, 2, 3, 5, 6, and pooled wetlands under the 2-yr scenario, and at wetlands 3 and 8 under the RSS scenario, but significantly positive at wetland 6 under the R10 scenario; trends at other wetlands, pooled wetlands, and scenarios were nonsignificant. Confidence intervals indicated that negative population trends did not differ from one another, but differed from the single positive trend at wetland 6 (Table 3). Statistical power ranged from 0.063 to 0.761 among individual wetlands and scenarios (Table 3); power models under the R10 scenario did not converge, likely due to multiple years when few or no *R. sphenocephalus* adults were captured. Statistical power (negative 0.05 effect size) for pooled wetlands ranged from 0.154 to 0.206 among tested scenarios (the power model for R10 scenario was not obtainable). Power curves for pooled wetlands were poor for all scenarios until the size effect became at least 0.15, and generally increased with the number of years included in the different sampling scenarios. Kendall's tau rank correlations indicated a significant, positive correlation between juvenile recruitment and adult *R. sphenocephalus* populations 1, 2, 3, 5, 6, and 7 years later (Table 4).

Adult *S. holbrookii* (Fig. 3) population trends were negative at wetlands 1, 2, 6, and pooled wetlands under the 5-yr scenario, and positive at wetland 8 under the RSS scenario. Confidence intervals overlapped among all negative population trends, but none overlapped with the single positive trend (Table 3). Statistical power (0.05 effect size) ranged from 0.019 to 0.420 among individual wetlands and scenarios (Table 3). Statistical power for pooled wetlands ranged from 0.080 to 0.348 among scenarios. Power curves for pooled wetlands were adequate for detecting a 0.10 change only for the All-yr and 5-yr scenarios. The 5-yr scenario ($n = 5$) power curve was roughly equivalent to the All-yr scenario ($n = 24$); the R10 scenario ($n = 10$) was much lower all other scenarios across all tested effect sizes. Kendall's tau rank correlations indicated a significant, negative relationship between juvenile recruitment and adult *S. holbrookii* populations 2 years later, a positive relationship 4 years later, and no relationship during other tested years (Table 4).

4. Discussion

Our results illustrate how estimates of species richness, annual probability of each species' presence, and population trend estimates can differ among similar wetlands within a landscape, and according to the interval or span of years sampled. In our study, none of the seven

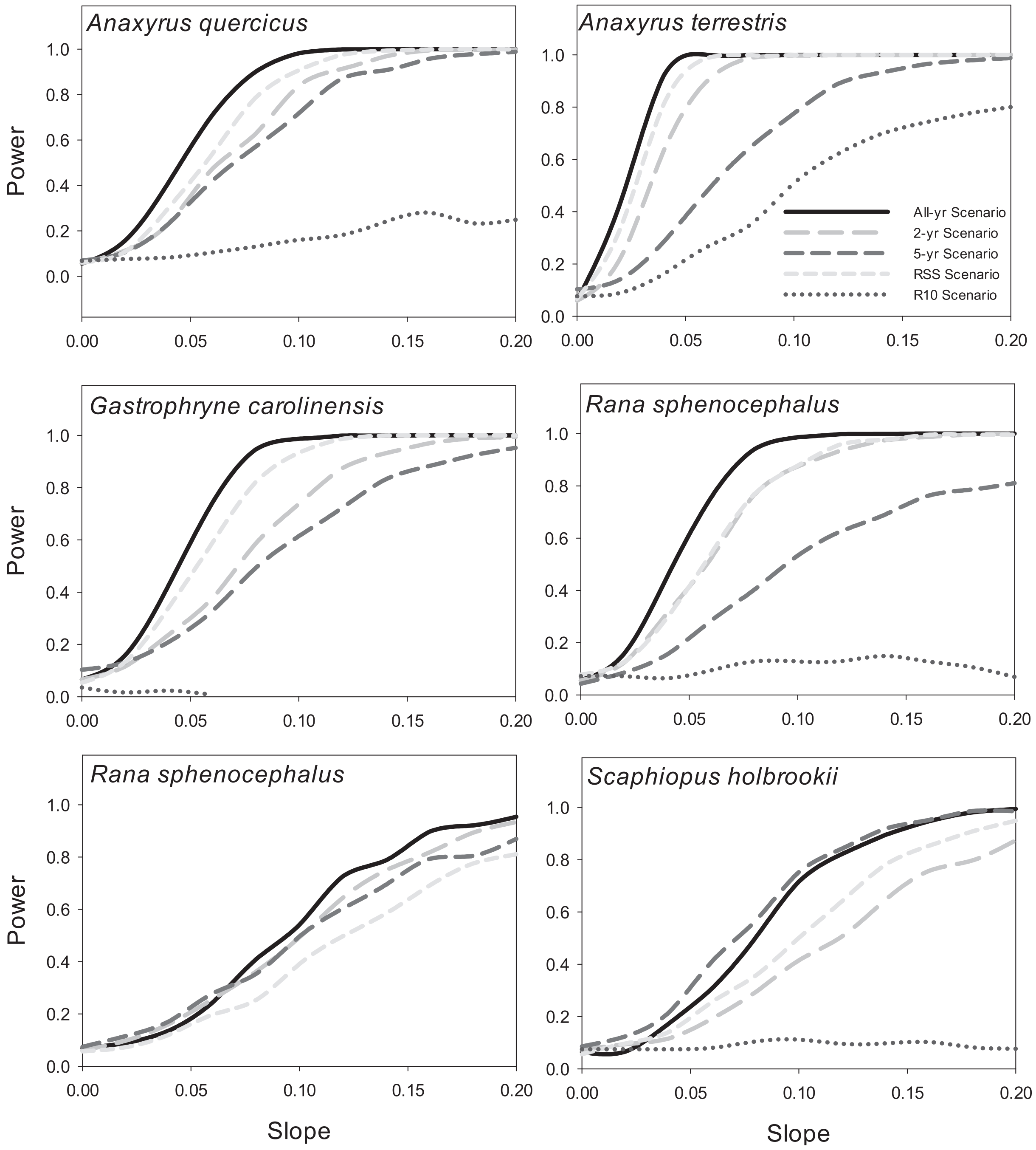

Fig. 4. Simulated power curves for *Anaxyrus quercicus*, *A. terrestris*, *Gastrophryne carolinensis*, *Rana capito*, *R. sphenocephalus*, and *Scaphiopus holbrookii* using pooled ($n = 7$) wetlands under five sampling scenarios: (1) all years (1994–2017; All-yr); (2) alternate-years starting 1994 (2-yr); (3) 5-year interval starting 1994 (5-yr); (4) all years, with a randomly selected start- and stop year (1996–2016; RSS), and; (5) a randomly selected span of 10 years (2007–2016; R10), Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017).

individual wetlands captured all 22 amphibian species trapped within the study period (all years and wetlands), and only 29–89% of total species ever captured in a given wetland (all years) was captured in any single year. Two additional amphibian species, *Amphiuma means* and *Pseudacris nigrita*, were captured at an eighth wetland within the study area (wetland 4; omitted from analyses due to prolonged flooding during several years), indicating that even 24 years of monitoring at multiple wetlands may not detect all species known to occur within a landscape. Additionally, the non-native invasive *Osteopilus septentrionalis* was first captured in the 23rd sampled year (2016), indicating it was recently introduced. Our results highlight the value of long-term monitoring at multiple sites for assessing biodiversity and detecting new potential threats.

In our study, average annual probability of presence based on all 24 years of data (All-yr scenario) ranged from 100% in all seven wetlands for abundant, commonly captured species such as *A. terrestris* and *H. femoralis*, to < 1% for infrequently captured (e.g., *P. crucifer* and *R. clamitans*) or recently introduced species (e.g., *O. septentrionalis*). Probabilities of presence increased for pooled wetlands, suggesting that presence should be monitored at multiple wetlands to better gauge amphibian persistence at a landscape level. Not surprisingly, sampling regime had little effect on the probability of presence for most commonly captured species (e.g., *A. quercicus*, *A. terrestris*, *G. carolinensis*, and *H. femoralis*) (e.g., MacKenzie and Bailey, 2004) but significantly

Table 4
Kendall's tau correlations between the number of juvenile recruits and the number of adults in the following eight years for six focal anuran species using data from seven wetlands during a 24-year period (1994–2017; $n = 168$ maximum possible pairs; for 1-year correlations)[a], Ocala National Forest, Marion and Putnam Counties, Florida (1994–2017).

Species	1-year	2-years	3-years	4-years	5-years	6-years	7 years	8 years
Anaxyrus quercicus	$p = 0.3348$ $n = 144$	$p = 0.1273$ $n = 138$	$p = 0.7422$ $n = 130$	$p = 0.0525$ $r = +0.13614$ $n = 123$	$p = 0.5552$ $n = 116$	$p = 0.8207$ $n = 109$	$p = 0.3291$ $n = 102$	$p = 0.0573$ $r = +0.15402$ $n = 95$
A. terrestris	$p = 0.2536$ $n = 142$	$p = 0.5949$ $n = 135$	$p = 0.6343$ $n = 128$	$p = 0.5638$ $n = 121$	$p = 0.4420$ $n = 116$	$p = 0.3194$ $n = 109$	$p = 0.4396$ $n = 104$	$p = 0.6240$ $n = 95$
Gastrophryne carolinensis	$p < 0.0001$ $r = +0.39426$ $n = 144$	$p < 0.0001$ $r = +0.29580$ n-131	$p < 0.0001$ $r = +0.32964$ $N = 123$	$p = 0.0015$ $r = +0.21726$ $n = 116$	$p = 0.2197$ $n = 109$	$p = 0.6497$ $n = 102$	$p = 0.3310$ $n = 95$	$p = 0.9641$ $n = 88$
Rana capito	$p = 0.0545$ $r = +0.13103$ $n = 137$	$p = 0.9540$ $n = 128$	$p = 0.5685$ $n = 121$	$p = 0.2484$ $n = 114$	$p = 0.0005$ $r = +0.26781$ $n = 109$	$p = 0.0049$ $r = +0.22159$ $n = 102$	$p = 0.7033$ $n = 95$	$p = 0.7136$ $n = 88$
R. sphenocephalus	$p < 0.0001$ $r = +0.38978$ $n = 137$	$p = 0.0341$ $r = +0.14536$ $n = 128$	$p = 0.0113$ $r = +0.18032$ $n = 121$	$p = 0.4536$ $n = 114$	$p = 0.0419$ $r = +0.15193$ $n = 109$	$p = 0.0006$ $r = +0.26492$ $n = 102$	$p = 0.0397$ $r = +0.16340$ $n = 95$	$p = 0.2985$ $n = 88$
Scaphiopus holbrookii	$p = 0.1058$ $n = 158$	$p = 0.0055$ $\mathrm{r} = -0.18129$ $n = 151$	$p = 0.1527$ $n = 144$	$p = 0.0011$ $r = +0.22134$ $n = 137$	$p = 0.4711$ $n = 130$	$p = 0.6225$ $n = 123$	$p = 0.4912$ $n = 116$	$p = 0.5289$ $n = 109$

[a] See Table 1 for years when data for either adults or juveniles was omitted for a given wetland, year, and species.

affected estimates for some less common (e.g., *Eurycea quadridigitata and P. crucifer*) and even some commonly captured (e.g., *A. gryllus* and *R. sphenocephalus*) species. The R10 often differed from the other sampling scenarios, suggesting that even 10 years may yield different results regarding probability of presence than longer-term monitoring (e.g., 24 years). Nonetheless, even significant differences in species' probability of presence among sampling scenarios were relatively minor. Other studies based on "snapshot" (one to a few visits annually) aquatic sampling also show inconsistent presence of amphibian species at breeding wetlands among years. Werner et al. (2007) reported that only about half of the larval amphibian species that could be present in a given wetland were in fact detected in any given year, based on sampling 37 wetlands for two days each summer for seven years. Skelly et al. (2003) reported that larval presence/absence-based estimates of amphibian declines and distributional changes are greatly reduced with increased number of years included in resurvey efforts. Greenberg et al. (2017a) found that larval amphibian species were often detected intermittently within wetlands even within a single breeding season, likely due to high mortality followed by deposition of new eggs and tadpole cohorts. Thus, probability of presence estimates in studies with intermittent sampling is likely influenced not only by the specific years, but also the specific days that are sampled. Our intensive, continuous, year-round terrestrial sampling likely increased the likelihood of capture for most species in any given year, and likely reflected species presence more reliably than studies using "snapshot" sampling regimes.

In our study, population trend estimates for all six focal species were inconsistently significant or nonsignificant among wetlands under at least some of the same sampling scenarios, suggesting that individual wetlands function independently as population sinks or sources within the larger landscape. Perhaps more importantly, population trends for each of our six focal species were inconsistently significant or nonsignificant among sampling scenarios within the same wetlands. For example, *G. carolinensis* significantly increased over time at six of the seven individual wetlands and overall (pooled wetlands) when a randomly selected subset of 10 years was analyzed (R10 scenario), but results were nonsignificant when all 24 years or other subsets of years (scenarios) were analyzed. Similarly, we found a significantly declining adult *R. capito* population in wetland 2 and overall (pooled wetlands) when all years (1994–2017; All-yr scenario) or alternate years (2-yr scenario) were included in analyses, but nonsignificant trends when all years with a different start- and end date (1996–2016; RSS scenario) were included. Generally, the direction of significant adult population trends for a given species was in agreement among wetlands and scenarios (e.g., all significant *A. quercicus* population trends were negative; all significant *A. terrestris* population trends were positive), but not always. For example, at wetland 6 adult *R. sphenocephalus* population trends were negative under the 2-yr scenario, but positive under the R10 scenario. Pechmann and Wilbur (1994) provided several examples in addition to their own 12-year data (Pechmann et al., 1991), showing that conclusions of amphibian population declines or extinctions were sometimes reversed with additional years of data due to highly variable breeding populations among years. Our results corroborate these observations, and illustrate how even intensive, long-term amphibian population monitoring can yield inconsistent, or even contradictory results according to the year monitoring begins or ends, the span of years monitored or, when sampling is intermittent, according to the specific subsets of years analyzed.

Extreme fluctuation in breeding amphibian populations among years reduces the statistical power to detect the presence or absence of population trends (e.g., Hayes and Steidl, 1997). This problem is amplified by a paucity of long-term studies, making it difficult to gauge how many years of data are required to assess population trends with confidence (Blaustein et al., 1994; Marsh, 2001; Meyer et al., 1998; Pechmann and Wilbur, 1994; Reed and Blaustein, 1995). In our 24-year study, low statistical power (at a negative 0.05 effect size) for virtually all population trend analyses regardless of species, wetland, or sampling scenario, indicated that most regression results were inconclusive, even when significant. Further, statistical power for pooled wetlands was not consistently or substantially improved relative to estimates for individual wetlands or sampling scenarios. Instances of adequate (≥ 0.80; Hayes and Steidl, 1997) power were rare, and inconsistent among wetlands and scenarios for any given species (e.g., *A. quercicus* only at wetland 1 under the 5-yr scenario; *A. terrestris*, only at wetlands 3 and 8 under the All-yr scenario), suggesting they were artifacts of the subsets of years analyzed for those wetlands and sampling scenarios, rather than affirmations of specific sampling scenarios that could be replicated with confidence in other monitoring efforts.

Pooled wetland power curves for most species indicated that generally, power increased with sampling scenarios that included more years. An exception was the R10 scenario (10 continuous years), where power was substantially lower (or could not be obtained due to many simulated replications having all zero values) than all other sampling scenarios, including the 5-yr scenario ($n = 4$ or 5 years, at 5-year intervals). Another notable exception was the 5-yr scenario for *S. holbrookii*, where power was greater than other scenarios, and roughly similar to the All-yr scenario (24 continuous years). These examples illustrate that statistical power of population trend analyses for species with highly variable interannual abundance can vary based not only on the number of years, but also according to the specific span or subset of years sampled. Our results indicate that even 24 years of continuous, concurrent monitoring of multiple amphibian species at multiple wetlands was insufficient to infer population trends due to low statistical power.

Accurate assessment of population trends based on amphibian monitoring at wetlands is confounded by multiple factors contributing to extreme fluctuations in the number of breeding adults among years and wetlands, thereby reducing the statistical power to detect them. Wetlands are a “magnet” for wetland-breeding amphibians, and therefore may not reflect their true population sizes at the larger landscape level. Infrequent, unpredictable breeding behavior (Skelly et al., 2003), among-year shifts in adult “choice” of breeding wetlands within a landscape (e.g. Greenberg and Tanner, 2005), different rates of sexual maturation – hence breeding activity - between males and females, frequency of breeding by individuals within the same population (e.g., Hansen, 1958), and edaphic factors such as weather and hydroregime that can additionally influence annual breeding populations at wetlands (e.g., Greenberg et al., 2017b; Semlitsch et al., 1996). Additionally, juvenile recruitment, also highly variable among years and wetlands (e.g., Greenberg et al., 2017a; Semlitsch et al., 1996), could presumably influence adult populations in subsequent years.

In our study, juvenile recruitment was correlated with adult breeding populations in some subsequent years for some focal species, but not for *A. quercicus* and *A. terrestris*. Species with significant correlations differed in the number of years after juvenile recruitment when significant relationships with adult populations were found. For example, *G. carolinensis* juvenile recruitment was correlated with adult populations during the following four years, whereas *R. capito* juvenile recruitment was correlated with adult populations only 5 and 6 years later, *R. sphenocephalus* juvenile recruitment was correlated with adult populations during most subsequent years, and *S. holbrookii* juvenile recruitment was negatively correlated with adults 2 years later, but positively correlated 4 years later. Differences among species likely reflected life history differences, such as age at sexual maturity and longevity. Within species, relationships between juvenile recruitment and adult breeding populations in subsequent years are likely confounded by differences in rates of juvenile survival to adulthood among years and wetlands, in addition to factors influencing adult breeding populations, discussed above. Semlitsch et al. (1996) reported positive relationships between juvenile recruits and the breeding adult population in subsequent years for 5 of 12 amphibian species, and positive trends for most others over 16 years at a single wetland. Berven (1990) found that variation in adult breeding populations of *R. sylvatica* was largely due to variation in juvenile recruitment one (for males) or two (for females) years prior. Beebee et al. (1996) reported a positive correlation between breeding adult *Bufo calamita* and average toadlet production over the previous decade. Our results indicate that potential relationships between juvenile recruitment and adult breeding populations in subsequent years can be detected for some amphibian species, but relationships may be confounded by multiple life history and edaphic factors.

5. Conclusion

Widespread destruction of breeding wetlands and the surrounding uplands required by many amphibians renders it irrefutable that populations of many species are declining (Ficetola et al., 2015); disease and multiple environmental stressors acting at local or regional scales are also driving continental or worldwide declines (Blaustein et al., 2011; Grant et al., 2016; Houlahan et al., 2000). We emphasize that our study does not prove or disprove that amphibian populations within our study area are declining, increasing, or stable, but rather illustrates the challenges in finding definitive trends for breeding populations that fluctuate widely among wetlands and from year to year. Our results illustrate how species richness, annual probability of presence, and population trend estimates can differ among wetlands, and according to the span of years monitored or the specific subsets of years analyzed. Additionally, we show that 24 years of intensive, continuous, concurrent monitoring of multiple amphibian species at multiple wetlands was insufficient to conclude that population trends did, or did not, exist due to low statistical power. Clearly, long-term monitoring at multiple wetlands has value in assessing biodiversity, detecting potential threats, and gauging amphibian species' persistence. However, our results indicate that amphibian monitoring at wetlands cannot conclusively gauge population trends at the landscape level due to multiple factors affecting their abundance among years and wetlands.

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