

GRAMP Amphibian Diversity Summary for 2011–2015

Submitted to:

M. Segura and B. Mitchell

I&M Network:

Gulf Coast

Submitted by:

Ellen Cheng, University of Georgia

Date:

September 19, 2016

Contents

Introduction	2
Methods	3
Results	4
Overview	4
JELA	6
GUIS	12
SAAN	18
VICK	22
Discussion	28
Summary of findings	28
Detection probabilities	28
Literature cited	29
Appendix A1: Additional site-specific results	30
JELA	30
GUIS	33
SAAN	36
VICK	37
Appendix A2: Measuring and understanding species diversity	40
Hill diversity numbers: A unifying concept of species diversity	40
Relationships between common diversity indices and Hill diversities	41
Species richness	41
Shannon-Wiener index	41
Simpson diversity index	41
Berger-Parker index	42
Examples	42
Example A2.1	42
Example A2.2	44
Example A2.3	45
Literature cited	48
Appendix A3: Comparing diversities based on standardized sample coverage	49
Rarefaction, extrapolation, and sample coverage	49
Examples	49
Example A3.1	49
Example A3.2	52
Literature cited	54

Introduction

In 2011, the Gulf Coast Inventory and Monitoring Network (GULN) of the National Park Service implemented its reptile and amphibian monitoring protocol (GRAMP) at six network parks. GRAMP surveys use terrestrial cover-board (CB), arboreal PVC pipe (PVC), and/or aquatic minnow-trap (MT) fixed-point sampling to assess temporal trends in herp relative abundance and assemblage composition. At each park (one site per park), herps captured during monthly surveys are counted and recorded to species. Data from these surveys are not intended to provide park-wide inference on herp composition or trend, but rather to support local condition and change assessment at sites considered representative of particular habitat types, or sites of interest to management. This report synthesizes the first five years of GRAMP amphibian diversity data from four parks (Table 1).

Table 1. GULN parks with amphibian data summarized in this report, and number of each trap type. Monthly herp surveys are conducted with fixed numbers and locations of traps at each park (one site per park). In this report, sites are referenced by their park acronym. At least two trap types are deployed at each site: ‘# CB’=number of coverboard traps; ‘# PVCsets’=number of PVC sets (one large and one small pipe on a tree constitute a set); ‘# MT’=number of minnow traps

Acronym	Park name	# CB	# PVCsets	# MT
JELA	Jean Lafitte National Historical Park and Preserve	30	26	25
GUIS	Gulf Islands National Seashore	30	24	25
SAAN	San Antonio Missions National Historical Park	60	10	0
VICK	Vicksburg National Military Park	60	15	0

The diversity of species at a site, and temporal trends in diversity, can be characterized in various ways. One of the simplest measures of diversity is a count of species detected in a community, i.e., species richness. Species evenness is another aspect of diversity that is frequently incorporated in measures. For example, given two sites with the same number of species, the Shannon-Wiener diversity index will yield a lower diversity score for a site with the majority of individuals in a single species compared to a site with individuals more evenly distributed among species.

Many diversity indices commonly used in ecology, including species richness and the Shannon-Wiener index, are related through a family of diversity measures known as Hill diversity numbers:

$${}^qD = \left(\sum_{i=1}^S p_i^q \right)^{1/(1-q)}$$

In this equation, D is the Hill diversity number, S is the total number of species observed in the sample, p_i is the proportional abundance of the i^{th} species, and q is the “order” of diversity. A larger D means higher diversity.

A key point is that the order of diversity (q , which can be any number from 0 to Infinity) in this function indicates how sensitive that particular order of diversity D is to the richness versus evenness of a community. When $q = 0$, D is the species richness of a community—an index that depends only on number of species and is insensitive to the evenness of species distributions. When $q = 1$, D is the exponent of the Shannon-Wiener diversity index (hereafter referred to as “Shannon diversity”). When $q = 2$, D is inverse Simpson diversity, which is the most sensitive of the three to changes in species evenness. Higher orders of Hill diversities are related to other (less frequently used) indices that place increasingly greater emphasis on species evenness as a determinant of biological diversity.

Many measures of diversity, including Hill diversities, do not measure species replacement (“true” species turnover). That is, if a species disappears from a site and is replaced in similar abundance by a species that did not previously occur, the species richness and Shannon diversity of the site would not change although the composition of the species assemblage has changed. Dynamics indices have been developed specifically to characterize changes in species composition due to replacement (e.g., Romanuk and Kolasa 2001). Dynamics indices can be useful indicators of transience in communities with many rare or difficult-to-detect species.

Diversity and dynamics indices condense large amounts of data into summary values that, when tracked over time, can reveal trends in diversity. However, knowing that diversity is increasing or decreasing tells us little about what changes are actually

occurring in a community, why they are occurring, or what to do about it. For example, diversity may increase (because species evenness will increase) if a common species declines while others either remain unchanged or decline at a slower rate. Diversity indices by themselves are limited indicators of ecosystem health. Examining changes at the species level, and linking these to changes in environmental conditions, remains an important complement to monitoring diversity indices.

To understand amphibian dynamics at GRAMP sites we present a composite of Hill diversity statistics, dynamics indices, and complementary species-level summaries for each site. Appendix A1 provides additional site-specific details on patterns of species detections by trap type, season (breeding versus non-breeding), and day-of-year. Appendices A2 and A3 provide explanations of many of the diversity calculations presented in the main report.

Methods

We summarized GRAMP amphibian diversity data from four Parks (i.e., sites), for monitoring years 2011 through 2015 (a monitoring year begins on October 1 of the previous calendar year). The surveyed sites differ in habitat and are widely separated geographically, so are not expected to exhibit similarities in amphibian composition or dynamics. At all sites, fewer surveys were conducted in 2011, the first year of monitoring. In some years at some sites, more than 12 surveys were conducted during a monitoring year. Detailed site and survey descriptions can be found in GRAMP annual reports. Species detections logged as “casual observation” during field sampling were excluded from analyses for this report. Except for “*Anaxyrus* spp.” at VICK, we also omitted any detection that was not recorded to species (e.g., entered as “Bufonidae” or “*Hyla* spp.”). For simplicity, the category “*Anaxyrus* spp.” for VICK was considered a single species.

We calculated various dynamics indices for each site. Dynamics indices characterize the average number of species detected during any single survey or monitoring year relative to the potential richness of the site (Romanuk and Kolasa 2001). Potential richness, defined as the pool of species capable of persisting at a site, was approximated by the list of all species ever recorded at each site during the five-year monitoring period. A low dynamics index (high potential richness, but in any single survey or year only a small proportion of species were detected) may indicate high species turnover at the site or a large number of species with low detection probabilities.

For each site and monitoring year, we estimated species richness, exponentiated Shannon-Wiener, and inverse Simpson diversity (Hill diversities of $q = 0-2$) based on the maximum single-survey count for each species in each year. The maximum single-survey count seemed more appropriate than the total number of animals captured for each species in a year, because the latter could be biased by multiple captures of the same individuals across repeated surveys of the same site. In addition to diversity based on sample data, we estimated diversity standardized to a common sample coverage for each site. Appendix A3 gives an overview of the method, which can reduce biases due to unequal detection probabilities or sampling among survey years (Chao and Jost 2012).

In separate analyses conducted for GRAMP (summarized in a different report), we evaluated effects of reduced sampling frequency on statistical power to detect population trends for the most common species at each site. To complement that power analysis, for this report we also estimated amphibian diversities using the same reduced sampling scenarios (6- or 3-month sampling), to assess influence on estimated diversities. To further understand sampling effects on estimated diversities, we present tables and figures of annual changes in species detections and the relative contributions of trap type (CB, PVC, MT) and season (non-breeding = October through March; breeding = April through September) to observed species richness.

Results

Overview

Thirty-two amphibian species were captured from four sites during 2011–2015 GRAMP surveys (Figure 1). *Hyla cinerea* was the only species detected at all sites (Figure 1). Every site included some species not detected at the other three sites. JELA and GUIS shared the greatest number of species ($N = 7$; in Figure 1, species with colored cells for both JELA and GUIS).

The number of amphibian species detected was similar for JELA, GUIS, and VICK ($N = 12$ – 15) and much lower for SAAN ($N = 5$; Table 2). On average, 52–74% of species at each site were detected during a monitoring year (I[my]) and 23–45% of species were detected during a single survey (I[t]). At JELA and GUIS, the majority of species were seen in at least three of the five survey years. At SAAN and VICK, most species were seen in less than three survey years.

	JELA	GUIS	SAAN	VICK
<i>Hyla_cinerea</i>	1	0.8	0.2	0.4
<i>Gastrophryne_carolinensis</i>	1	1		0.2
<i>Lithobates_clamitans</i>	1	0.4		0.8
<i>Acris_blanchardi</i>	0.6			0.2
<i>Eleutherodactylus_planirostris</i>	0.8	0.8		
<i>Eurycea_quadridigitata</i>	0.8	0.2		
<i>Hyla_squirella</i>	1	1		
<i>Incilius_nebulifer</i>	1		1	
<i>Lithobates_catesbeianus</i>	0.4	0.6		
<i>Lithobates_sphenocephalus</i>		0.6		0.2
<i>Acris_crepitans</i>	0.2			
<i>Hyla_avivoca</i>	1			
<i>Notophthalmus_viridescens</i>	1			
<i>Pseudacris_crucifer</i>	0.2			
<i>Siren_intermedia</i>	0.4			
<i>Acris_gryllus</i>		1		
<i>Amphiuma_means</i>		0.4		
<i>Anaxyrus_quercicus</i>		0.8		
<i>Anaxyrus_terrestris</i>		1		
<i>Hyla_femoralis</i>		1		
<i>Lithobates_grylio</i>		0.6		
<i>Plethodon_grobmani</i>		0.6		
<i>Eleutherodactylus_cystignathoides</i>			0.8	
<i>Gastrophryne_olivacea</i>			0.4	
<i>Lithobates_berlandieri</i>			0.2	
<i>Ambystoma_maculatum</i>				0.4
<i>Anaxyrus_sp_VICK</i>				1
<i>Desmognathus_conanti</i>				1
<i>Eurycea_guttolineata</i>				1
<i>Hyla_versicolor_chrysoscelis</i>				0.8
<i>Plethodon_mississippi</i>				0.8
<i>Scaphiopus_holbrookii</i>				0.4

Figure 1. Species detections by site. For each species and site, the proportion of survey years in which that species was detected. For example, at JELA, *Hyla cinerea* was detected every monitoring year; at VICK, it was detected in only 2 of 5 monitoring years. Cell colors range from purple (low proportion of detections) to red (high proportion). A white cell means the species was not detected at that site.

Table 2. Summary diversity statistics for all sites. N = number of species known to exist at site (“potential species”, i.e., species detected at least once since the start of monitoring); $S.my$ = average (and SE) number of species detected during a monitoring year; $I(my)$ = Dynamics Index by monitoring year is the average proportion of species (out of species known to exist at site) detected in a monitoring year, calculated as $S.my(mean)/N$; $S.t$ = average (and SE) number of species detected during a single survey event; $I(t)$ = Dynamics Index by survey event is the average proportion of species (out of species known to exist at site) detected during a single survey event. *EXAMPLE: At JELA, 14 species were recorded. In a monitoring year, on average, 10.4 (SE = 0.68) of the 14 species were detected—this represented 74% of the potential species at JELA. In a single survey, on average, 6.30 (SE = 0.24) of the 14 species were detected—this represented 45% of the potential species at JELA.*

Site	N	S.my(mean)	S.my(SE)	I(my)	S.t(mean)	S.t(SE)	I(t)
JELA	14	10.4	0.68	0.74	6.30	0.24	0.45
GUIS	15	10.8	0.66	0.72	4.26	0.17	0.28
SAAN	5	2.6	0.68	0.52	2.00	0.06	0.40
VICK	12	7.2	0.58	0.60	2.81	0.15	0.23

JELA

JELA had the highest species dynamics index of the four sites (Table 2). On average, 74% of JELA’s species were detected in each monitoring year and 45% were detected during each survey event. JELA’s dynamics index by monitoring year increased steadily from 2011 to 2015, consistent with an increase in number of species detected each year (Figure 2 and Table 3). More species ($N = 12$) were detected in 2015 than in any other year at JELA. Shannon and inverse Simpson diversity—both of which account for species evenness—were highest in 2012 and lowest in 2013 (Figure 3).

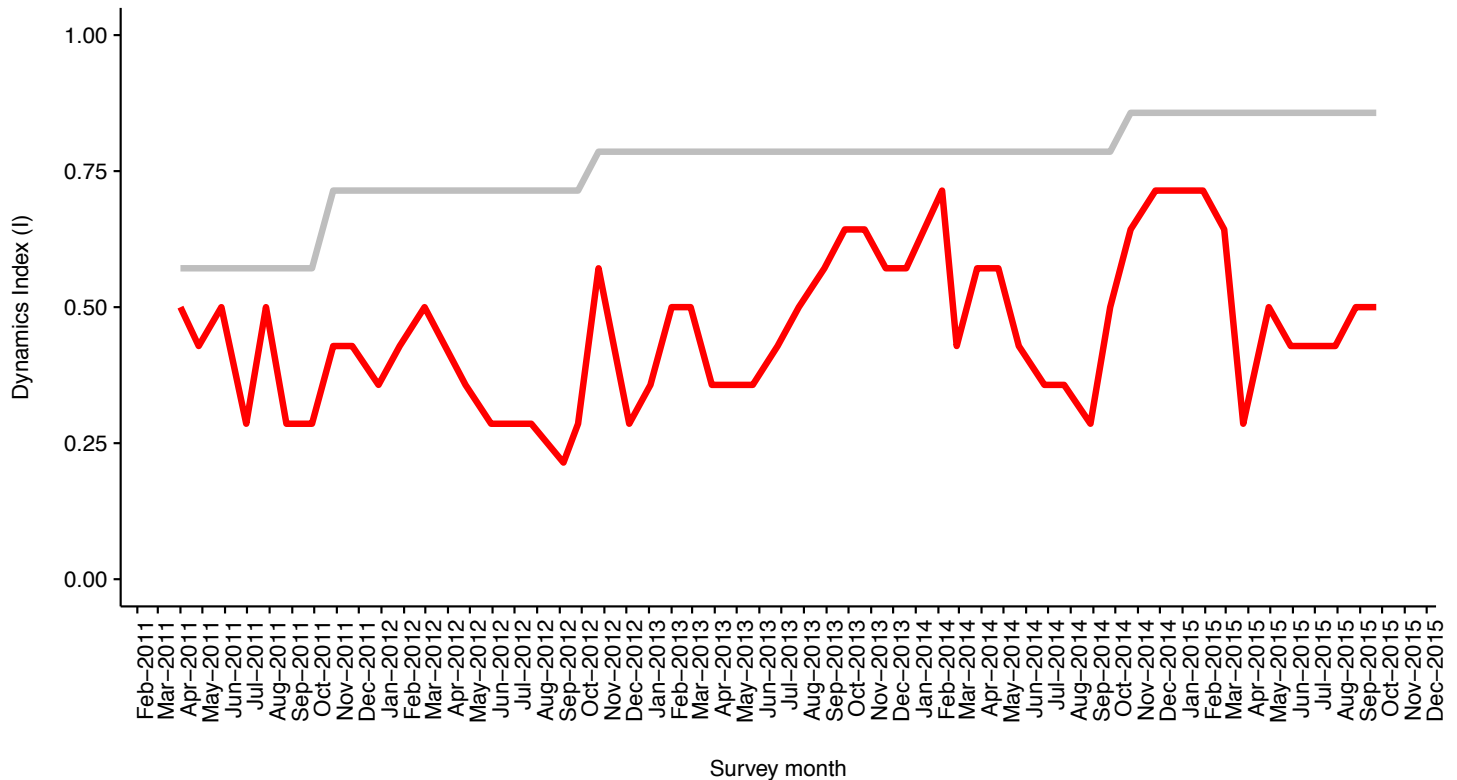


Figure 2. (JELA) Dynamics plot. Gray line represents the Dynamics Index for each monitoring year, i.e., the proportion of species (out of the 14 species known to occur at JELA) recorded in that monitoring year. Red line represents the Dynamics Index for each survey event, i.e., the proportion of species (out of 14) recorded in that survey event.

Table 3. (JELA) Species diversity and turnover statistics. *Appear* = proportion of species detected in that year relative to the combined number of species detected in that or the previous monitoring year; *Disappear* = proportion of species not detected in that year relative to the combined number of species detected in that or the previous monitoring year.

Monitoring year	Number of surveys	Species richness	Appear	Disappear
2011	7	8	NA	NA
2012	12	10	0.20	0.00
2013	12	11	0.09	0.00
2014	12	11	0.08	0.08
2015	11	12	0.15	0.08

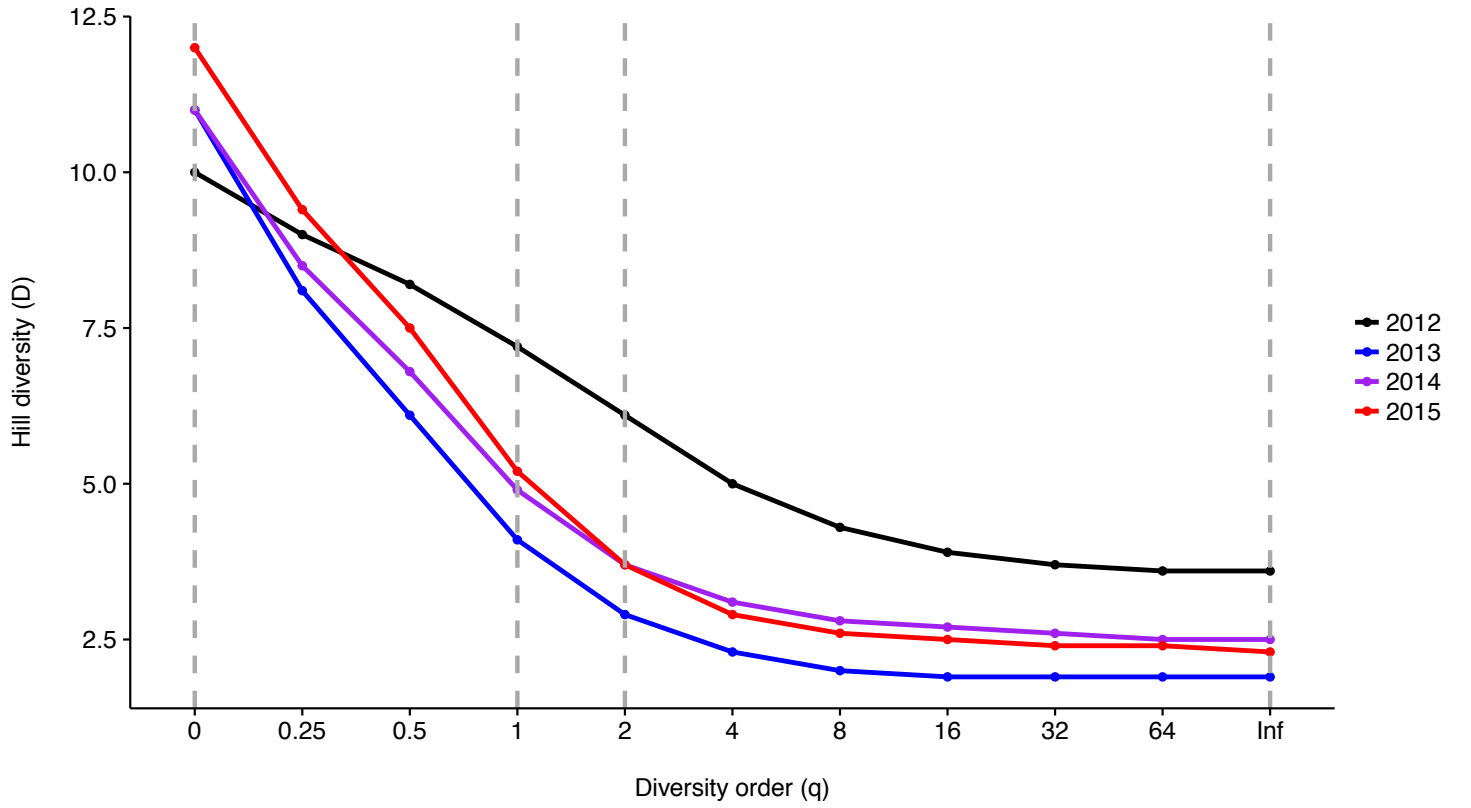


Figure 3. (JELA) Hill diversities. Colored lines represent different monitoring years (2011 omitted due to few surveys). Diversity estimates for each year are based on the maximum single-survey count for each species. Orders of diversity (q) are presented sequentially, but not to scale, on the x-axis. The vertical gray dashed lines identify orders of diversity that are commonly used ($q = 0$ is species richness, $q = 1$ is Shannon diversity, $q = 2$ is inverse Simpson diversity, $q = \text{Infinity}$ is exponentiated Berger-Parker index). See Appendix A2 for an overview of Hill diversities.

Regardless of diversity order, JELA diversity did not exhibit any clear monotonic trend from 2012 to 2015 (Figure 4). With the full data, 95% confidence intervals for species richness overlapped considerably across years. With reduced sampling frequencies, estimates were more variable and for some years confidence intervals did not overlap. In most cases, the decline in species richness due to reduced sampling was due to uncommon species (maximum number of individuals in a single survey was one or two) that were detected with 12-month sampling but not with 6- or 3-month sampling (Table 4). For example, in 2013, three uncommon species were detected with full sampling but not with reduced sampling.

Shannon and inverse Simpson diversities for 2012 and 2013 were markedly lower with reduced sampling compared to estimates based on the full data. Inverse Simpson is a higher order of diversity than Shannon diversity, so it is more sensitive to changes in species evenness, and therefore more influenced by changes in relative abundance of common species. In 2012, two of the most common species detected with full sampling (*Eleutherodactylus planirostris* = 19 individuals and *Lithobates climatanus* = 17 individuals) were much less prevalent with reduced sampling (maximum counts declining to $N = 0$ and $N = 5$, respectively). In 2013, maximum counts of *Eleutherodactylus planirostris* also declined considerably with reduced sampling (from $N = 54$ to $N = 12$). Thus, these changes in diversity estimates with reduced sampling may be due to the choice of which months were included in subsets, rather than the fact that sampling effort was reduced by half. An examination of the full data supports this idea. For example, in both 2012 and 2013, the maximum count of *Eleutherodactylus planirostris* was made on September 26, but the reduced sampling subsets spanned October to March of the monitoring year, therefore “missing” the maximum count survey by four days. With sparse and highly variable count data, seemingly significant differences (non-overlapping 95% confidence intervals) in diversity may be largely due to random chance. The highest proportion of species at JELA was detected by minnow traps (71%); the lowest, by PVC traps (21%; Table 5). Almost all species were detected during both non-breeding and breeding season surveys.

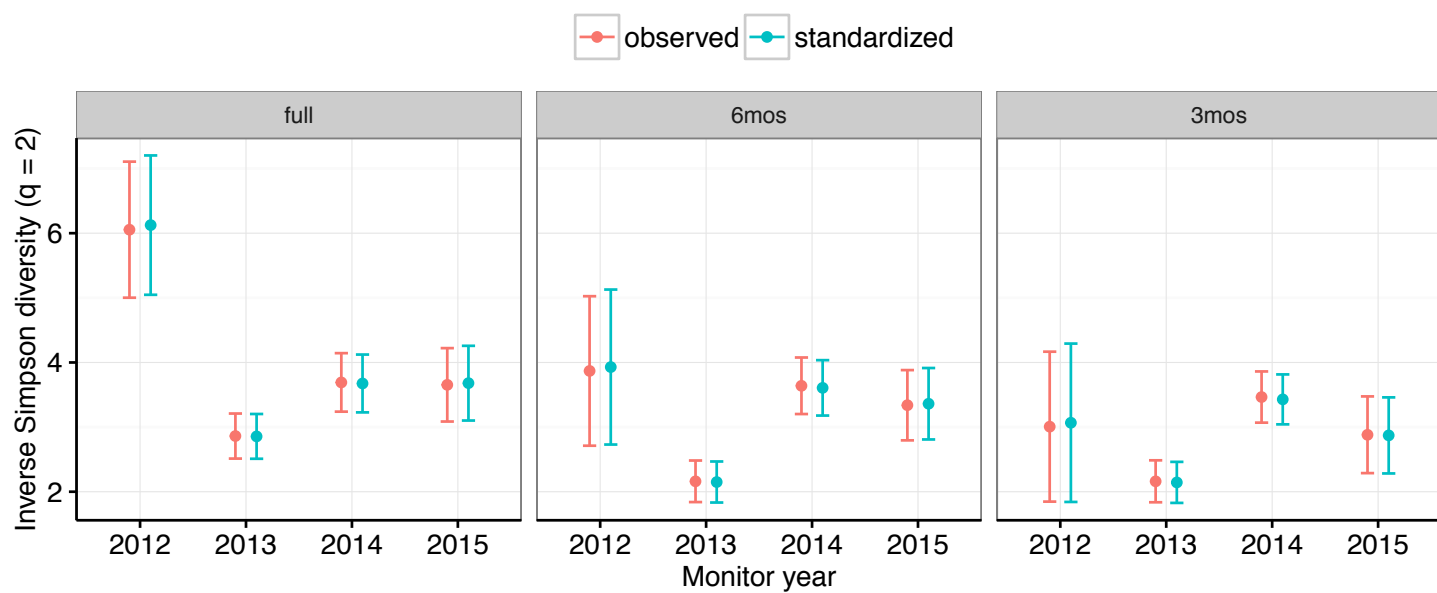
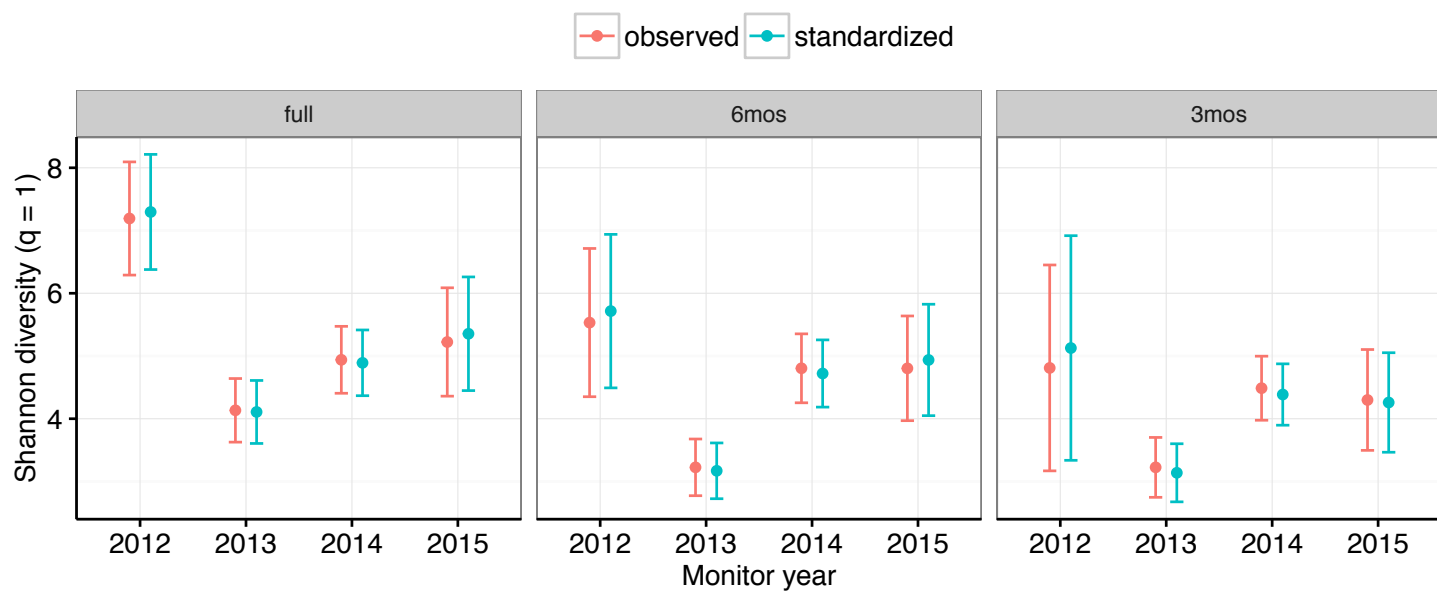
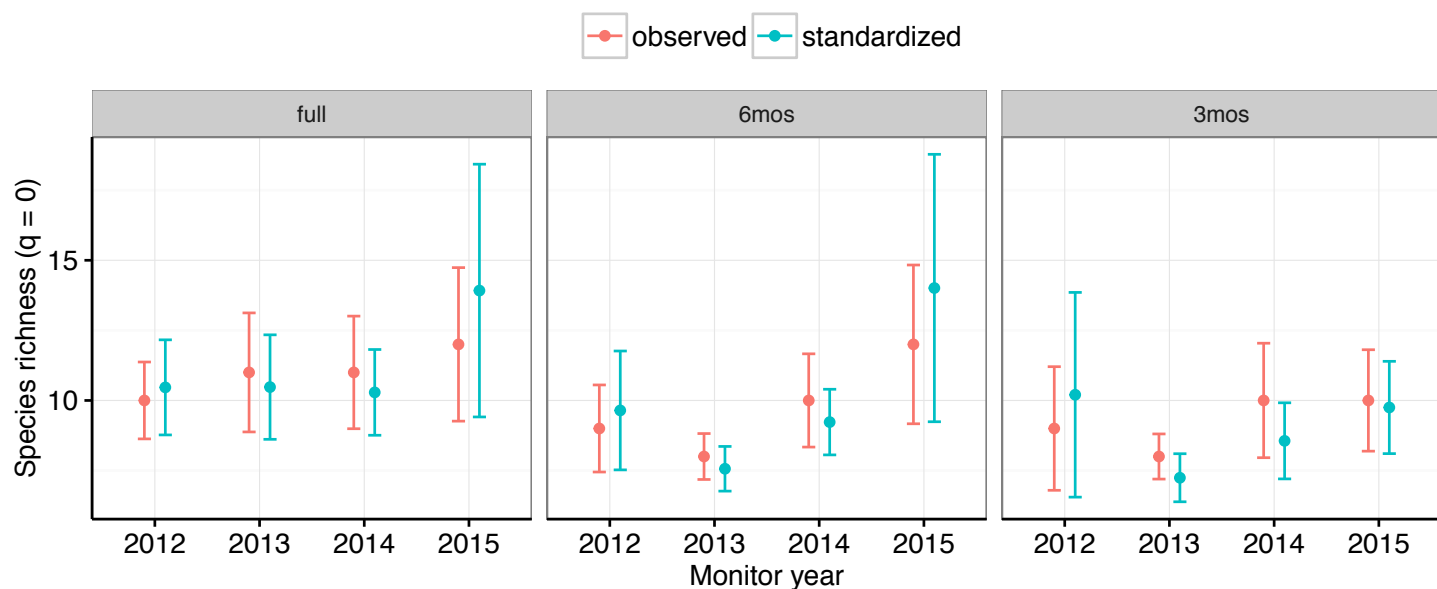


Figure 4. (JELA) Observed and standardized Hill diversities for full and reduced sampling frequencies. Hill diversities and bootstrapped 95% confidence intervals for 2012–2015 at three sampling frequencies (vertical panels; full = 12 months/yr; 6mos = Oct–Mar each year; 3mos = Oct, Dec, Feb each year). The top row of graphs is species richness (Hill order 0); middle row, Shannon diversity (Hill order 1); bottom row, inverse Simpson diversity (Hill order 2). Diversity estimates for each year are based on the maximum single-survey count for each species. Observed diversities (red) are based on the sample data. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage (99% for full and 6-month sampling, 98.5% for 3-month sampling), determined according to Chao et al. (2014). See Appendix A3 for an overview of standardizing and bootstrapping Hill diversities.

Table 4. (JELA) Maximum single-survey count for each species, for full and reduced sampling frequencies. For each species known to occur at JELA, the maximum single-survey count for different sampling frequencies is presented in columns immediately following each monitoring year. These data are the basis for diversity estimates in Figure 4. *EXAMPLE:* In 2012, the highest count of *Acris blanchardi* in a single survey was 10 individuals. Using the subset of surveys conducted from October to March (non-breeding season), the highest count was 4 individuals. Using the subset of surveys conducted in just three non-breeding months (October, December, February), the highest count in any survey was 4 individuals.

species	monitor.yr	full	6mos	3mos	monitor.yr	full	6mos	3mos
<i>Acris blanchardi</i>	2012	10	4	4	2013	3	2	2
<i>Acris crepitans</i>	2012	0	0	0	2013	0	0	0
<i>Eleutherodactylus planirostris</i>	2012	19	0	0	2013	54	12	12
<i>Eurycea quadridigitata</i>	2012	1	1	1	2013	1	0	0
<i>Gastrophryne carolinensis</i>	2012	9	5	4	2013	15	13	13
<i>Hyla avivoca</i>	2012	1	1	1	2013	3	3	3
<i>Hyla cinerea</i>	2012	30	30	28	2013	8	8	8
<i>Hyla squirella</i>	2012	10	10	3	2013	153	153	153
<i>Incilius nebulifer</i>	2012	3	3	3	2013	2	0	0
<i>Lithobates catesbeianus</i>	2012	0	0	0	2013	1	0	0
<i>Lithobates clamitans</i>	2012	17	5	5	2013	39	39	39
<i>Notophthalmus viridescens</i>	2012	7	7	2	2013	5	4	4
<i>Pseudacris crucifer</i>	2012	0	0	0	2013	0	0	0
<i>Siren intermedia</i>	2012	0	0	0	2013	0	0	0

species	monitor.yr	full	6mos	3mos	monitor.yr	full	6mos	3mos
<i>Acris blanchardi</i>	2014	0	0	0	2015	0	0	0
<i>Acris crepitans</i>	2014	0	0	0	2015	1	1	0
<i>Eleutherodactylus planirostris</i>	2014	39	39	39	2015	12	3	3
<i>Eurycea quadridigitata</i>	2014	6	6	6	2015	5	5	5
<i>Gastrophryne carolinensis</i>	2014	10	10	8	2015	16	16	12
<i>Hyla avivoca</i>	2014	6	6	1	2015	3	3	3
<i>Hyla cinerea</i>	2014	14	14	14	2015	4	4	3
<i>Hyla squirella</i>	2014	79	79	79	2015	47	47	27
<i>Incilius nebulifer</i>	2014	2	2	2	2015	2	2	2
<i>Lithobates catesbeianus</i>	2014	1	1	1	2015	0	0	0
<i>Lithobates clamitans</i>	2014	109	109	109	2015	74	74	67
<i>Notophthalmus viridescens</i>	2014	5	4	4	2015	7	7	2
<i>Pseudacris crucifer</i>	2014	0	0	0	2015	1	1	0
<i>Siren intermedia</i>	2014	1	0	0	2015	1	1	1

Table 5. (JELA) Proportion of species detected by each trap type or season. EXAMPLE: 64% of the potential species at JELA were detected by coverboard traps and 100% of the species were detected during non-breeding season surveys.

Method	Proportion.detected
CB	0.64
PVC	0.21
MT	0.71
Nonbreeding season (0)	1.00
Breeding season (1)	0.93

Out of fourteen amphibian species known to occur at JELA, seven were commonly observed, having been reported in the majority of surveys during all five monitoring years (Figures 5 and 6). On the other hand, four species (*Lithobates catesbeianus*, American Bullfrog; *Siren intermedia*, Lesser Siren; *Acris crepitans*, Northern Cricket Frog; *Pseudacris crucifer*, Spring Peeper) were detected in three or fewer surveys over the five years of monitoring. The latter two species (*Acris crepitans* and *Pseudacris crucifer*) were observed only in 2015. Two other species appeared to exhibit strong but opposite trends in detection. *Acris blanchardi* (Blanchard’s Cricket Frog) was recorded during all surveys in 2011, but only in 33% of surveys in 2012 and was not observed at all in 2014 or 2015. In contrast, detections of *Eurycea quadridigitata* (Dwarf Salamander) increased from 2011 to 2015. Detections of *Eleutherodactylus planirostris* (Greenhouse Frog) generally increased over the years, but somewhat erratically.

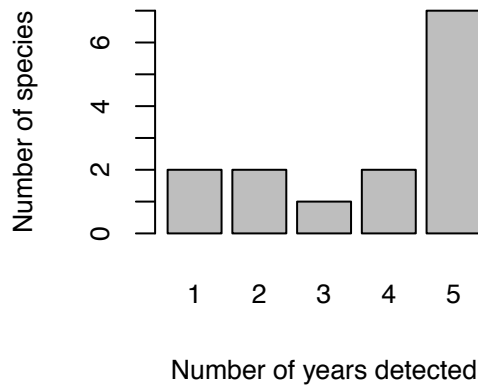


Figure 5. (JELA) Frequency of species detections. Frequency of detection for species known to occur at JELA, e.g., 2 species were detected in only one monitoring year; 7 species were detected in all five monitoring years.

	2011	2012	2013	2014	2015
Gastrophryne_carolinensis	0.86	0.75	1	0.92	1
Hyla_squirella	0.57	0.92	1	0.92	0.91
Hyla_cinerea	1	0.75	0.58	0.92	0.64
Lithobates_clamitans	0.29	0.83	0.92	1	0.82
Incilius_nebulifer	1	0.58	0.25	0.83	1
Notophthalmus_viridescens	0.43	0.5	0.67	0.75	0.82
Eleutherodactylus_planirostris		0.08	1	0.5	0.91
Hyla_avivoca	0.43	0.17	0.5	0.5	0.45
Acris_blanchardi	1	0.33	0.25		
Eurycea_quadridigitata		0.08	0.08	0.42	0.45
Lithobates_catesbeianus			0.08	0.17	
Acris_crepitans					0.18
Siren_intermedia				0.08	0.09
Pseudacris_crucifer					0.09

Figure 6. (JELA) Proportion of surveys in which each species was detected. For each species and monitoring year, proportion of surveys in which that species was detected. For example, *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad) was detected in 86% of surveys in monitoring year 2011 and in 75% of surveys in 2012. Cell colors range from purple (low proportion of detections) to red (high proportion). White cells represent years in which the species was not detected at all.

GUIS

Among the four sites, the largest number of amphibian species ($N = 15$) was reported from GUIS. On average, 72% of GUIS' species were detected in each monitoring year but during a single survey only 28% were detected on average (Table 2). GUIS' dynamics index by monitoring year generally increased from 2011 to 2015 (Figure 7), but no such trend was apparent in the dynamics index by survey. Similar to JELA, more species ($N = 13$) were detected at GUIS in 2015 than in any other monitoring year and Shannon and inverse Simpson diversity were highest in 2012 (Table 6; Figure 8). By these two indices, diversity was lowest at GUIS in 2014.

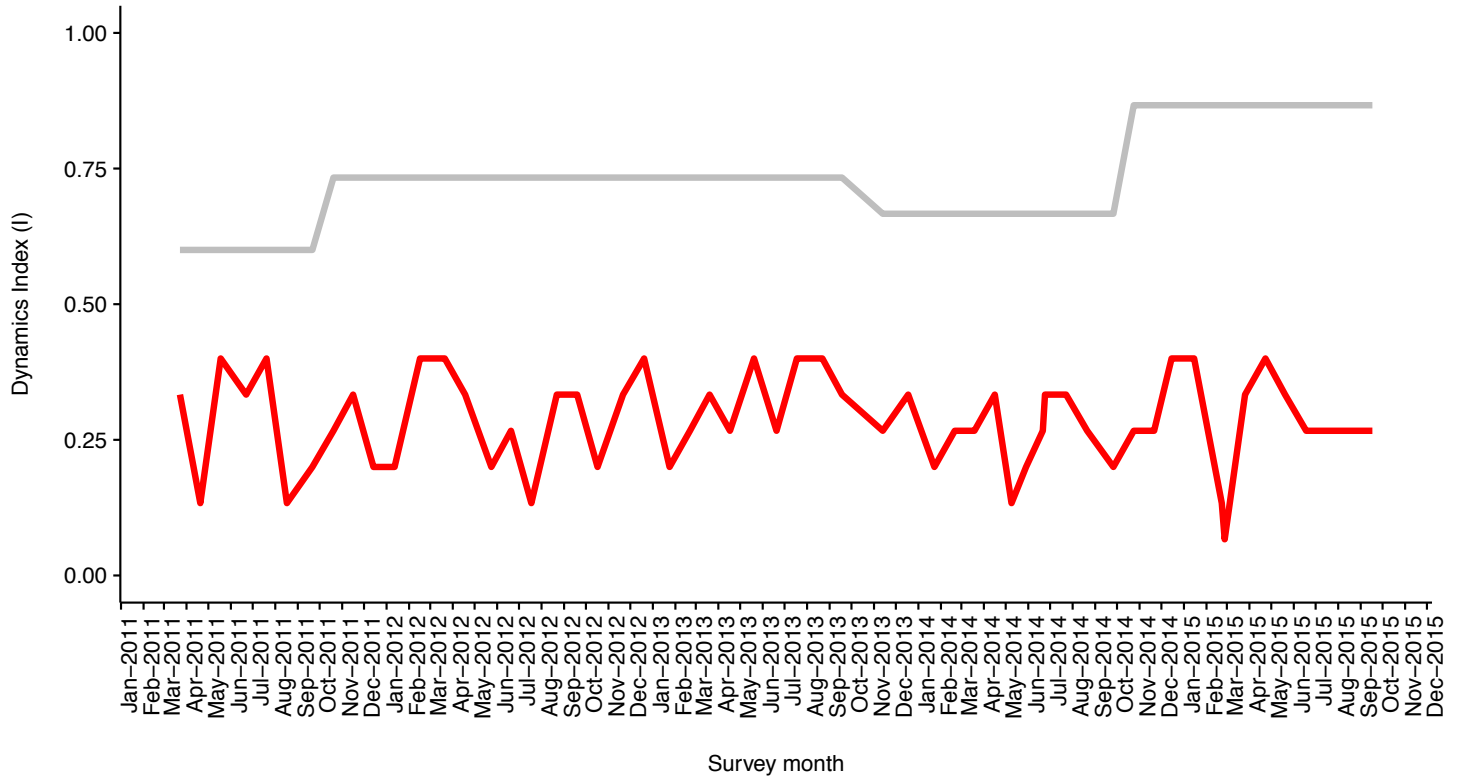


Figure 7. (GUIS) Dynamics plot. Gray line represents the Dynamics Index for each monitoring year, i.e., the proportion of species (out of the 15 species known to occur at GUIS) recorded in that monitoring year. Red line represents the Dynamics Index for each survey event, i.e., the proportion of species (out of 15) recorded in that survey event.

Table 6. (GUIS) Species diversity and turnover statistics. *Appear* = proportion of species detected in that year relative to the combined number of species detected in that or the previous monitoring year; *Disappear* = proportion of species not detected in that year relative to the combined number of species detected in that or the previous monitoring year.

Monitoring year	Number of surveys	Species richness	Appear	Disappear
2011	7	9	NA	NA
2012	12	11	0.25	0.08
2013	12	11	0.21	0.21
2014	14	10	0.15	0.23
2015	14	13	0.23	0.00

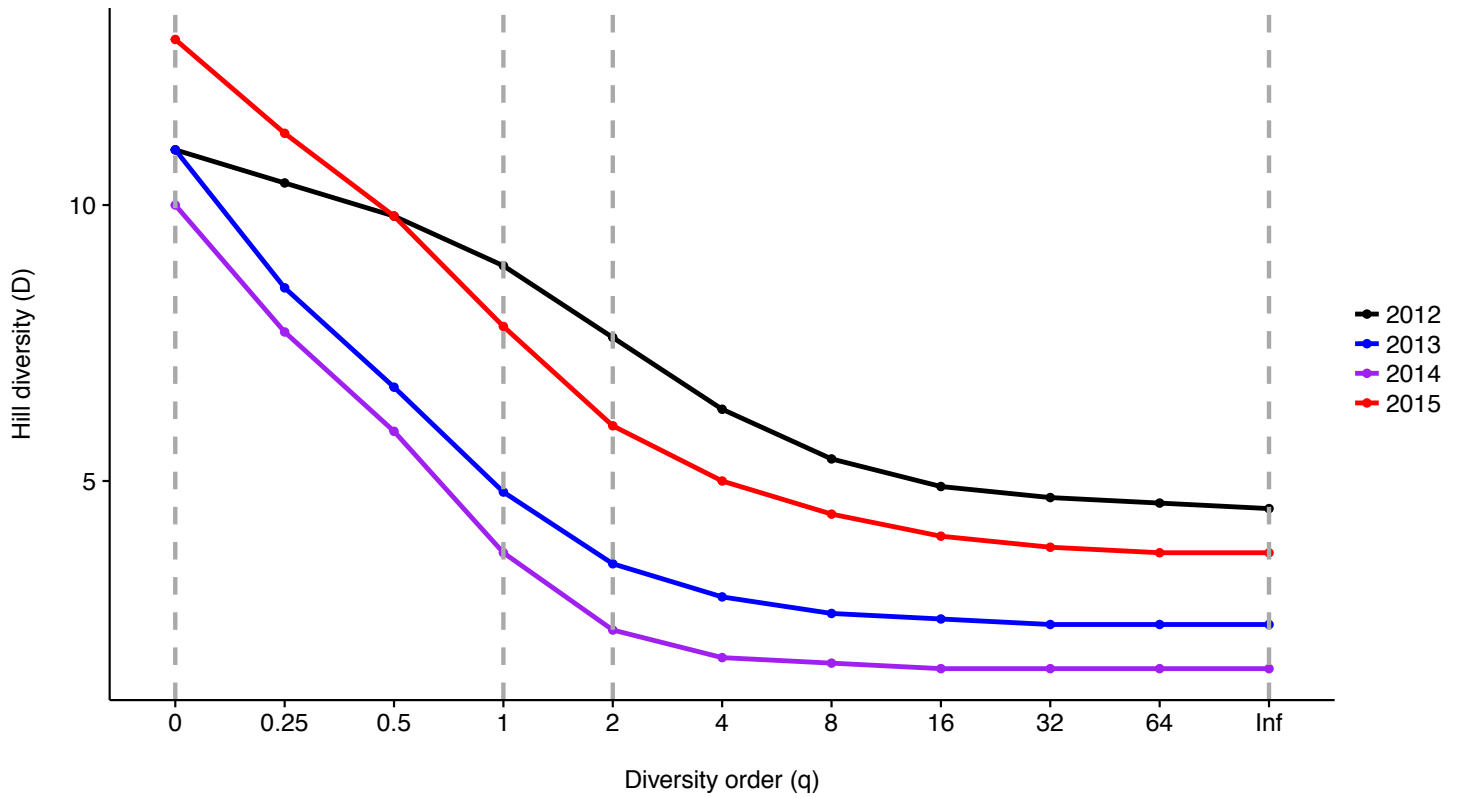


Figure 8. (GUIS) Hill diversities. Colored lines represent different monitoring years (2011 omitted due to few surveys). Diversity estimates for each year are based on the maximum single-survey count for each species. Orders of diversity (q) are presented sequentially, but not to scale, on the x-axis. The vertical gray dashed lines identify orders of diversity that are commonly used ($q = 0$ is species richness, $q = 1$ is Shannon diversity, $q = 2$ is inverse Simpson diversity, $q = \text{Infinity}$ is exponentiated Berger-Parker index). See Appendix A2 for an overview of Hill diversities.

Regardless of diversity order, GUIS diversity did not exhibit any clear monotonic trend from 2012 to 2015 (Figure 9). The ranking of monitoring years based on species richness changed with sampling frequency. Differences in species richness with sampling frequency were due to uncommon species (maximum survey count of five individuals in any year) that were detected with full sampling, but not with reduced sampling restricted to the non-breeding season (Table 7). Confidence intervals for standardized species richness estimates sometimes did not overlap those for observed estimates. Observed and standardized estimates were similar in all cases for Shannon and inverse Simpson diversities. For these two higher orders of diversity, full and reduced sampling estimates exhibited similar patterns—higher diversities in 2012 and 2015, and lower diversities in 2013 and 2014. The highest proportion of species was detected by minnow traps (67%); the lowest, by PVC traps (20%; Table 8). All species were detected at least once during breeding season surveys. A high proportion (87%) were also detected during non-breeding season surveys.

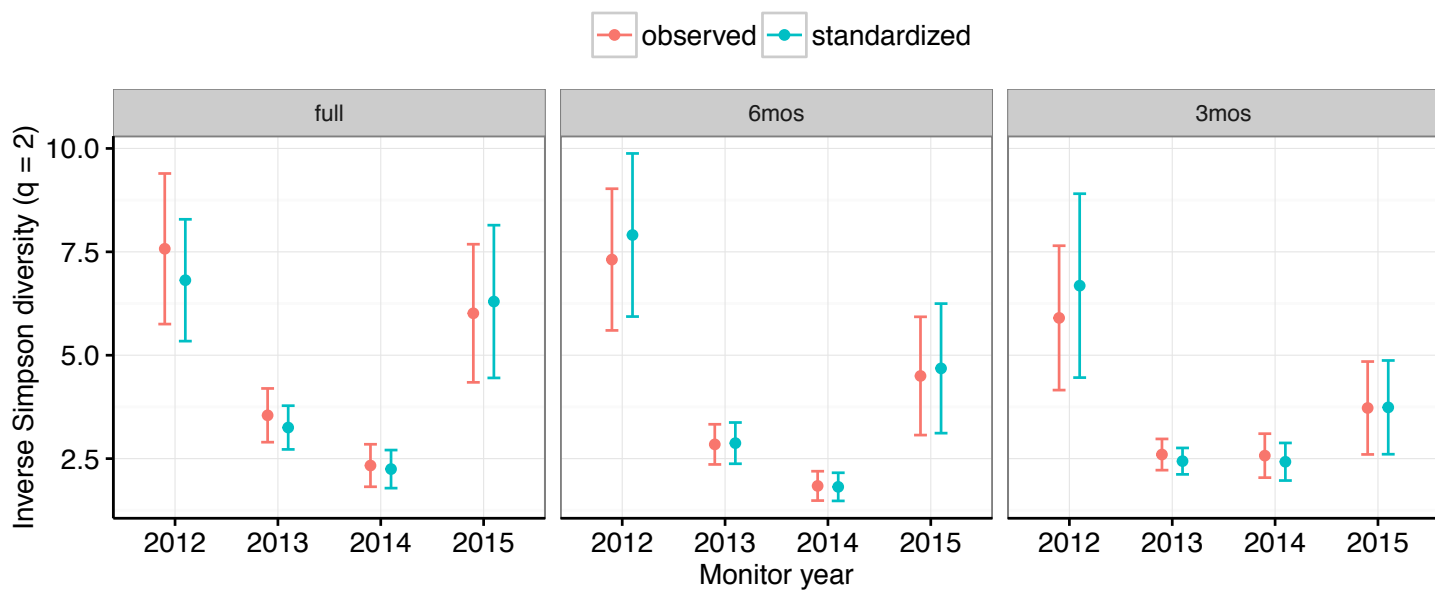
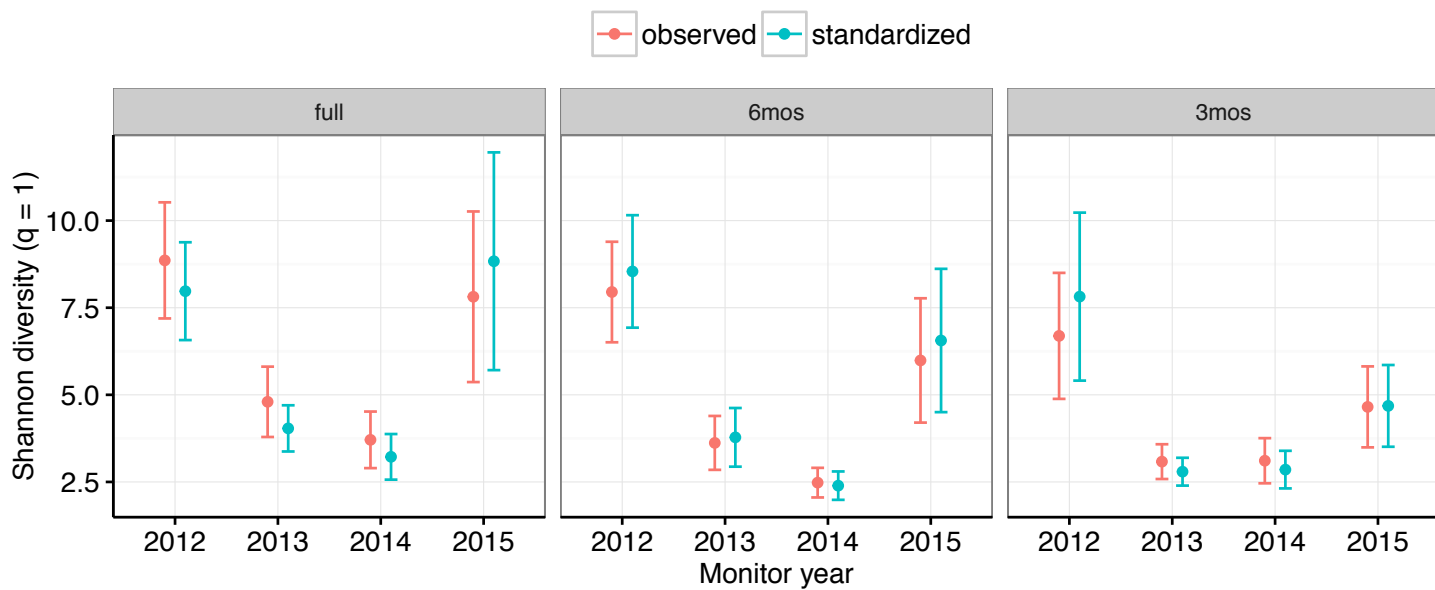
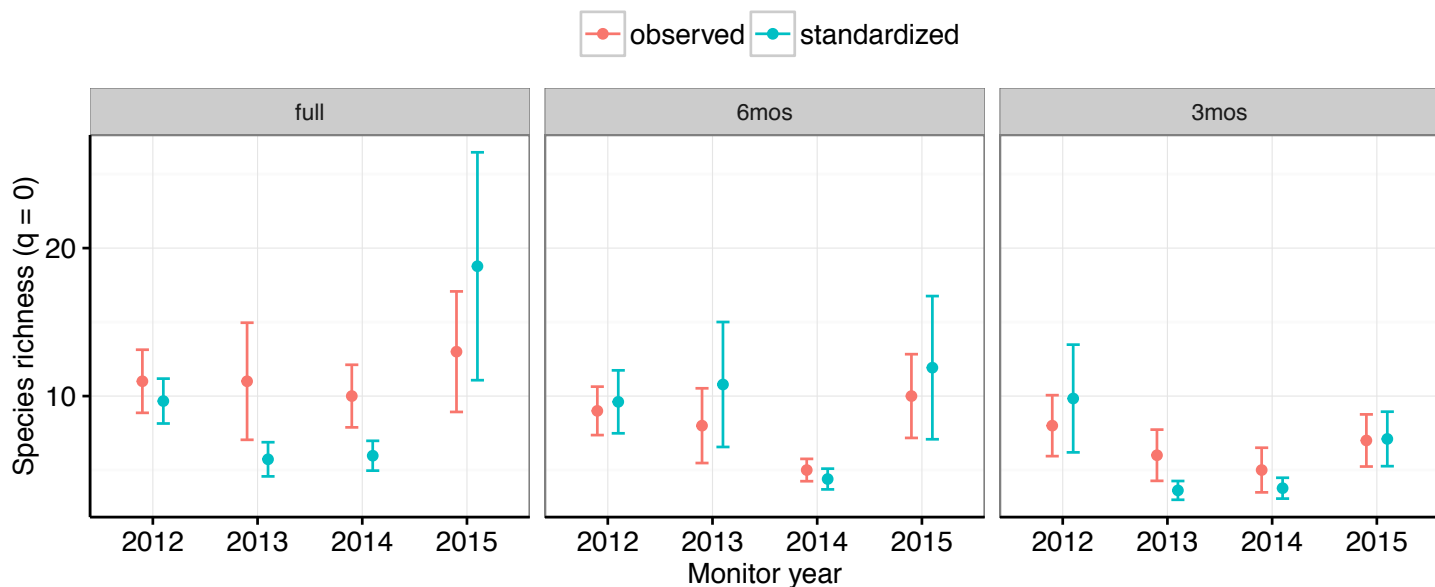


Figure 9. (GUIS) Observed and standardized Hill diversities for full and reduced sampling frequencies. Hill diversities and bootstrapped 95% confidence intervals for 2012–2015 at three sampling frequencies (vertical panels; full = 12 months/yr; 6mos = Oct–Mar each year; 3mos = Oct, Dec, Feb each year). The top row of graphs is species richness (Hill order 0); middle row, Shannon diversity (Hill order 1); bottom row, inverse Simpson diversity (Hill order 2). Diversity estimates for each year are based on the maximum single-survey count for each species. Observed diversities (red) are based on the sample data. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage (90% for full data, 97.6% for 6-month sampling, 95.2% for 3-month sampling), determined according to Chao et al. (2014). See Appendix A3 for an overview of standardizing and bootstrapping Hill diversities.

Table 7. (GUIS) Maximum single-survey count for each species, for full and reduced sampling frequencies. For each species known to occur at GUIS, the maximum single-survey count for different sampling frequencies is presented in columns immediately following each monitoring year. These data are the basis for diversity estimates in Figure 9. *EXAMPLE:* In 2012, the highest count of *Acris gryllus* in a single survey was 5 individuals. Using the subset of surveys conducted from October to March (non-breeding season), the highest count was 3 individuals. Using the subset of surveys conducted in just three non-breeding months (October, December, February), the highest count in any survey was 1 individual.

species	monitor.yr	full	6mos	3mos	monitor.yr	full	6mos	3mos
<i>Acris_gryllus</i>	2012	5	3	1	2013	10	3	0
<i>Amphiuma_means</i>	2012	0	0	0	2013	1	1	0
<i>Anaxyrus_quercicus</i>	2012	1	1	1	2013	1	0	0
<i>Anaxyrus_terrestris</i>	2012	2	0	0	2013	1	0	0
<i>Eleutherodactylus_planirostris</i>	2012	9	6	6	2013	28	28	28
<i>Eurycea_quadridigitata</i>	2012	0	0	0	2013	0	0	0
<i>Gastrophryne_carolinensis</i>	2012	1	1	0	2013	1	1	1
<i>Hyla_cinerea</i>	2012	3	3	3	2013	3	1	1
<i>Hyla_femoralis</i>	2012	5	4	4	2013	10	10	10
<i>Hyla_squirella</i>	2012	11	3	3	2013	42	42	42
<i>Lithobates_catesbeianus</i>	2012	4	0	0	2013	0	0	0
<i>Lithobates_clamitans</i>	2012	3	3	3	2013	0	0	0
<i>Lithobates_grylio</i>	2012	0	0	0	2013	1	0	0
<i>Lithobates_sphenocephalus</i>	2012	5	5	1	2013	0	0	0
<i>Plethodon_grobmani</i>	2012	0	0	0	2013	1	1	1

species	monitor.yr	full	6mos	3mos	monitor.yr	full	6mos	3mos
<i>Acris_gryllus</i>	2014	4	3	1	2015	9	2	2
<i>Amphiuma_means</i>	2014	0	0	0	2015	0	0	0
<i>Anaxyrus_quercicus</i>	2014	0	0	0	2015	1	0	0
<i>Anaxyrus_terrestris</i>	2014	2	2	2	2015	1	1	1
<i>Eleutherodactylus_planirostris</i>	2014	75	75	33	2015	7	7	7
<i>Eurycea_quadridigitata</i>	2014	0	0	0	2015	2	2	2
<i>Gastrophryne_carolinensis</i>	2014	2	0	0	2015	4	2	0
<i>Hyla_cinerea</i>	2014	0	0	0	2015	1	1	1
<i>Hyla_femoralis</i>	2014	10	8	8	2015	15	15	15
<i>Hyla_squirella</i>	2014	17	17	17	2015	11	11	8
<i>Lithobates_catesbeianus</i>	2014	1	0	0	2015	1	0	0
<i>Lithobates_clamitans</i>	2014	0	0	0	2015	0	0	0
<i>Lithobates_grylio</i>	2014	2	0	0	2015	1	0	0
<i>Lithobates_sphenocephalus</i>	2014	5	0	0	2015	1	1	0
<i>Plethodon_grobmani</i>	2014	1	0	0	2015	1	1	0

Table 8. (GUIS) Proportion of species detected by each trap type or season. *EXAMPLE: 60% of the potential species at GUIS were detected by coverboard traps and 87% of the species were detected during non-breeding season surveys.*

Method	Proportion.detected
CB	0.60
PVC	0.20
MT	0.67
Nonbreeding season (0)	0.87
Breeding season (1)	1.00

Out of 15 amphibian species known to occur at GUIS, five were observed during all monitoring years (Figures 10 and 11). Three species (*Lithobates clamitans*, Green Frog; *Amphiuma means*, Two-toed Amphiuma; *Eurycea quadridigitata*) were detected in only one or two years. Ten species were never detected in more than 50% of surveys in any monitoring year. *Eleutherodactylus planirostris* was not detected in 2011, but was found during most surveys in 2012 through 2015.

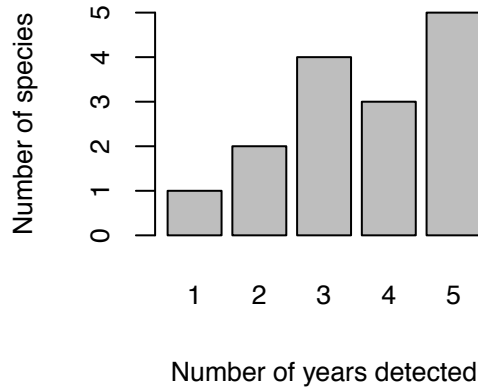


Figure 10. (GUIS) Frequency of species detections. *Frequency of detection for species known to occur at GUIS, e.g., 1 species was detected in only one monitoring year; 5 species were detected in all five monitoring years.*

	2011	2012	2013	2014	2015
Hyla_femoralis	0.86	0.75	1	0.86	0.79
Eleutherodactylus_planirostris		0.75	1	0.86	0.86
Hyla_squirella	0.71	0.58	0.92	0.71	0.43
Acris_gryllus	0.71	0.67	0.5	0.29	0.43
Hyla_cinerea	0.71	0.25	0.25		0.29
Gastrophryne_carolinensis	0.29	0.17	0.33	0.21	0.29
Anaxyrus_terrestris	0.29	0.08	0.25	0.43	0.14
Lithobates_sphenocephalus		0.5		0.07	0.07
Lithobates_clamitans	0.29	0.33			
Anaxyrus_quercicus	0.14	0.08	0.08		0.07
Lithobates_grylio			0.08	0.07	0.21
Amphiuma_means	0.14		0.17		
Plethodon_grobmani			0.17	0.07	0.07
Lithobates_catesbeianus		0.08		0.07	0.14
Eurycea_quadridigitata					0.14

Figure 11. (GUIS) Proportion of surveys in which each species was detected. For each species and monitoring year, proportion of surveys in which that species was detected. For example, *Hyla femoralis* (Pine Woods Treefrog) was detected in 86% of surveys conducted in monitoring year 2011 and in 75% of surveys in 2012. Cell colors range from purple (low proportion of detections) to red (high proportion). White cells represent years in which the species was not detected at all.

SAAN

SAAN had the fewest observed species ($N = 5$) of any site, and the lowest average dynamics index by monitoring year (52%; Table 2 and Figure 12). The average proportion of species detected during any survey event was 40% ($N = 2$). All potential species ($N = 5$) were detected in 2014, and Shannon diversity was also highest in this year (Table 9; Figure 13).

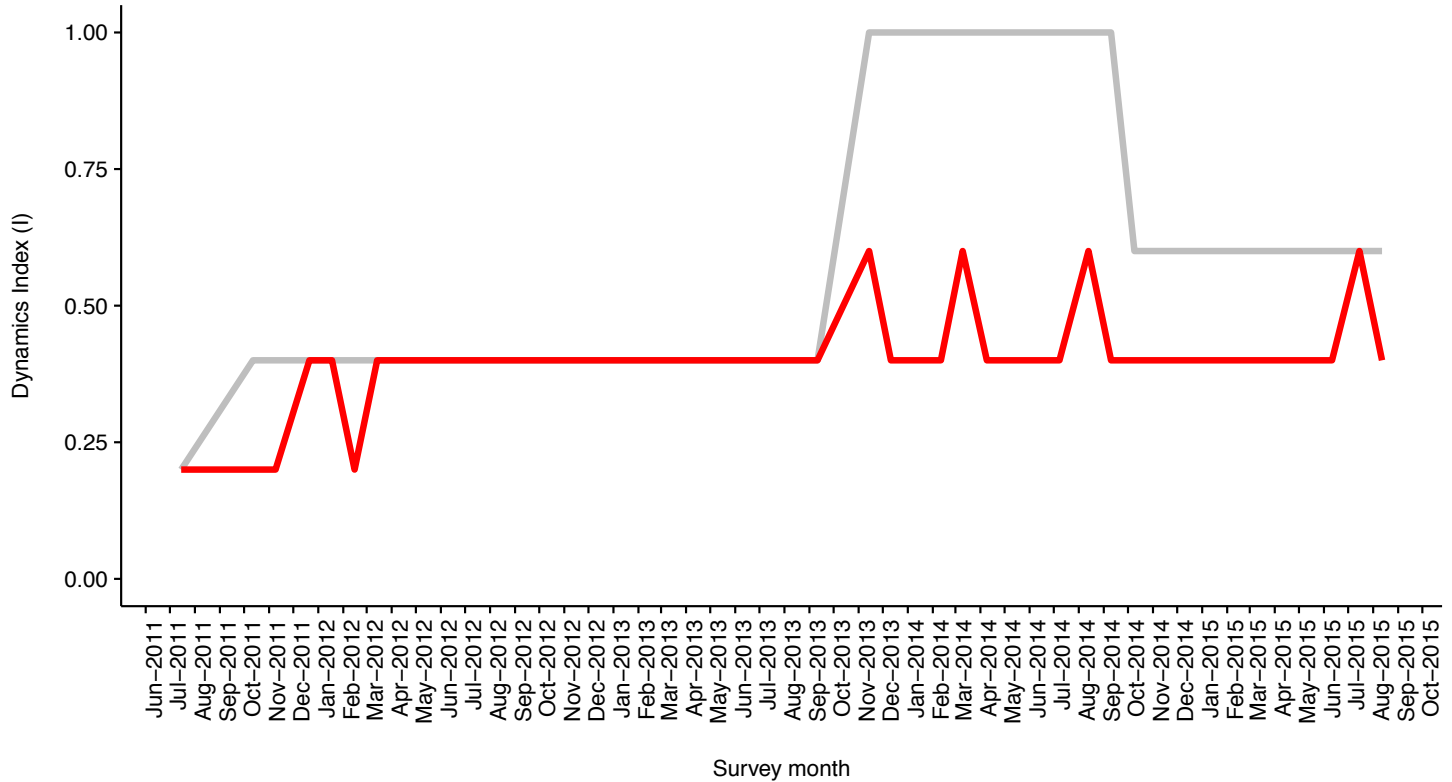


Figure 12. (SAAN) Dynamics plot. Gray bars represent the Dynamics Index for each monitoring year, i.e., the proportion of species (out of the 5 species known to occur at SAAN) recorded in that monitoring year. Red line represents the Dynamics Index for each survey event, i.e., the proportion of species (out of 5) recorded in that survey event.

Table 9. (SAAN) Species diversity and turnover statistics. Appear = proportion of species detected in that year relative to the combined number of species detected in that or the previous monitoring year; Disappear = proportion of species not detected in that year relative to the combined number of species detected in that or the previous monitoring year.

Monitoring year	Number of surveys	Species richness	Appear	Disappear
2011	4	1	NA	NA
2012	13	2	0.5	0.0
2013	12	2	0.0	0.0
2014	11	5	0.6	0.0
2015	13	3	0.0	0.4

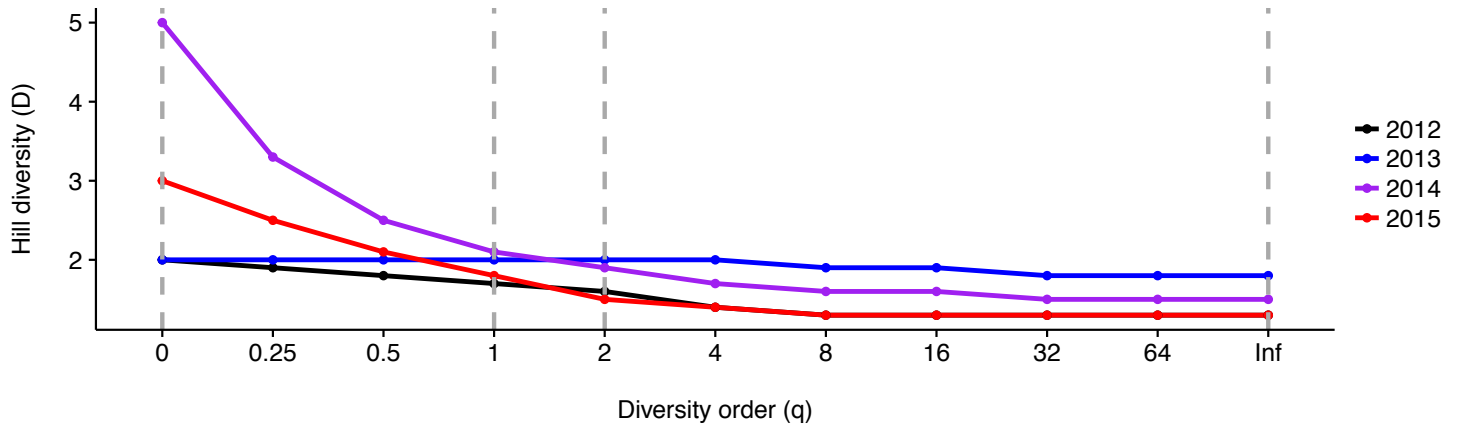


Figure 13. (SAAN) Hill diversities. Colored lines represent different monitoring years (2011 omitted due to few surveys). Diversity estimates for each year are based on the maximum single-survey count for each species. Orders of diversity (q) are presented sequentially, but not to scale, on the x-axis. The vertical gray dashed lines identify orders of diversity that are commonly used ($q = 0$ is species richness, $q = 1$ is Shannon diversity, $q = 2$ is inverse Simpson diversity, $q = \text{Infinity}$ is exponentiated Berger-Parker index). See Appendix A2 for an overview of Hill diversities.

Of the five species known to occur at SAAN, two (*Eleutherodactylus cystignathoides* and *Incilius nebulifer*) accounted for almost all captures in any given year (Table 10). In 2014, the year with highest species richness, three additional species were captured but the maximum count for each of these species was one individual. In 2012 and 2013, captures of the relatively common *Eleutherodactylus cystignathoides* were considerably less with 6-month compared to full sampling, as reflected in the lower Shannon and inverse Simpson diversities for reduced sampling (Figure 14). Eighty percent ($N = 4$) of potential species were detected by coverboard traps and 20% ($N = 1$) by PVC traps (Table 11).

Table 10. (SAAN) Maximum single-survey count for each species, for full and reduced sampling frequencies. For each species known to occur at SAAN, the maximum single-survey count for different sampling frequencies is presented in columns immediately following each monitoring year. These data are the basis for diversity estimates in Figure 14. *EXAMPLE:* In 2012, the highest count of *Eleutherodactylus cystignathoides* in a single survey was 30 individuals. With reduced sampling (6 months per year, alternate months), the highest count was 9 individuals.

species	monitor.yr	full	6mos	monitor.yr	full	6mos
<i>Eleutherodactylus_cystignathoides</i>	2012	30	9	2013	106	71
<i>Gastrophryne_olivacea</i>	2012	0	0	2013	0	0
<i>Hyla_cinerea</i>	2012	0	0	2013	0	0
<i>Incilius_nebulifer</i>	2012	9	8	2013	133	120
<i>Lithobates_berlandieri</i>	2012	0	0	2013	0	0

species	monitor.yr	full	6mos	monitor.yr	full	6mos
<i>Eleutherodactylus_cystignathoides</i>	2014	131	131	2015	96	71
<i>Gastrophryne_olivacea</i>	2014	1	1	2015	2	0
<i>Hyla_cinerea</i>	2014	1	0	2015	0	0
<i>Incilius_nebulifer</i>	2014	65	35	2015	25	23
<i>Lithobates_berlandieri</i>	2014	1	0	2015	0	0

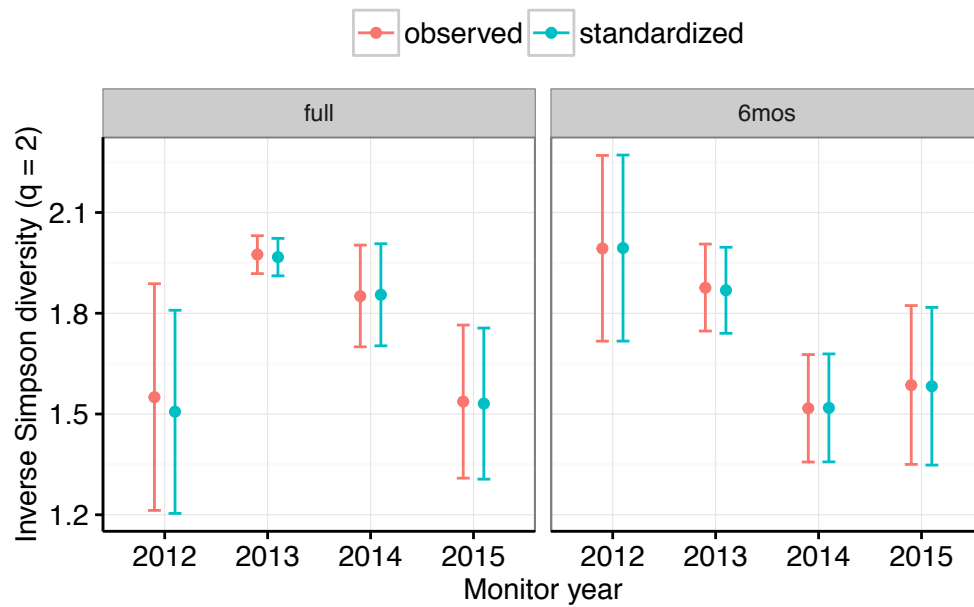
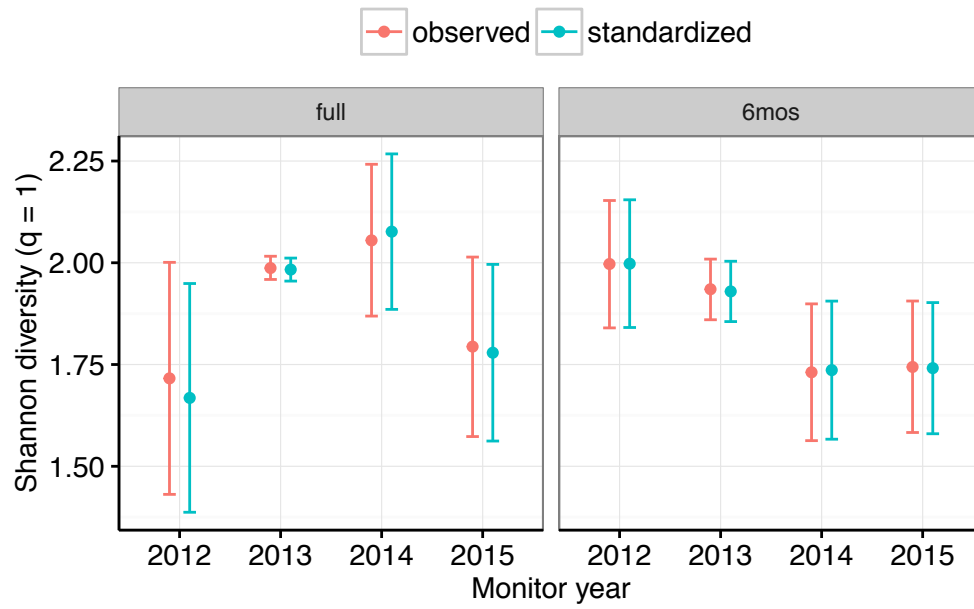
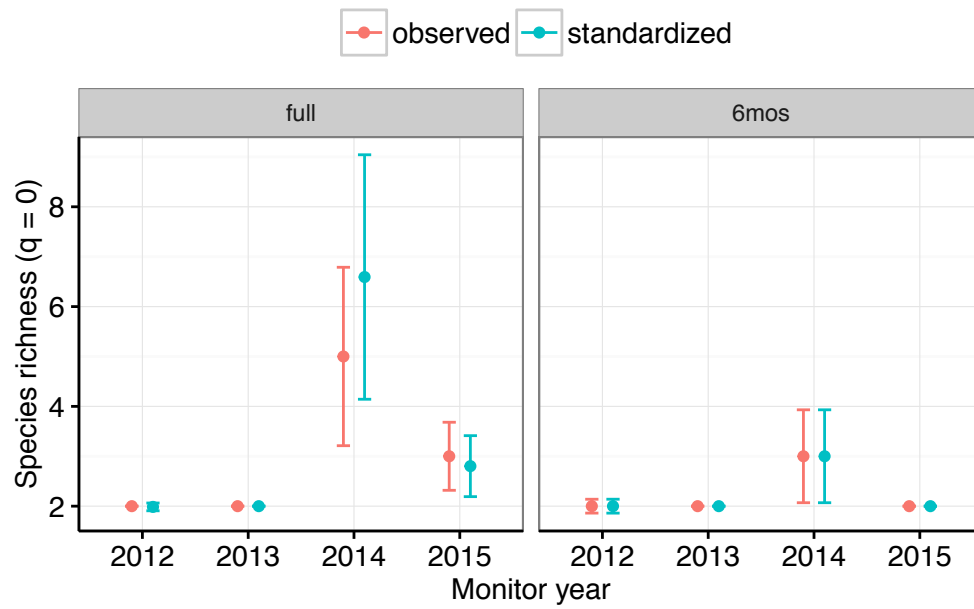


Figure 14. (SAAN) Observed and standardized Hill diversities for full and reduced sampling frequencies. Hill diversities and bootstrapped 95% confidence intervals for 2012–2015 at two sampling frequencies (vertical panels; full = 12 months/yr; 6mos = alternate months). The top row of graphs is species richness (Hill order 0); middle row, Shannon diversity (Hill order 1); bottom row, inverse Simpson diversity (Hill order 2). Diversity estimates for each year are based on the maximum single-survey count for each species. Observed diversities (red) are based on the sample data. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage (99.3% for full data, 99.8% for 6-month sampling), determined according to Chao et al. (2014). See Appendix A3 for an overview of standardizing and bootstrapping Hill diversities.

Table 11. (SAAN) Proportion of species detected by each trap type or season. EXAMPLE: 80% of the potential species at SAAN were detected by coverboard traps and 80% of the species were detected during non-breeding season surveys.

Method	Proportion.detected
CB	0.8
PVC	0.2
MT	NA
Nonbreeding season (0)	0.8
Breeding season (1)	0.6

Only one species, *Incilius nebulifer* (Gulf Coast Toad), was detected in all survey years (Figures 15 and 16). *Incilius nebulifer* and *Eleutherodactylus cystignathoides* (Rio Grande Chirping Frog) made up almost all amphibian detections at SAAN. Three additional species were detected in 2014. *Gastrophryne olivacea* (Great Plains Narrow-Mouthed Toad) was also detected in 2015.

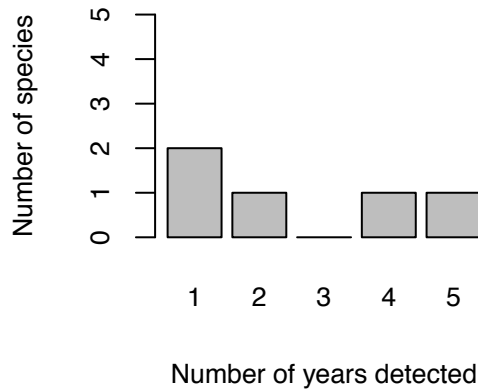


Figure 15. (SAAN) Frequency of species detections. Frequency of detection for species known to occur at SAAN, e.g., 2 species were detected in only one monitoring year; no species were detected for exactly 3 years.

	2011	2012	2013	2014	2015
Incilius_nebulifer	0.25	0.92	1	1	0.92
Eleutherodactylus_cystignathoides		0.69	1	1	0.92
Gastrophryne_olivacea				0.09	0.08
Hyla_cinerea				0.09	
Lithobates_berlandieri				0.09	

Figure 16. (SAAN) Proportion of surveys in which each species was detected. For each species and monitoring year, proportion of surveys in which that species was detected. For example, *Incilius nebulifer* (Gulf Coast Toad) was detected in 25% of surveys in 2011 and 92% of surveys in 2012. Cell colors range from purple (low proportion of detections) to red (high proportion). White cells represent years in which the species was not detected at all.

VICK

VICK straddles a potential zone of hybridization for the genus *Anaxyrus*, with many captured individuals exhibiting characteristics of more than one species. Therefore, individuals of this genus are combined in the category “*Anaxyrus* spp.”, which is considered a single species in VICK analyses.

The number of amphibian species detected at VICK was comparable to JELA and GUIB, but dynamics index by monitoring year was lower, with only 60% of potential species detected each year, on average (Table 2). VICK had the lowest species dynamics index by survey event (23%) among the four sites. These data suggest a relatively high number of amphibian species are found at VICK, but they occur at low abundances or with low detection probabilities. There was no obvious temporal trend in dynamics index by monitoring year or survey (Figure 17). The largest number of species recorded for a monitoring year was in 2013 ($N = 9$), corresponding with the lowest Shannon and inverse Simpson diversity indices among years (Table 12; Figure 18).

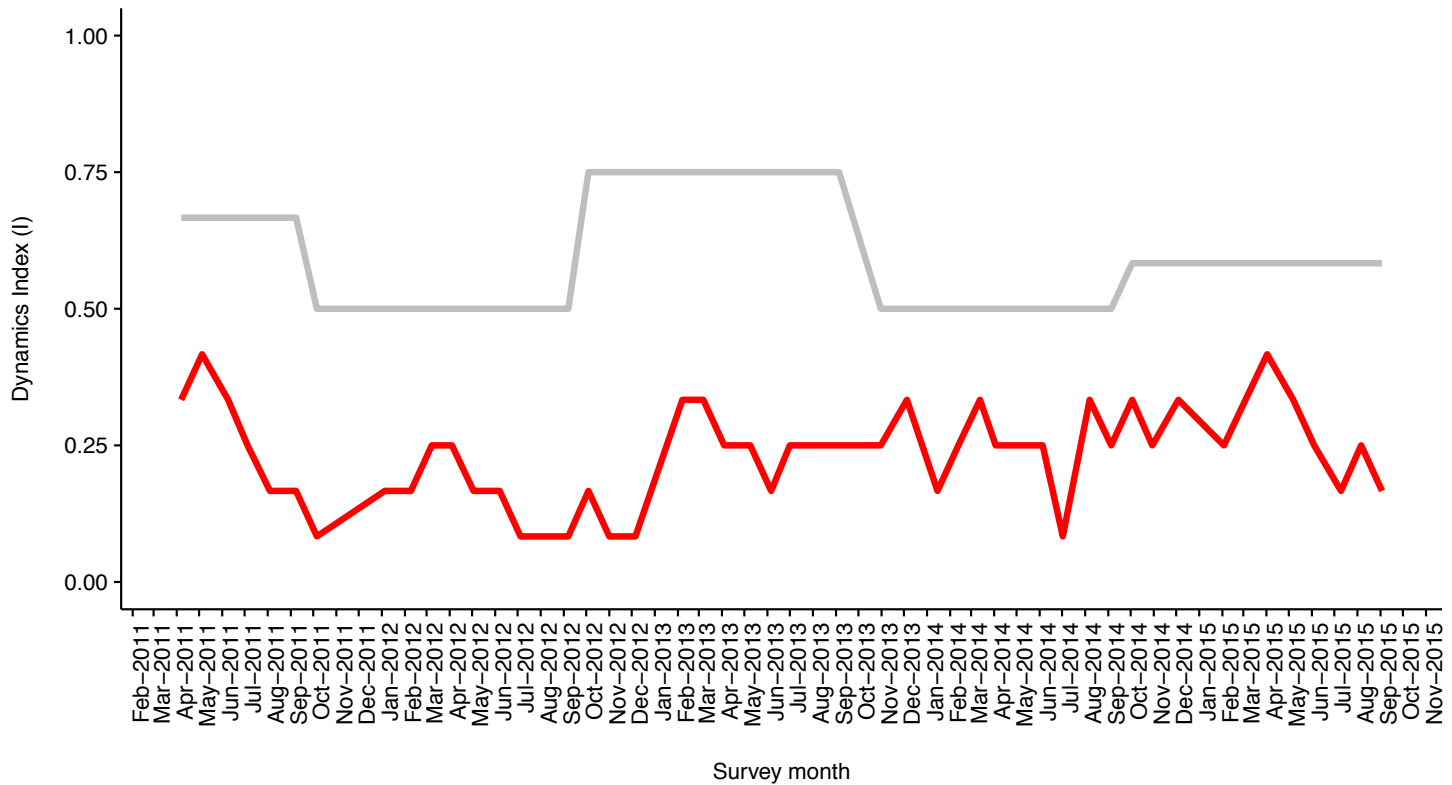


Figure 17. (VICK) Dynamics plot. Gray bars represent the Dynamics Index for each monitoring year, i.e., the proportion of species (out of the 12 species known to occur at VICK) recorded in that monitoring year. Red line represents the Dynamics Index for each survey event, i.e., the proportion of species (out of 12) recorded in that survey event.

Table 12. (VICK) Species diversity and turnover statistics. Appear = proportion of species detected in that year relative to the combined number of species detected in that or the previous monitoring year; Disappear = proportion of species not detected in that year relative to the combined number of species detected in that or the previous monitoring year.

Monitoring year	Number of surveys	Species richness	Appear	Disappear
2011	6	8	NA	NA
2012	12	6	0.20	0.40
2013	11	9	0.40	0.10
2014	11	6	0.00	0.33
2015	11	7	0.25	0.12

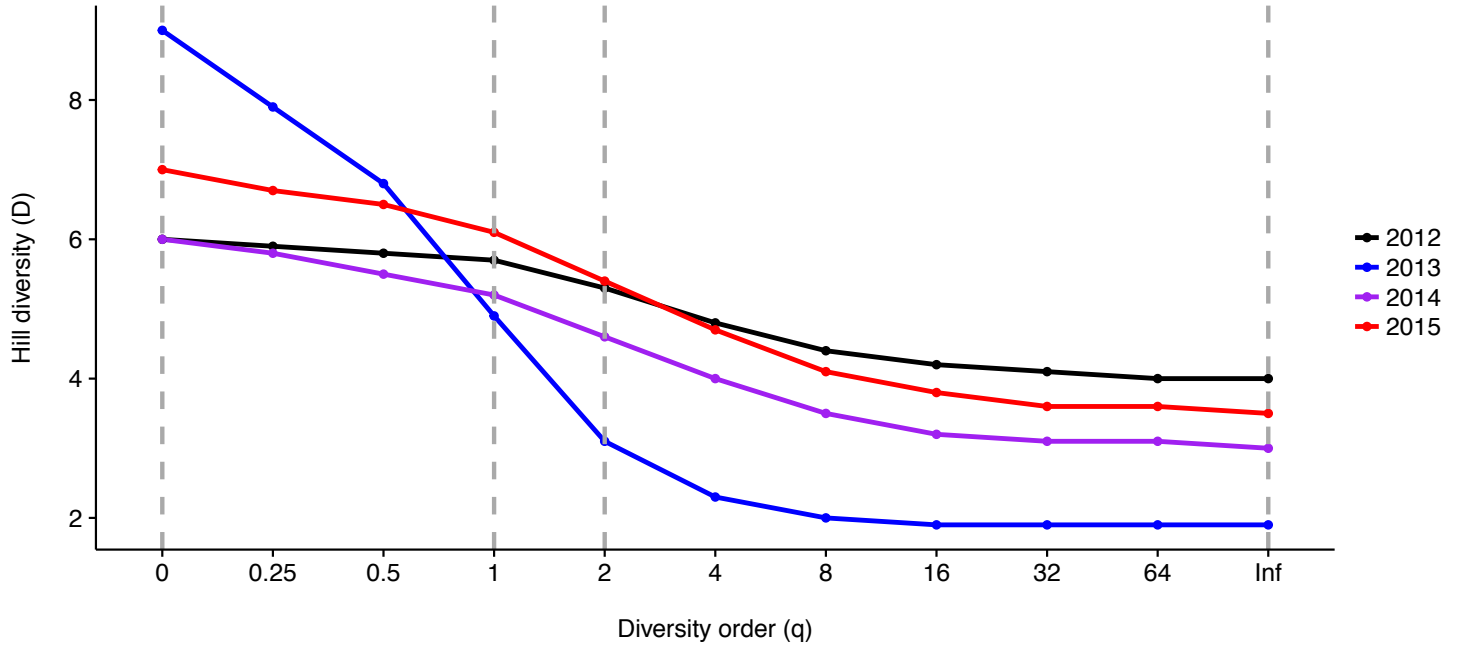


Figure 18. (VICK) Hill diversities. Colored lines represent different monitoring years (2011 omitted due to few surveys). Diversity estimates for each year are based on the maximum single-survey count for each species. Orders of diversity (q) are presented sequentially, but not to scale, on the x-axis. The vertical gray dashed lines identify orders of diversity that are commonly used ($q = 0$ is species richness, $q = 1$ is Shannon diversity, $q = 2$ is inverse Simpson diversity, $q = \text{Infinity}$ is exponentiated Berger-Parker index). See Appendix A2 for an overview of Hill diversities.

At VICK, the maximum single-survey count of any species was 15 individuals captured in 2013 from the combined category *Anaxyrus* spp. (Table 13). Regardless of diversity order, VICK diversity did not exhibit any clear monotonic trend from 2012 to 2015 (Figure 19). For Shannon and inverse Simpson diversities, 95% confidence intervals overlapped extensively in most cases except for differences between 2013 and 2015 diversities based on standardized estimates. Most species (83%) were detected by coverboard traps, and all were detected at least once during breeding season surveys (Table 14).

Table 13. (VICK) Maximum single-survey count for each species, for full and reduced sampling frequencies. For each species known to occur at VICK, the maximum single-survey count for different sampling frequencies is presented in columns immediately following each monitoring year. These data are the basis for diversity estimates in the Figure 19. *EXAMPLE: In 2012, the highest count of Acris blanchardi in a single survey was 1 individual. With reduced sampling (6 months per year, alternate months), the highest count was still 1 individual.*

species	monitor.yr	full	6mos	monitor.yr	full	6mos
Acris_blanchardi	2012	1	1	2013	0	0
Ambystoma_maculatum	2012	2	2	2013	1	1
Anaxyrus_sp	2012	1	1	2013	15	15
Desmognathus_conanti	2012	1	1	2013	2	2
Eurycea_guttolineata	2012	2	2	2013	3	2
Gastrophryne_carolinensis	2012	0	0	2013	0	0
Hyla_cinerea	2012	0	0	2013	1	0
Hyla_versicolor_chrysoscelis	2012	0	0	2013	3	1
Lithobates_clamitans	2012	0	0	2013	1	0
Lithobates_sphenocephalus	2012	0	0	2013	0	0
Plethodon_mississippi	2012	1	0	2013	1	1
Scaphiopus_holbrookii	2012	0	0	2013	1	1

species	monitor.yr	full	6mos	monitor.yr	full	6mos
Acris_blanchardi	2014	0	0	2015	0	0
Ambystoma_maculatum	2014	0	0	2015	0	0
Anaxyrus_sp	2014	4	2	2015	2	1
Desmognathus_conanti	2014	2	2	2015	4	4
Eurycea_guttolineata	2014	7	2	2015	6	5
Gastrophryne_carolinensis	2014	0	0	2015	0	0
Hyla_cinerea	2014	3	3	2015	0	0
Hyla_versicolor_chrysoscelis	2014	4	4	2015	4	4
Lithobates_clamitans	2014	1	1	2015	1	0
Lithobates_sphenocephalus	2014	0	0	2015	2	2
Plethodon_mississippi	2014	0	0	2015	2	2
Scaphiopus_holbrookii	2014	0	0	2015	0	0

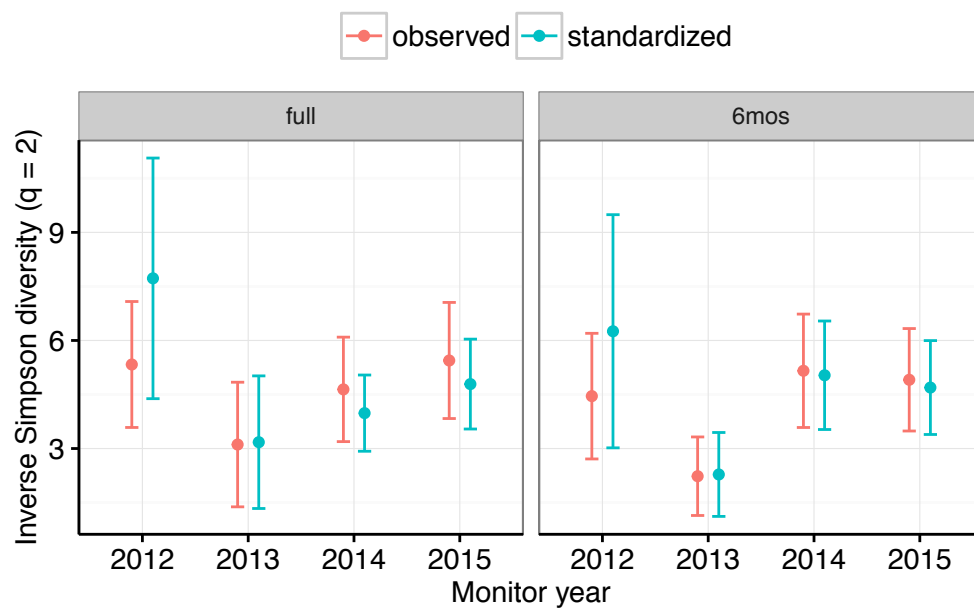
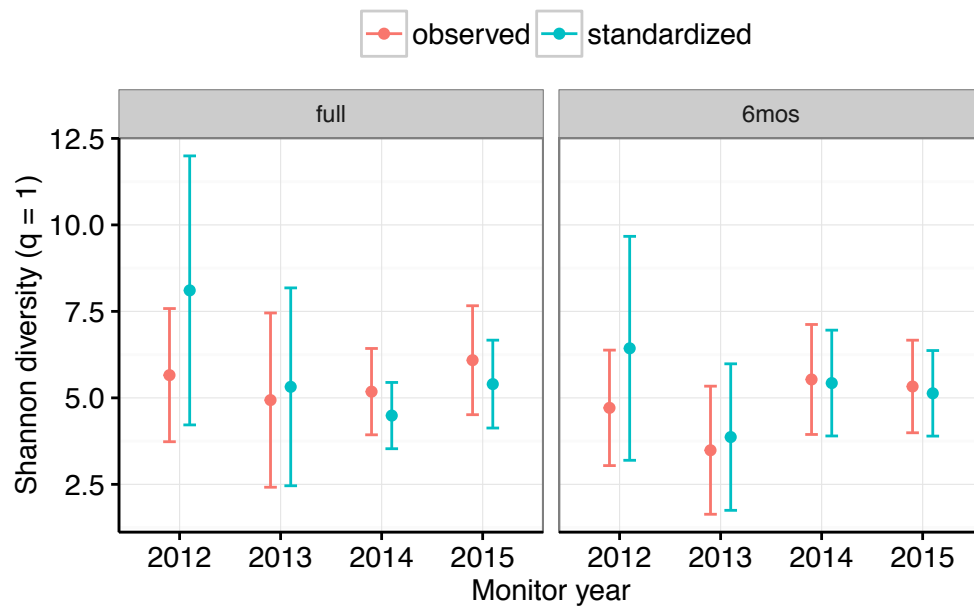
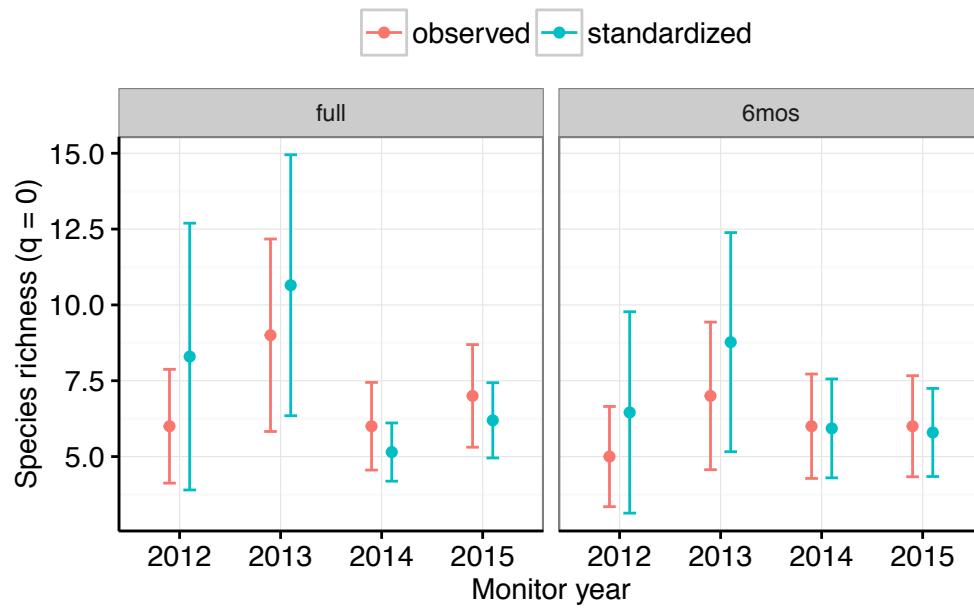


Figure 19. (VICK) Observed and standardized Hill diversities for full and reduced sampling frequencies. Hill diversities and bootstrapped 95% confidence intervals for 2012–2015 at two sampling frequencies (vertical panels; full = 12 months/yr; 6mos = alternate months). The top row of graphs is species richness (Hill order 0); middle row, Shannon diversity (Hill order 1); bottom row, inverse Simpson diversity (Hill order 2). Diversity estimates for each year are based on the maximum single-survey count for each species. Observed diversities (red) are based on the sample data. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage (84.9% for full data, 65.7% for 6-month sampling), determined according to Chao et al. (2014). See Appendix A3 for an overview of standardizing and bootstrapping Hill diversities.

Table 14. (VICK) Proportion of species detected by each trap type or season. EXAMPLE: 83% of the potential species at VICK were detected by coverboard traps and 75% of the species were detected during non-breeding season surveys.

Method	Proportion.detected
CB	0.83
PVC	0.17
MT	NA
Nonbreeding season (0)	0.75
Breeding season (1)	1.00

Only one-quarter of species at VICK were detected in all monitoring years (Figure 20). One-third of species were regularly detected in a majority of surveys each year (Figure 21). Half of all species were observed during only one or two monitoring years. Given these low and sporadic detections, the gain or loss of species at VICK may be difficult to confirm without relatively long time series.

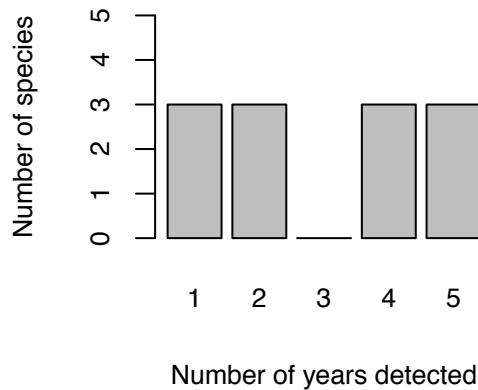


Figure 20. (VICK) Frequency of species detections. Frequency of detection for species known to occur at VICK, e.g., 3 species were detected in only one monitoring year; 3 species were detected in all five monitoring years.

	2011	2012	2013	2014	2015
Eurycea_guttolineata	0.83	0.58	0.45	0.82	0.91
Anaxyrus_sp	0.67	0.25	0.91	0.45	0.45
Desmognathus_conanti	0.5	0.25	0.36	0.73	0.64
Hyla_versicolor_chrysoscelis	0.17		0.18	0.55	0.55
Plethodon_mississippi	0.33	0.17	0.27		0.27
Hyla_cinerea			0.09	0.36	
Lithobates_clamitans	0.17		0.09	0.09	0.09
Scaphiopus_holbrookii	0.33		0.09		
Gastrophryne_carolinensis	0.33				
Ambystoma_maculatum		0.08	0.18		
Lithobates_sphenocephalus					0.09
Acris_blanchardi		0.08			

Figure 21. (VICK) Proportion of surveys in which each species was detected For each species and monitoring year, proportion of surveys in which that species was detected. For example, *Eurycea guttolineata* (Three-lined Salamander) was detected in 83% of surveys in monitoring year 2011 and 58% of surveys in 2012. Cell colors range from purple (low proportion of detections) to red (high proportion). White cells represent years in which the species was not detected at all.

Discussion

Summary of findings

The findings in this report are preliminary. With only five years of GRAMP data available, diversity estimates are highly sensitive to variation in the relative numbers of species detected in particular years. In addition, perceived trends may be biased by the smaller number of surveys conducted in the first year of GRAMP monitoring (2011).

Based on these preliminary data, SAAN was the least diverse of the four sites assessed. It had by far the fewest species recorded ($N = 5$, compared to $N = 12\text{--}15$ for other sites), with captures almost entirely dominated by two common species, *Eleutherodactylus cystignathoides* and *Incilius nebulifer*. The remaining three species at SAAN were each detected in only one or two of SAAN's 53 surveys. Similar to SAAN, captures at JELA and GUIS were highly skewed toward a few species. At JELA the numerically dominant species were *Hyla squirella*, *Eleutherodactylus planirostris*, and *Lithobates clamitans*; at GUIS, *Hyla squirella* and *Eleutherodactylus planirostris*. Individuals were more evenly distributed at VICK, but the total number of individuals detected at VICK in any survey was almost an order of magnitude lower than at the other sites. The four sites had very different species compositions—only *Hyla cinerea* was found at all sites. More than a third of recorded species at each site were not found at the other three sites.

High turnover rates (indicated by dynamics indices) at GRAMP sites suggested low and variable detection probabilities or high levels of transience (species moving on and off site). Either factor could make it difficult to identify temporal trends in diversity. On average, slightly over 70% of species known to occur at JELA and GUIS were detected at least once in a year of monthly surveys. At VICK, only 60% were detected in an average year. On average in a single survey, less than 30% of known species were detected at GUIS and VICK; at JELA, 45%. Dynamics indices for SAAN reflect that only the two most common species were detected in most surveys.

No GRAMP site exhibited a clear monotonic trend in diversity (species richness, Shannon diversity, or inverse Simpson diversity) from 2012 to 2015 (2011 data were excluded due to fewer surveys and potential bias due to “settling in” of coverboard traps). Observed (but not standardized) species richness was always the same or lower with sampling frequencies of 3 or 6 months per year compared to 12 months per year. Reduced sampling frequencies had inconsistent effects on Shannon and inverse Simpson diversity, sometimes causing diversity to increase and sometimes to decrease.

Some sampling factors had species-specific impacts on detection rates. For example, the species captured by PVC traps (mostly treefrogs) were distinct from the species primarily detected by coverboard traps. Therefore, the relative abundances of species at each site was influenced by the relative abundances of different trap types. The number of species detected during breeding versus non-breeding season surveys did not differ in any consistent way across sites, but certain taxa (e.g., *Acris spp.*) tended to be captured during breeding season months while others (e.g., *Hyla spp.*) were more frequently captured during non-breeding season months (Appendix A1).

Detection probabilities

Uncertainties exist regarding factors that influence detection probabilities in GRAMP studies. Recent statistical advances have focused on improving diversity estimates and trend detection by addressing species-specific detection probabilities in survey design and analyses (Kery et al. 2009; Iknayan et al. 2014). Detection probability could not be accounted for in our assessment of the first five years of GRAMP data, but it is an important issue to consider when interpreting data produced by any species monitoring program so is briefly expanded upon here.

Detection probability—the probability of detecting an animal that is present—can vary among species, sites, survey methods, survey events (e.g., due to weather or other survey conditions), and observers. Variation in detection probabilities, if uncorrected in analyses, can bias diversity estimates (Kellner and Swihart 2014). For example, in refuge choice field studies, Hoffmann et al. (2009) found that *Osteopilus septentrionalis* (Cuban Treefrogs) are eight times more likely to choose plants over PVC pipes for refuge, *Hyla squirella* (Squirrel Treefrogs) choose PVC pipes more than twice as often as plants, and *Hyla cinerea* (Green Treefrogs) exhibit no obvious preference. If these three species occurred at similar abundances at a site, differences in their detection probabilities would lead to underestimates in diversity due to observed (but not actual) low species evenness.

Diversity estimates uncorrected for detection probabilities can also lead to wrong conclusions about temporal trends in diversity. For example, for some amphibian species, use of artificial cover such as coverboards and PVC pipes is inversely related to the availability of natural cover (e.g., Grover 1998). If natural cover at a site declines over time, researchers may observe an increase in species richness, through greater detection of rare species. However, the mechanism underlying this apparent increase in diversity would be reduced habitat quality (loss of natural cover) leading to greater use of artificial covers (traps) by amphibians, rather than an increase in the species diversity of the site.

To minimize biases due to incomplete and variable detection, species- and survey-specific detection probabilities can be incorporated in hierarchical models for species diversity (Dorazio et al. 2006; Kery and Royle 2016). Information from replicate surveys of populations, conducted over short time periods, are used to determine detection probabilities. Observed counts are then adjusted based on these estimates. Additional factors potentially influencing species occupancy, abundance, or detection are incorporated in the hierarchical model as covariates. For amphibian monitoring, relevant covariates may include time-of-year, temperature, precipitation, vegetation structure and type, and availability of freshwater bodies including coverage, proximity, and transience (Grover 1998; Brodman 2009; Smith et al. 2014). The application of hierarchical models with covariate data emphasizes a biologically oriented, process-driven approach to estimating diversity, in contrast to traditional extrapolation and standardization methods (Appendix A3) that use probability theory to correct for sampling bias. Some recent studies of amphibian diversity that incorporate detection probabilities provide insights on relevant covariates and appropriate sampling frequencies for hierarchical models (Weir et al. 2005; Gomez-Rodriguez et al. 2010; Smith et al. 2014).

Literature cited

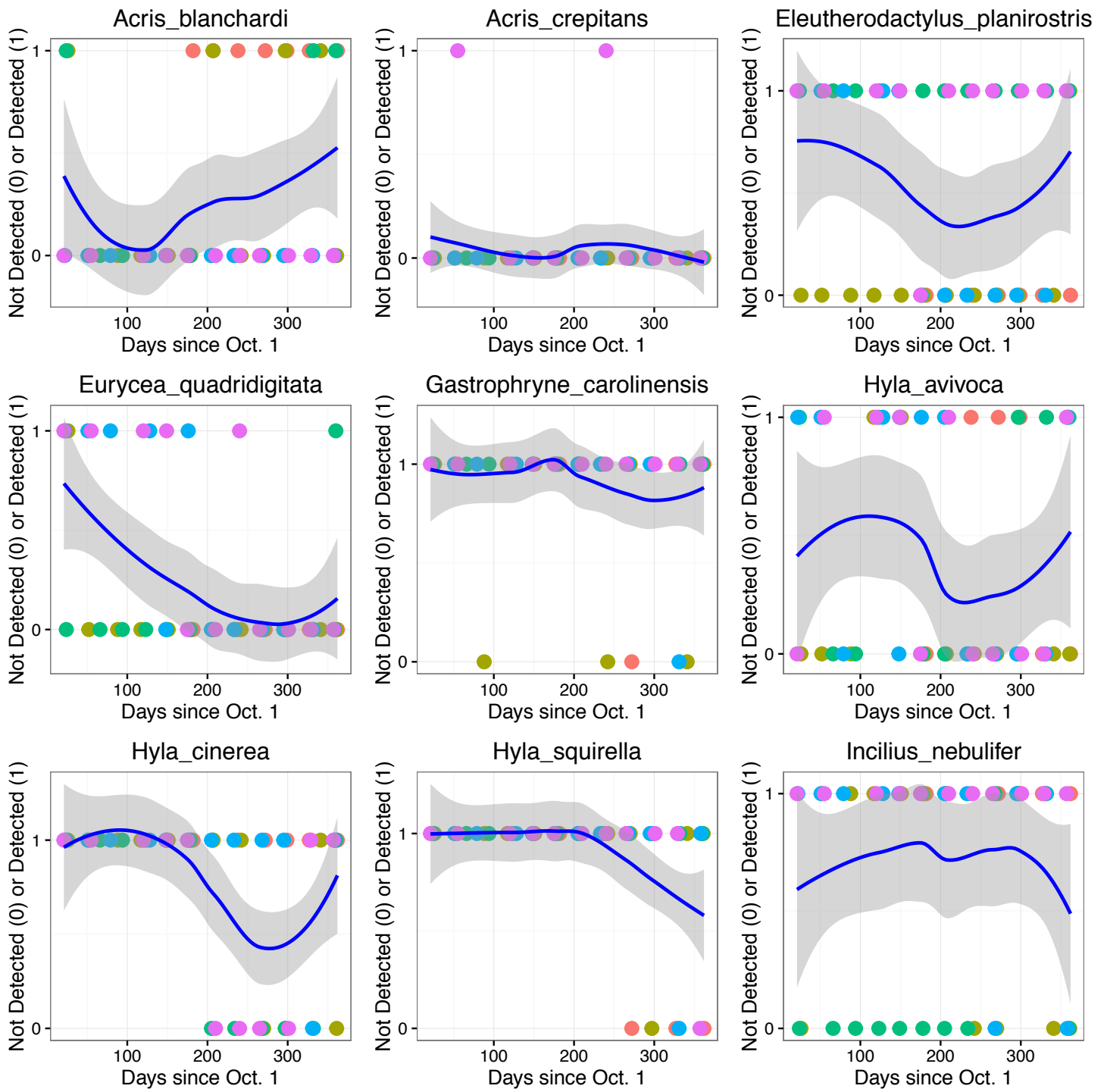
- Boughton, R.G., J. Staiger, and R. Franz. 2000. Use of PVC pipe refugia as a sampling technique for hyliid treefrogs. *American Midland Naturalist* 144:168–177.
- Brodman, R. 2009. A 14-year study of amphibian populations and metacommunities. *Herpetological Conservation and Biology* 4:106–119.
- Chao, A., N.J. Gotelli, T.C. Hsieh, E.L. Sander, K.H. Ma, R.K. Colwell, and A.M. Ellison. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* 84:45–67.
- Chao, A. and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93: 2533–2547.
- Dorazio, R.M., J.A. Royle, B. Söderström, and A. Glimskär. 2006. Estimating species richness and accumulation by modeling species occurrence and detectability. *Ecology* 87:842–854.
- Gomez-Rodriguez C., A. Guisan, C. Diaz-Paniagua, and J. Bustamante. 2010. Application of detection probabilities to the design of amphibian monitoring programs in temporary ponds. *Annales Zoologici Fennici* 47(5):306–322.
- Grover, M.C. 1998. Influence of cover and moisture on abundances of the terrestrial salamanders *Plethodon cinereus* and *Plethodon glutinosus*. *Journal of Herpetology* 32:489–497.
- Hoffman, K.E., S.A. Johnson, and M.E. McGarrity. 2009. Interspecific variation in use of polyvinyl chloride (PVC) pipe refuges by hyliid treefrogs: a potential source of capture bias. *Herpetological Review* 40:423–426.
- Iknayan, K.J., M.W. Tingley, B.J. Furnas, and S.R. Beissinger. 2014. Detecting diversity: emerging methods to estimate species diversity. *Trends in Ecology & Evolution* 29:97–106.
- Kellner, K.F., and R.K. Swihart. 2014. Accounting for imperfect detection in ecology: A quantitative review. *PLoS ONE* 9(10):e111436.
- Kery, M., and J.A. Royle. 2016. *Applied Hierarchical Modeling in Ecology: Analysis of distribution, abundance and species richness in R and BUGS*. 1st Edition. Academic Press & Elsevier. 808 pages.
- Kery, M., J.A. Royle, M. Plattner, and R.M. Dorazio. 2009. Species richness and occupancy estimation in communities subject to temporary emigration. *Ecology* 90:1279–1290.
- Romanuk, T.N. and J. Kolasa. 2001. Simplifying the complexity of temporal diversity dynamics: a differentiation approach. *Ecoscience* 8:259–263.
- Smith, D.H.V., L. Randall, B.L. Jones, and D.R.C. Prescott. 2014. Difference in detection and occupancy between two anurans: The importance of species-specific monitoring *Herpetological Conservation and Biology* 9:267–277.
- Weir, L.A., J.A. Royle, P. Nanjappa, and R.E. Jung. 2005. Modeling anuran detection and site occupancy on North American Amphibian Monitoring Program (NAAMP) routes in Maryland. *Journal of Herpetology* 39(4): 627–639.

Appendix A1: Additional site-specific results

JELA

Table A1.1. (JELA) *For each species, proportion of detections by trap type and season. For each species, proportion of detections by each combination of trap type and season, and total number of detections (“Total detections”) summed over surveys. Non-breeding season = 0 and breeding season = 1, e.g., “CB_0” is proportion of detections at coverboard traps during non-breeding season surveys.*

Species	CB_0	CB_1	PVC_0	PVC_1	MT_0	MT_1	Total detections
<i>Hyla_squirella</i>	-	-	0.84	0.16	-	-	1123
<i>Lithobates_clamitans</i>	0.02	0.02	-	-	0.74	0.22	930
<i>Hyla_cinerea</i>	0.01	-	0.66	0.31	-	0.02	343
<i>Eleutherodactylus_planirostris</i>	0.52	0.48	-	-	-	-	292
<i>Gastrophryne_carolinensis</i>	0.51	0.47	-	-	-	0.02	284
<i>Notophthalmus_viridescens</i>	0.09	0.17	-	-	0.4	0.34	95
<i>Incilius_nebulifer</i>	0.41	0.59	-	-	-	-	63
<i>Acris_blanchardi</i>	-	0.18	-	-	0.12	0.71	51
<i>Hyla_avivoca</i>	-	-	0.52	0.24	-	0.24	42
<i>Eurycea_quadridigitata</i>	0.92	0.08	-	-	-	-	37
<i>Lithobates_catesbeianus</i>	-	-	-	-	0.33	0.67	3
<i>Acris_crepitans</i>	0.5	-	-	-	-	0.5	2
<i>Siren_intermedia</i>	-	-	-	-	0.5	0.5	2
<i>Pseudacris_crucifer</i>	-	-	-	-	1	-	1



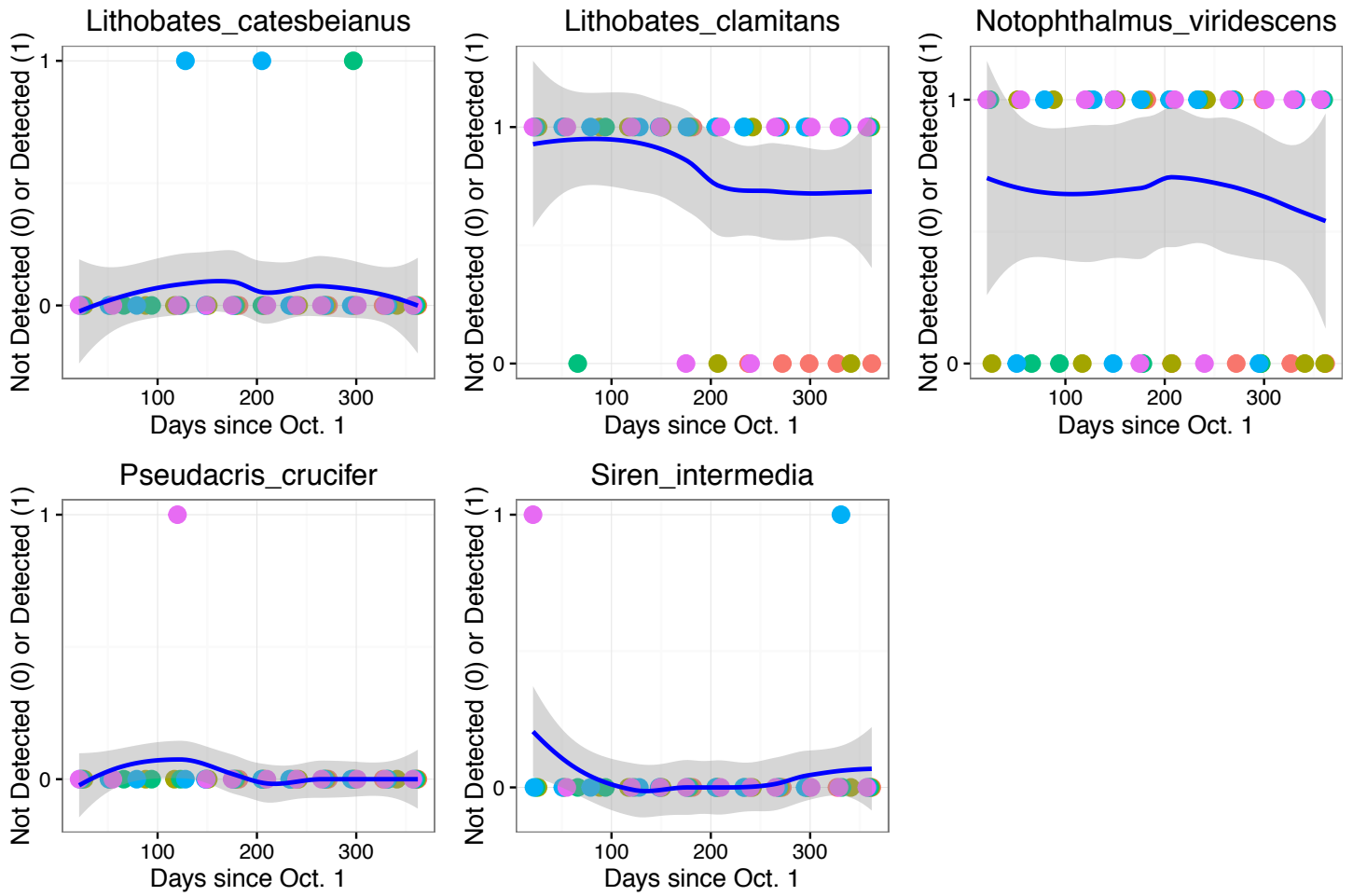
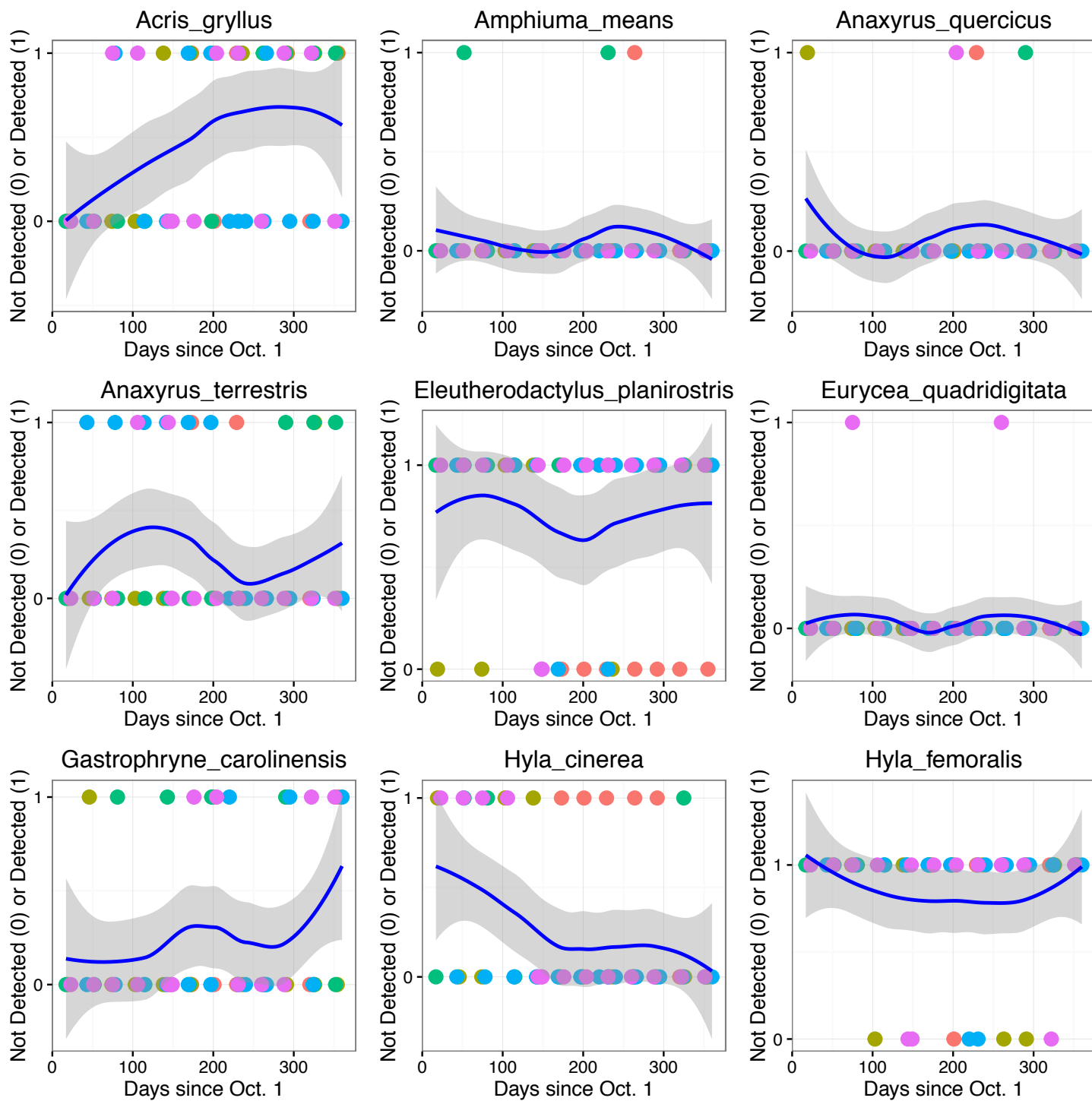


Figure A1.1. (JELA) For each species, detection as a function of day-of-year (start date, Oct. 1). Each point represents a survey event—“1” if the species was detected during that survey and “0” if it was not detected. LOESS curve added to aid with visualization of trend, because overlapping points are difficult to see. Colors represent monitoring year: 2011 = orange, 2012 = bronze, 2013 = green, 2014 = blue, 2015 = pink.

GUIS

Table A1.2. (GUIS) For each species, proportion of detections by trap type and season. For each species, proportion of detections by each combination of trap type and season, and total number of detections (“Total detections”) summed over surveys. Non-breeding season = 0 and breeding season = 1, e.g., “CB_0” is proportion of detections at coverboard traps during non-breeding season surveys.

Species	CB_0	CB_1	PVC_0	PVC_1	MT_0	MT_1	Total detections
Eleutherodactylus_planirostris	0.66	0.34	-	-	-	-	434
Hyla_squirella	-	0.01	0.78	0.21	-	-	356
Hyla_femoralis	0.04	-	0.46	0.42	-	0.08	250
Acris_gryllus	0.03	0.05	-	-	0.13	0.79	101
Hyla_cinerea	-	-	0.38	0.5	0.05	0.08	40
Anaxyrus_terrestris	0.41	0.26	-	-	-	0.33	27
Gastrophryne_carolinensis	0.21	0.79	-	-	-	-	24
Lithobates_sphenocephalus	-	-	-	-	0.38	0.62	21
Lithobates_clamitans	-	-	-	-	0.67	0.33	9
Lithobates_catesbeianus	-	-	-	-	-	1	7
Lithobates_grylio	-	-	-	-	-	1	6
Anaxyrus_quercicus	0.25	0.5	-	-	-	0.25	4
Plethodon_grobmani	0.75	0.25	-	-	-	-	4
Amphiuma_means	-	-	-	-	0.33	0.67	3
Eurycea_quadridigitata	0.67	0.33	-	-	-	-	3



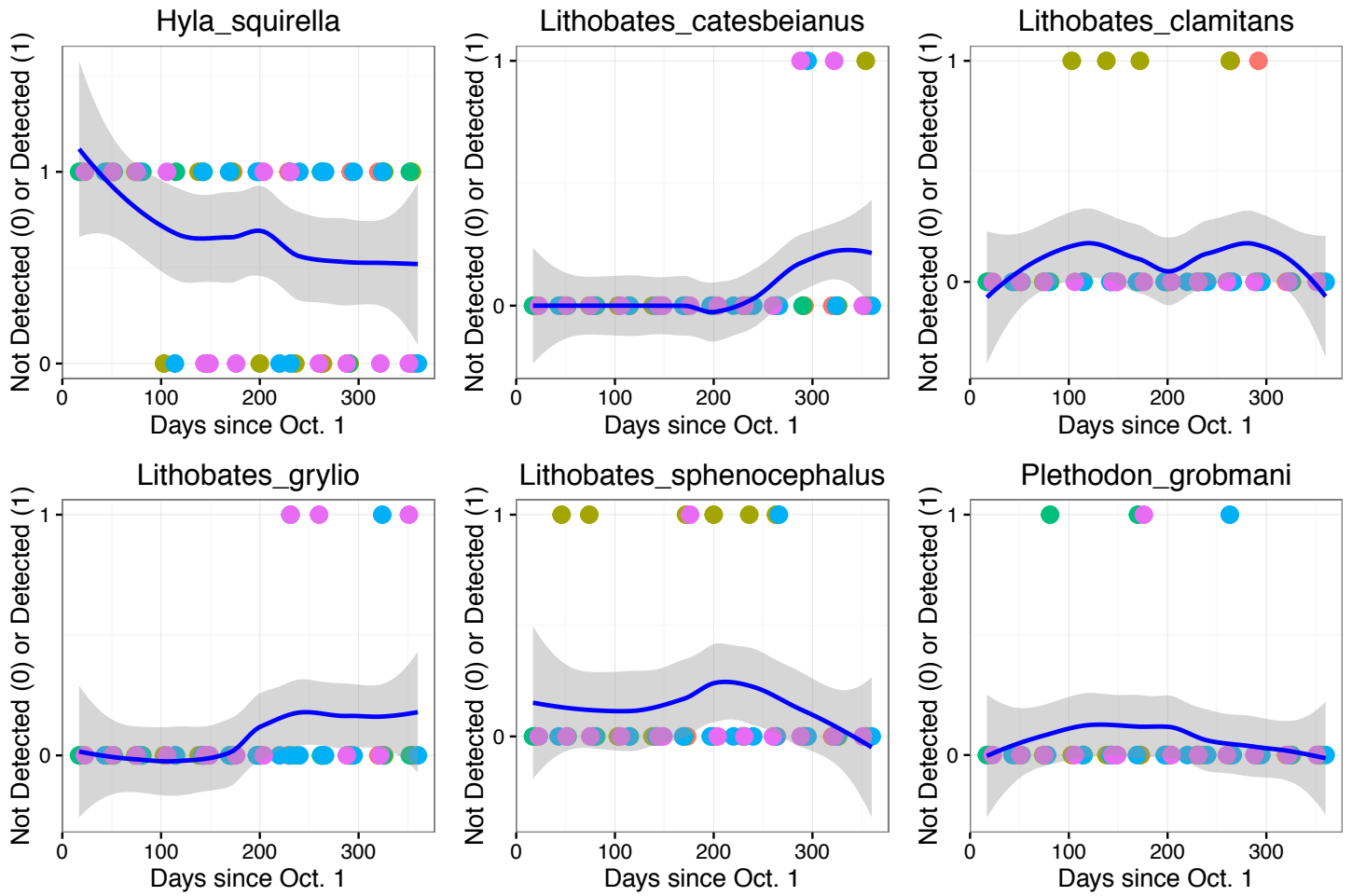


Figure A1.2. (GUIS) For each species, detection as a function of day-of-year (start date, Oct. 1). Each point represents a survey event—“1” if the species was detected during that survey and “0” if it was not detected. LOESS curve added to aid with visualization of trend, because overlapping points are difficult to see. Colors represent monitoring year: 2011 = orange, 2012 = bronze, 2013 = green, 2014 = blue, 2015 = pink.

SAAN

Table A1.3. (SAAN) For each species, proportion of detections by trap type and season. For each species, proportion of detections by each combination of trap type and season, and total number of detections (“Total detections”) summed over surveys. Non-breeding season = 0 and breeding season = 1, e.g., “CB_0” is proportion of detections at coverboard traps during non-breeding season surveys.

Species	CB_0	CB_1	PVC_0	PVC_1	MT_0	MT_1	Total detections
<i>Eleutherodactylus_cystignathoides</i>	0.33	0.67	-	-	NA	NA	1869
<i>Incilius_nebulifer</i>	0.64	0.36	-	-	NA	NA	1288
<i>Gastrophryne_olivacea</i>	-	1	-	-	NA	NA	3
<i>Hyla_cinerea</i>	-	-	1	-	NA	NA	1
<i>Lithobates_berlandieri</i>	1	-	-	-	NA	NA	1

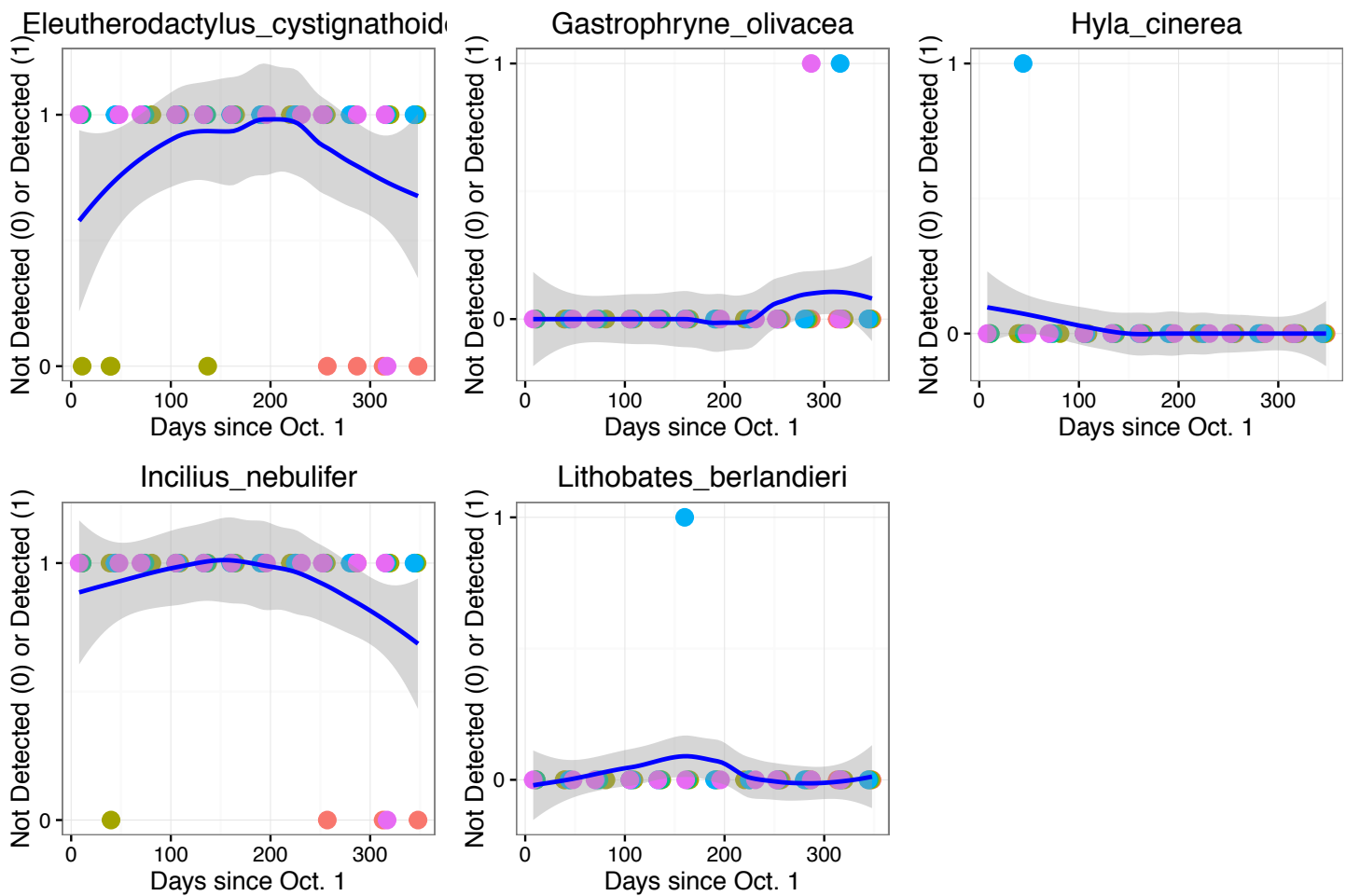
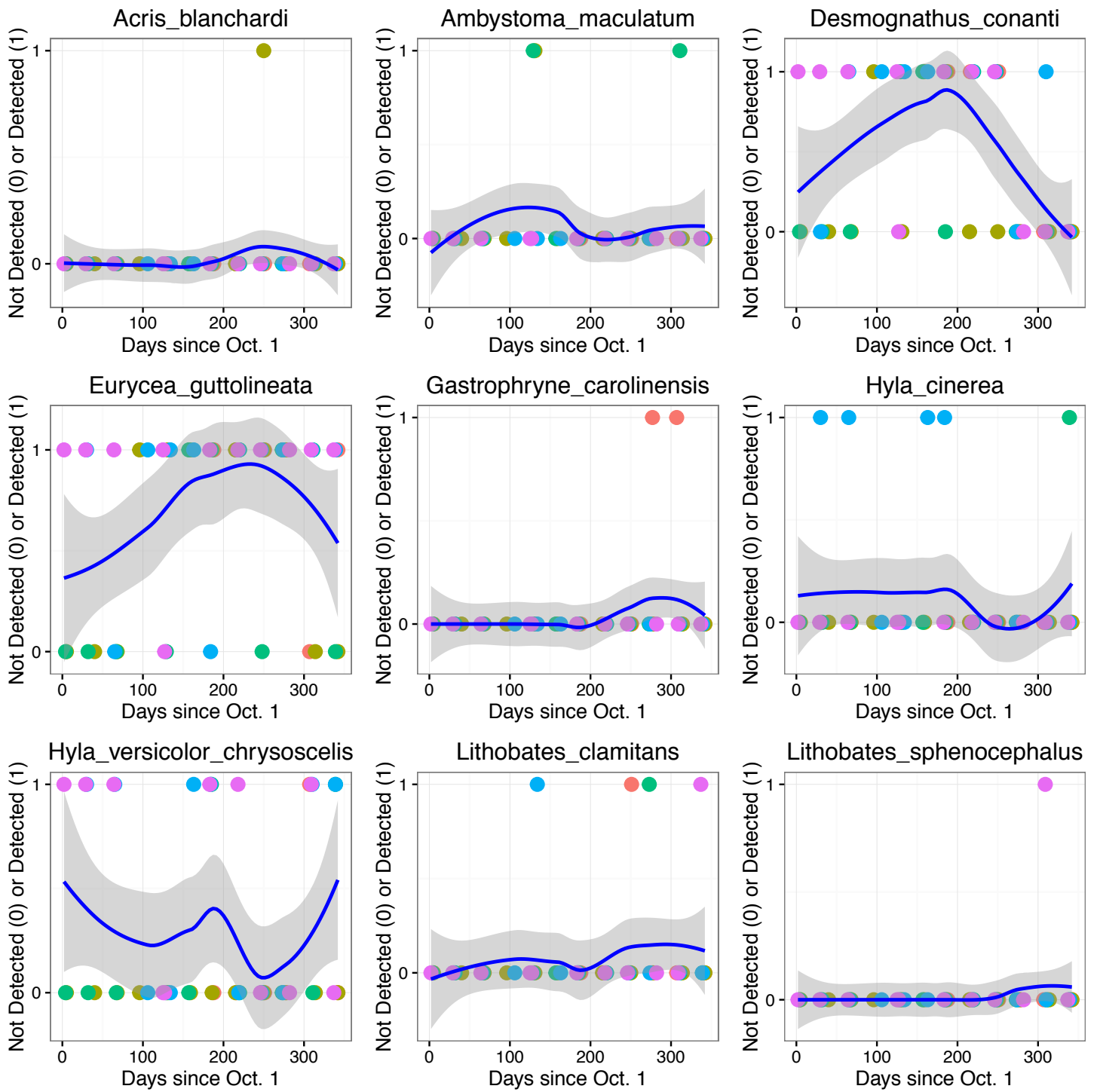


Figure A1.3. (SAAN) For each species, detection as a function of day-of-year (start date, Oct. 1). Each point represents a survey event—“1” if the species was detected during that survey and “0” if it was not detected. LOESS curve added to aid with visualization of trend, because overlapping points are difficult to see. Colors represent monitoring year: 2011 = orange, 2012 = bronze, 2013 = green, 2014 = blue, 2015 = pink.

VICK

Table A1.4. (VICK) For each species, proportion of detections by trap type and season. For each species, proportion of detections by each combination of trap type and season, and total number of detections (“Total detections”) summed over surveys. Non-breeding season = 0 and breeding season = 1, e.g., “CB_0” is proportion of detections at coverboard traps during non-breeding season surveys.

Species	CB_0	CB_1	PVC_0	PVC_1	MT_0	MT_1	Total detections
Eurycea_guttolineata	0.24	0.76	-	-	NA	NA	86
Anaxyrus_sp	0.2	0.8	-	-	NA	NA	50
Desmognathus_conanti	0.45	0.55	-	-	NA	NA	38
Hyla_versicolor_chrysoscelis	-	-	0.42	0.58	NA	NA	31
Plethodon_mississippi	0.5	0.5	-	-	NA	NA	12
Hyla_cinerea	-	-	0.6	0.4	NA	NA	10
Lithobates_clamitans	0.25	0.75	-	-	NA	NA	4
Ambystoma_maculatum	0.75	0.25	-	-	NA	NA	4
Scaphiopus_holbrookii	0.33	0.67	-	-	NA	NA	3
Gastrophryne_carolinensis	-	1	-	-	NA	NA	2
Lithobates_sphenocephalus	-	1	-	-	NA	NA	2
Acris_blanchardi	-	1	-	-	NA	NA	1



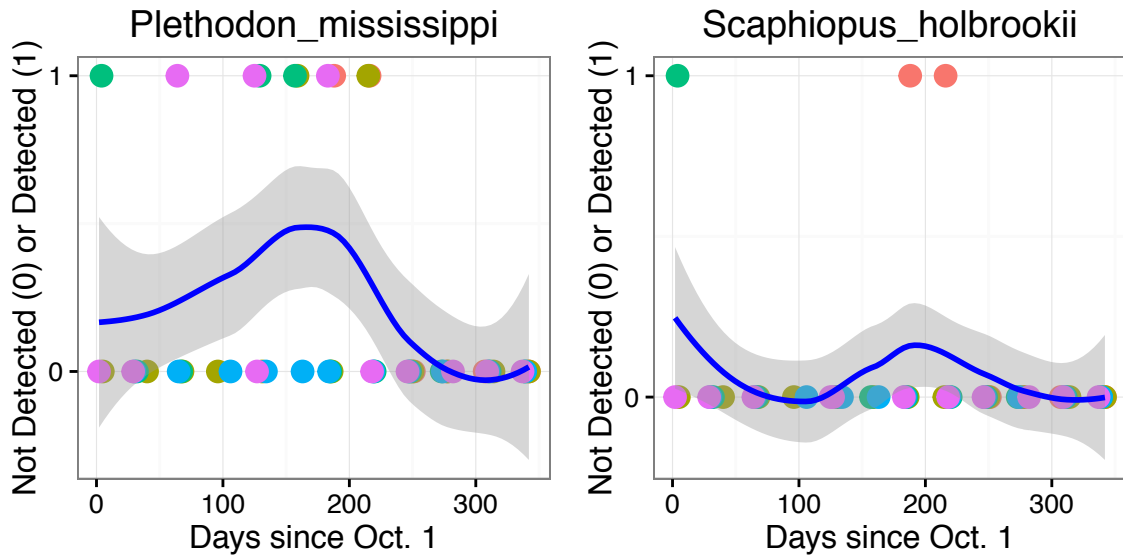


Figure A1.4. (VICK) For each species, detection as a function of day-of-year (start date, Oct. 1). Each point represents a survey event—“1” if the species was detected during that survey and “0” if it was not detected. LOESS curve added to aid with visualization of trend, because overlapping points are difficult to see. Colors represent monitoring year: 2011 = orange, 2012 = bronze, 2013 = green, 2014 = blue, 2015 = pink.

Appendix A2: Measuring and understanding species diversity

This Appendix gives an overview of commonly applied indices of species diversity, how the indices are related to Hill diversity numbers, and how Hill diversity profiles can be used to gain a better understanding of the richness and evenness of a community. The meaning of species richness—the number of unique species in a community—is easy to grasp. But if we were told that the Shannon-Wiener index for a community increased while the inverse Simpson diversity index remained the same, what could we say about how the community has changed and whether or not we should be alarmed?

This brief guidance document is intended to assist resource managers in understanding and applying diversity statistics relevant to their management objectives. We present highlights of ideas that are covered in much greater depth in Hill (1973) and Jost (2006). Our discussion focuses on site-specific species diversity. In this Appendix we do not address how sample size and coverage affect diversity estimates (see Appendix A3). We also do not consider biases caused by unequal detection probabilities (see main document). Instead, our examples in this Appendix assume we have complete knowledge about the species composition of hypothetical communities. The R code for examples in Appendix A2 is provided as accompanying script.

Hill diversity numbers: A unifying concept of species diversity

More than 200 indices have been developed to measure biological diversity (Laffan et al. 2010). No doubt, this plethora of indices has been both a source and a consequence of confusion on how to monitor diversity for conservation and management purposes. Hill (1973) presented a unified concept of species diversity, which clarified a relationship and continuity among many diversity indices frequently used in ecology. Thirty years later, Jost (2006) reintroduced Hill diversities in one of the most highly cited (> 1100 times) diversity publications of the past decade, attesting to the intuitive appeal of the Hill diversity concept to ecologists.

In his derivation of “Hill diversities” (aka “true diversities” or “effective species richness”), Hill proposed that many indices of diversity are simply transformations or limits of the function:

$${}^qD = \left(\sum_{i=1}^S p_i^q \right)^{1/(1-q)}$$

In this equation, which we will call the “Hill diversity equation”, D is the Hill diversity, S is the total number of species observed in the sample, p_i is the proportional abundance of the i^{th} species, and q is the “order” of diversity. The notation of the Hill diversity equation applies to all equations in this Appendix.

The Hill diversity (D) for a community can be estimated for a continuum of diversity orders, from $q = 0$ to Infinity. The order of diversity (q) indicates how sensitive D is to the richness versus evenness of a community. At lower orders of diversity (low q -values), D primarily reflects the richness of a community—a large D means a community has many species. At higher orders, a large D means the individuals of a community are distributed more equally among species, i.e., the community has high evenness. Mathematically speaking, when $q = 1$, D weighs species proportional to their frequencies (note that the Hill diversity equation is undefined for $q = 1$, but its limit exists). At $q < 1$, D gives disproportionately greater weight to rare species. For $q > 1$, D gives disproportionately greater weight to common species. We can monitor trends in diversity at an order that best fits our beliefs about the relative importance of richness versus evenness in the diversity of a community. For example, if we want to understand changes in diversity with more emphasis on species evenness, we might calculate diversity at $q = 4$. If we are more concerned with how the number of species may be changing, we can estimate diversity at $q = 0$. For a more comprehensive understanding of the diversity of a community, Hill diversities can be calculated at a range of orders including at least $q = 0$, 1, and 2 (Chao et al. 2014).

But what does Hill diversity really mean? A community in which all species are equally abundant has a Hill diversity equal to the total number of species in that community, regardless of diversity order. For example, given a community of five species that occur in equal abundances (each making up 20% of the community), ${}^0D = {}^1D = {}^2D \dots = {}^{Inf}D = 5$. As mentioned, at higher orders, D gives greater weight to the proportional abundances of more common species. It makes sense then that when species occur in equal proportions, D does not change with diversity order. In a community where species have unequal abundances, Hill diversity at $q = 0$ is still the number of species but D declines as the order of diversity increases. For example, given a community with five species that occur at the proportions (0.70, 0.21, 0.06, 0.02, 0.01), ${}^0D = 5$, ${}^1D = 2.39$, ${}^2D = 1.86$, and ${}^{Inf}D = 1.43$. The Hill diversity (D) at a particular order can be thought of as the community’s “effective species richness”, i.e., the species richness of a diversity-equivalent community (at that order) in which species are equally abundant. In other words, Hill diversities are standardized to the species richness of “reference” communities with perfect evenness.

Relationships between common diversity indices and Hill diversities

How are Hill diversities related to the diversity indices we are most familiar with? Species richness is Hill diversity at $q = 0$, exponentiating the Shannon-Wiener index gives us $q = 1$ diversity (aka “Shannon diversity”), the inverse of the Simpson diversity index is $q = 2$ diversity, and the inverse of the Berger-Parker index is $q = \text{Infinity}$ diversity. In fact, most diversity indices used in ecology are simply transformations of Hill diversities at different orders—hence, the recognition of the Hill diversity function as a unifying concept of diversity.

Species richness

Hill diversity of order 0 is the species richness of the community:

$${}^0D = \left(\sum_{i=1}^S p_i^0 \right)^{1/(1-0)}$$

When quantifying species richness, consideration is not given to how common or rare a species is. A species that makes up 95% of the population is counted equally with a species that makes up 1% of the population. Species richness represents one end of the continuum in Hill diversity orders.

Species richness as an index of diversity has well-known weaknesses, so is generally not recommended as a standalone measure of system diversity. First, species richness is highly sensitive to sample coverage and the number of individuals captured in a survey (Palmer 1990). Even if sample effort is consistent at a site (e.g., same numbers and types of traps) year-after-year, any factor that may influence capture success during a survey (e.g., weather, time-of-day, etc.) can affect the ratio of true to observed species richness. This occurs because most communities have a few common species and many very rare species. Common species can be detected with low sample effort, but detection of rare species is heavily dependent on sample effort and other factors that influence capture success. If these biases are not accounted for in sampling and analyses (see Appendix A3), estimates of species richness cannot be validly compared. Relative to species richness, diversities measured at higher orders converge asymptotically at lower sample sizes, so are less impacted by sample coverage. A second, related, issue with species richness is that its precision is low compared with higher orders of diversity. Third, species richness is not considered an ecologically meaningful measure of system diversity because rare species usually contribute much less to ecosystem function and ecological interactions than do common species, yet these are weighted equally.

Shannon-Wiener index

The Shannon-Wiener index (H') is a measure of the entropy in a system. This and other measures of entropy tell us about the uncertainty in predicting the species identity of an individual sampled from the community. If two communities have the same number of species, the community in which individuals are more evenly distributed among species would have a larger Shannon-Wiener index because it would be more difficult to predict the species of an individual sampled from that community. The Shannon-Wiener index is a monotonic function of the Hill diversity equation at $q = 1$, and is related by a simple conversion:

$$H' = - \sum_{i=1}^S p_i \ln(p_i)$$

$${}^1D = \exp(H')$$

(Note that the Hill diversity equation is undefined for $q = 1$, but its limit exists)

Simpson diversity index

The Simpson diversity index gives the probability that two randomly chosen individuals belong to the same species, as can be seen from its equation:

$$D_{Si} = \sum_{i=1}^S p_i^2$$

In a large population, the probability that two randomly chosen individuals belong to species i is the square of the proportional abundance of that species. The sum of these squared proportions is the Simpson diversity index. A modification of this equation corrects for small sample bias. The reciprocal of this equation is the inverse Simpson diversity index, which is equal to Hill diversity at $q = 2$:

$${}^2D = \left(\sum_{i=1}^S p_i^2 \right)^{1/(1-2)} = 1/D_{Si}$$

Berger-Parker index

The Berger-Parker index is the proportion of the most common species in the community:

$$D_{BP} = p_{max}$$

Its reciprocal is Hill diversity at $q = \text{Infinity}$:

$${}^{Inf}D = \left(\sum_{i=1}^S p_i^{Inf} \right)^{1/(1-Inf)} = 1/D_{BP}$$

The inverse Berger-Parker index sits at the opposite end of the Hill diversity continuum from species richness. While species richness counts every species in the community, no matter how rare, the inverse Berger-Parker index considers only the most common species, regardless of how many other species occur in the community.

At $q = \text{Infinity}$, it's mathematically simple to demonstrate the concept of Hill diversity as the effective species richness of a community. Given a community with eight species that occur at the proportions (0.50, 0.30, 0.10, 0.04, 0.02, 0.02, 0.01, 0.01), ${}^{Inf}D = 2$. This result is what we would expect when D is defined as the species richness of a diversity-equivalent community in which species are equally abundant. A diversity-equivalent community, at $q = \text{Infinity}$, must have its most common species making up 50% of the community. A community with the most common species making up 50% of the community and with equally abundant species would have two species that occur at the proportions (0.50, 0.50). The species richness of this diversity-equivalent reference community is 2, which matches ${}^{Inf}D$ for our hypothetical community of eight species.

Examples

Example A2.1

Let us compare the Hill diversities (D) for four communities (Table A2.1 and Figure A2.1) that differ in species richness and evenness, to understand how these twin aspects of diversity influence D at different orders.

Table A2.1. Four hypothetical communities for Example A2.1.

Community	Number of species	Number of individuals	Abundance distribution
A	20	140	Equal (7 individuals per species)
B	30	210	Equal (7 individuals per species)
C	20	140	Unequal (see R code in box)
D	30	210	Unequal (see R code in box)

Hand-calculating Hill diversities is a useful exercise for understanding the mathematics of diversity at different orders. Hill diversities can also be calculated with the free statistical program 'R', using the community ecology package 'vegan' (Oksanen et al. 2016):

```
### R code for calculating Hill diversities for the four hypothetical
### communities presented in Table A2.1. Install and load the package 'vegan'
```

```

### before running this code.

### Create vectors of species abundances. Each number in the vector is the
### number of individuals for a single species. For example, Community C has
### 20 species, the most common species had 47 individuals; second most
### common, 22 individuals, etc. The order of numbers in the vector does not
### matter.

A <- rep(7, 20) # repeat the number '7' twenty times (once for each of the 20 species)
B <- rep(7, 30)
C <- c(47, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1)
D <- c(70, 32, 22, 16, 12, 8, 6, 4, 3, 3, 3, 3, 3, 3, 3, 2, 2, 2, 2, 1, 1, 1,
      1, 1, 1, 1, 1, 1, 1, 1, 1)

### Calculate Hill diversities for the range of default orders q = c(0, 0.25,
### 0.5, 1, 2, 4, 8, 16, 32, 64, Inf). The function name is 'renyi' because
### Hill diversities are the exponent of the Renyi entropy. To obtain the Hill
### diversity for a specific order, use the argument 'scales' as in 'renyi(A,
### scales = 3, hill = TRUE)' to calculate diversity at order 3. In the output
### for each community, the diversity order (q) is specified in the first row,
### and D is in the second row.

round(renyi(A, hill = TRUE), 1)

##      0 0.25 0.5      1      2      4      8     16     32     64    Inf
##      20      20      20      20      20      20      20      20      20      20
## attr(,"class")
## [1] "renyi"      "numeric"

round(renyi(B, hill = TRUE), 1)

##      0 0.25 0.5      1      2      4      8     16     32     64    Inf
##      30      30      30      30      30      30      30      30      30      30
## attr(,"class")
## [1] "renyi"      "numeric"

round(renyi(C, hill = TRUE), 1)

##      0 0.25 0.5      1      2      4      8     16     32     64    Inf
## 20.0 16.7 13.9  9.7  6.0  4.2  3.5  3.2  3.1  3.0  3.0
## attr(,"class")
## [1] "renyi"      "numeric"

round(renyi(D, hill = TRUE), 1)

##      0 0.25 0.5      1      2      4      8     16     32     64    Inf
## 30.0 23.7 18.4 11.4  6.3  4.2  3.5  3.2  3.1  3.1  3.0
## attr(,"class")
## [1] "renyi"      "numeric"

```

Figure A2.1 shows that Community B (red line), with 30 equally abundant species, is the most diverse of the four communities. For a given number of species, the maximum Hill diversity—at any order—is achieved when the species are equally abundant, as is the case with Communities A and B. Communities C (blue line) and D (orange line) have the same numbers of species as A and B, respectively, as reflected in their diversity values at $q = 0$ (which is simply species richness). However, their declining diversities with increasing order indicate that these communities have low “evenness”, i.e., they are numerically dominated by

a few common species. Remember that lower orders of Hill diversities measure richness, and higher orders measure evenness. The rate at which Hill diversity declines with increasing order gives us an idea of how unevenly the individuals of a community are distributed among its species.

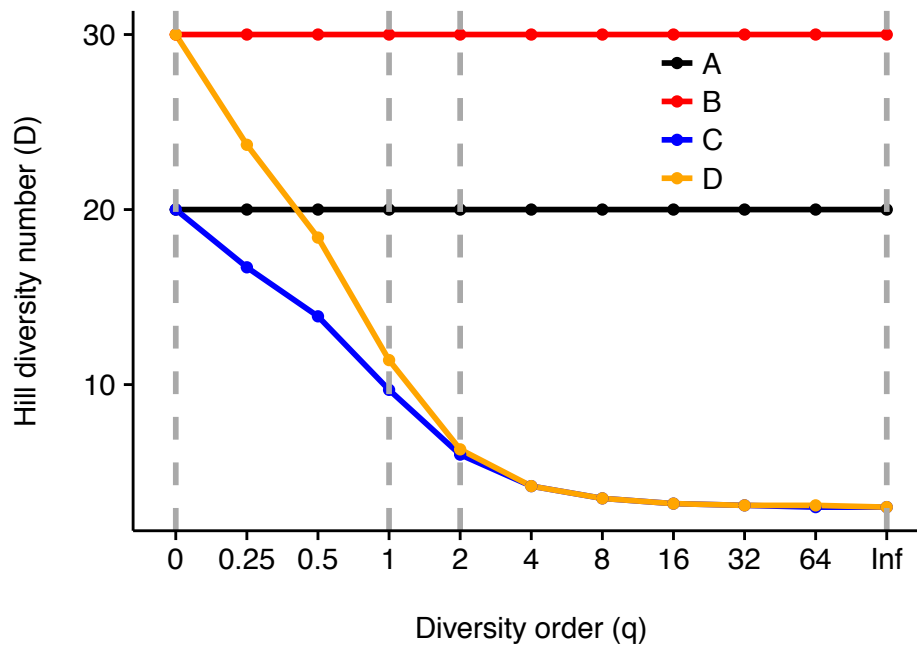


Figure A2.1. *Hill diversities (D) for four hypothetical communities that differ in species richness or evenness. The orders of diversity (q) are presented sequentially, but not to scale, on the x -axis. The vertical gray dashed lines identify four commonly used orders of diversity ($q^* = 0$ for species richness, $q = 1$ for Shannon diversity, $q = 2$ for inverse Simpson diversity index, $q = \text{Infinity}$ for inverse Berger-Parker index).**

Example A2.2

In this example we look more closely at how the proportional abundances of species in a community influence the slope of D in a Hill diversity profile. We compare three communities that have the same species richness, but differ in their species evenness (Figures A2.2 and A2.3).

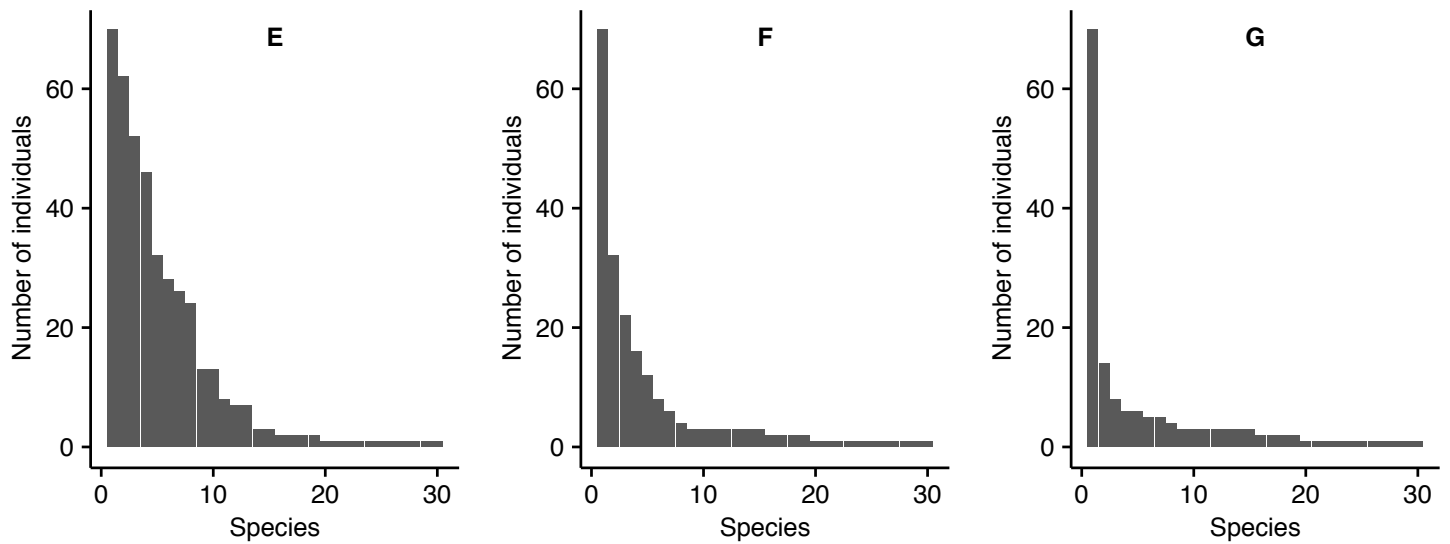


Figure A2.2. Species rank-abundance distributions of three hypothetical communities for Example A.2. The height of each bar marks the number of individuals for a single species. Each community has 30 species (30 bars). Among the three, Community E has the highest species evenness; Community G, the lowest.

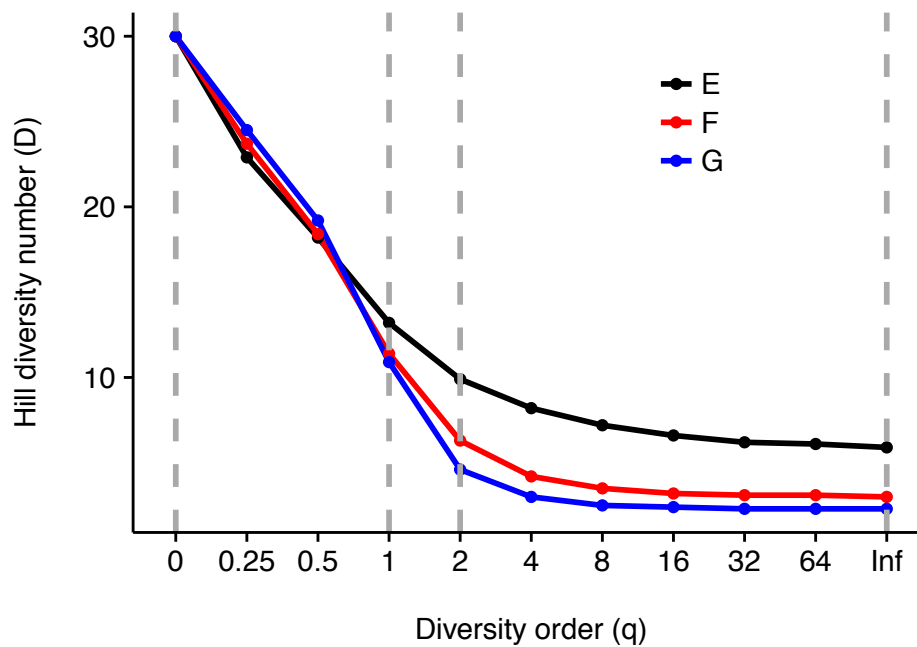


Figure A2.3. Hill diversities (D) for three hypothetical communities that differ in species evenness.

Example A2.3

By examining Hill diversity profiles for different years from the same site we may discern between changes in abundances of common versus rare species.

Table A2.2. Scenarios of changing species diversity for Example A2.3.

Scenario	Number of species	Number of individuals	Change from base scenario
Base.pop	20	140	NA
D.common (decline common)	20	117	The single most common species declines to approximately half its former abundance (changed from $N = 47$ to $N = 24$)
I.common (increase common)	20	187	The single most common species increases to double its former abundance (changed from $N = 47$ to $N = 94$)
D.rare (decline rare)	16	136	Four rarest species disappear (changed each from $N = 1$ to $N = 0$ individuals)
I.rare (increase rare)	24	144	Four rare species newly appear (changed each from $N = 0$ to $N = 1$ individuals)

The corresponding vectors of species abundances, as coded in R, are:

```
Base.pop <- c(47, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1)
D.common <- c(24, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1)
I.common <- c(94, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1)
D.rare <- c(47, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2)
I.rare <- c(47, 22, 17, 10, 8, 6, 4, 3, 3, 3, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1,
1, 1, 1, 1)
```

Figure A2.4 demonstrates that if common species increase in abundance at faster rates than do other species in the community, then species diversity may decline (I.common has lower diversity than Base.pop). Similarly, if many species are declining at similar rates but the most common species are declining at faster rates, then species diversity may increase (D.common has higher diversity than Base.pop). These results are a reminder that most diversity measures are not impacted by changes in absolute numbers of individuals—only by changes in proportional abundances. Even if all species are declining in abundance, the diversity of a site may be increasing.

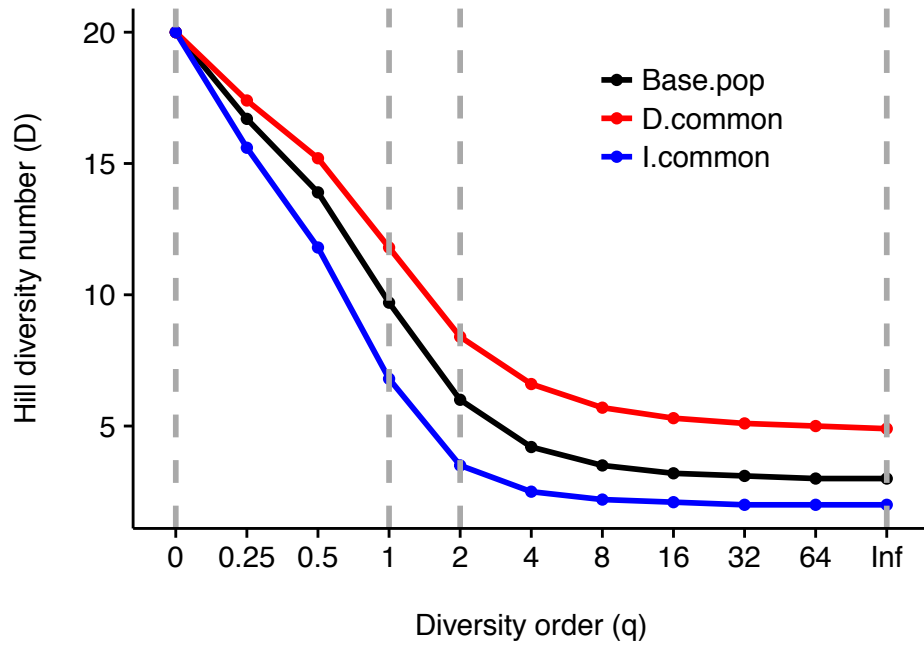


Figure A2.4. Hill diversities (D) for scenarios of changing abundance in the most common species. *Base.pop* = a starting scenario; *D.common* = a scenario in which the base population's single most common species is at half its former abundance; and *I.common* = a scenario in which the base population's single most common species is at twice its former abundance

Changes in abundances of common species are often more apparent at higher orders of diversity because as q increases, D is weighted more heavily on the proportional abundance of common species. Therefore, when examining trends in Hill diversities over time for a community (graphed as in Figure A2.4), lines that diverge at higher orders suggest the proportional abundances of common species may be changing.

Figure A2.5 shows that changes in abundances of rare species are often more apparent when D is measured at lower orders of diversity. Compared to $q = 2$ (inverse Simpson diversity index), $q = 1$ (Shannon diversity) shows a greater difference in D among the three scenarios. The differences are most apparent at $q = 0$ (species richness), because in this example the changes in abundance actually led to the loss or addition of species in the community. Even when changes in abundance of rare species do not impact species richness, the general rule stands that lower orders of diversity are more sensitive to changes in abundances of rare species.

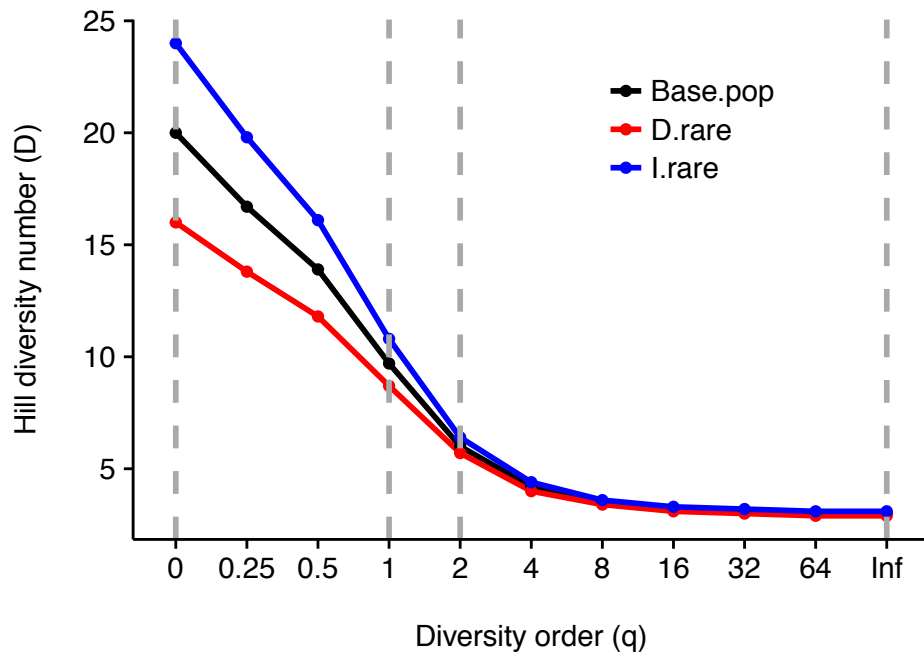


Figure A2.5. Hill diversities (D) for scenarios of changing abundance in rare species. *Base.pop* = a starting scenario; *D.rare* = a scenario in which the base population's four rarest species are gone (each from $N = 1$ in the base population to $N = 0$); and *I.rare* = a scenario in which four additional (rare) species are present (each from $N = 0$ in the base population to $N = 1$).

Literature cited

- Chao, A., N.J. Gotelli, T.C. Hsieh, E.L. Sander, K.H. Ma, R.K. Colwell, and A.M. Ellison. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* 84:45–67.
- Hill, M.O. 1973. Diversity and evenness: A unifying notation and its consequences. *Ecology* 54: 427–432.
- Jost, L. 2006. Diversity and entropy. *Oikos* 113(2): 363–375.
- Laffan, S.W., E. Lubarsky, and D.F. Rosauer. 2010. Biodiverse, a tool for the spatial analysis of biological and related diversity. *Ecography* 33, 643–647.
- Oksanen, J., F. Guillaume Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, and W. Wagner. 2016. *vegan*: Community Ecology Package. R package version 2.3-3. <https://CRAN.R-project.org/package=vegan>
- Palmer, M.W. 1990. The estimation of species richness by extrapolation. *Ecology* 71:1195–1198.

Appendix A3: Comparing diversities based on standardized sample coverage

Estimates of species diversity are known to be influenced by sample effort or sample coverage because rarer species are usually detected only with considerable sampling. Some researchers think that samples should be standardized to account for differences in the numbers of individuals detected. In this Appendix we demonstrate the use of the R package ‘iNEXT’ (Hsieh et al. 2016) to calculate diversities and to generate bootstrapped confidence intervals for standardized samples. There is no single ‘right’ way to define or measure diversity, but rather a continuum of definitions that we are constantly striving to encapsulate in summary statistics. The methods in this Appendix represent a snapshot and a single line of thought in the rapid evolution of such statistical methods.

Rarefaction, extrapolation, and sample coverage

A common approach for comparing species richness of different communities is rarefaction (Sanders 1968). Given three communities (A, B, C) for which $A = 100$, $B = 150$, and $C = 180$ individuals are detected, the richness of the communities can be compared by standardizing to the smallest sample size—in this case, standardized to 100 individuals. With rarefaction, standardization is achieved by randomly resampling (via computer) each community to generate a plot of the number of species (y-axis) against the number of sampled individuals (x-axis), for a range of sample sizes. In theory, the resulting rarefaction curve for a community demonstrates the number of species that likely would have been recorded if, for example, 100 individuals had been detected in the survey for Community C instead of the 180 individuals actually detected. Species richness can then be compared conditional on 100 individuals sampled from each community.

One downside of rarefaction is that data from communities with larger sample sizes are “thrown away” when standardizing to the smallest sample size. Therefore, extrapolation is sometimes used instead to extend sample sizes in the opposite direction, i.e., projecting forward along the species accumulation curve. Whereas rarefaction is a backward interpolation from the endpoint of a species accumulation curve, extrapolation is used to predict and then compare species richness for larger sample sizes than actually obtained (Colwell and Coddington 1994).

Whether rarefaction or extrapolation is used for comparisons of species richness, differences among communities are often biased low when sample size is the basis for standardization. Chao and Jost (2012) showed that standardizing data by sample coverage, or completeness, sometimes provides less biased comparisons of richness. Coverage is the proportion of the true population size (number of individuals) that is represented by species detected in a survey. The opposite of coverage—coverage deficit—is the probability of detecting a new species if sample size is increased by one. The concept of coverage was initially proposed by Turing and Good (Good 1953). They estimated coverage as one minus the proportion of singletons (species represented by only one individual) in a survey.

The above concepts—rarefaction, extrapolation, and sample coverage—have been integrated in a generalized framework for standardizing and comparing species diversity using Hill diversities of any order, for a wide range of sample sizes or sample coverages (Chao et al. 2014). Although species richness (Hill diversity of order zero) is most influenced by sampling, higher orders of diversity also depend to some degree on the completeness of sampling. The standardization method proposed by Chao et al. (2014) has been automated with the R package ‘iNEXT’, for Hill diversities of orders 0–3. We demonstrate the use of this package with examples. The ‘iNEXT’ manual (Hsieh et al. 2016) and Chao et al. (2014) provide details on underlying theory and equations.

Examples

In Appendix A2 we assumed complete knowledge about the species composition of hypothetical communities. In the following examples we demonstrate how sampling (as opposed to censusing, which is rarely possible) can influence diversity estimates, and how to compare diversities at standardized sample coverages. The R code for this example is provided as accompanying script.

Example A3.1

We begin with a known community (Comm) from which we take three samples. For Samp45, we randomly sample 45% of the individuals from Comm; Samp30, 30% of individuals; Samp10, 10% of individuals. The resulting vectors of species abundances are:

```
Comm <- c(600, 80, 70, 50, 50, 40, 40, 30, 30, 30, 30, 30, 30, 20, 20, 20, 20, 5,
5, 5)
# 19 species in the community. The most abundant species has 600
```

```
# individuals; the rarest has 5 individuals. Total number of individuals is
# 1175.
Samp45 <- c(276, 37, 34, 20, 20, 18, 15, 14, 14, 14, 13, 11, 10, 10, 9, 8, 3,
2, 1) # 19 species in the sample.
Samp30 <- c(180, 24, 22, 14, 14, 13, 11, 10, 10, 9, 8, 8, 8, 7, 5, 3, 3, 2,
1) # 19 species in the sample.
Samp10 <- c(56, 12, 7, 6, 6, 5, 4, 4, 4, 2, 2, 2, 2, 1, 1) # 16 species in the sample.
### KEEP IN MIND that this example represents one iteration of random
### sampling---if sampled again, results could be somewhat different.###
```

Diversity measures estimated from samples are expected to reflect the diversities of their underlying populations. Samp45, Samp30, and Samp10 are random samples from the same underlying population (Comm), so their estimated diversities should not be significantly different.

We use the method presented in Chao et al. (2014) to compare Hill diversities and bootstrapped confidence intervals at similar levels of sample coverage for the three samples. Here we use abundance data, but diversity can also be estimated from sample-based (incidence) data, e.g., when individual captures may be correlated by sample unit or when data are presence-by-sample-unit rather than counts of individuals (Chao et al. 2014). The results for the three samples, at diversity orders 0–2, are graphed in Figure A3.1. For these diversity orders, the 95% confidence intervals overlap extensively at sample coverages > 75% (this includes base coverage).

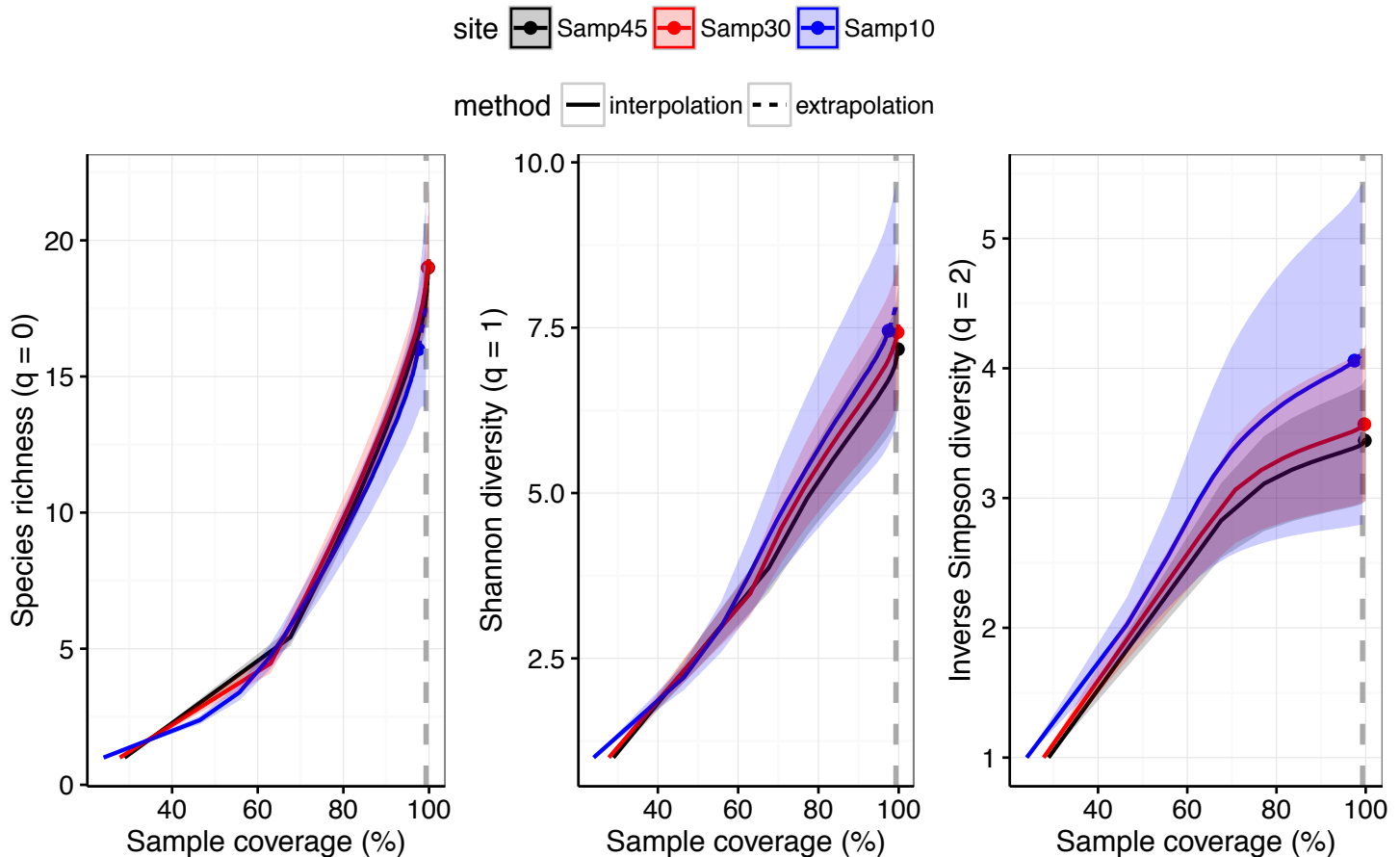


Figure A3.1. Estimated diversity as a function of sample coverage for three samples with moderate proportion of population observed. Samp45 (black) = 45% of the population observed, Samp30 (red) = 30% observed, and Samp10 (blue) = 10% observed. Each of the vertical panels shows the diversity accumulation curves at a specified diversity order: 0 = species richness, 1 = Shannon diversity, and 2 = inverse Simpson diversity (note differences in y-axes and see Appendix A2 for an explanation of diversity orders). The vertical gray dashed lines identify the base coverage (100%, in this case—determination of base coverage is explained below) at which to compare diversities for the three samples. The shaded polygons represent 95% bootstrapped confidence intervals. For each sample, the colored dot represents the sample coverage and estimated diversity

for the observed data. The line extended to the left of the dot is interpolated data (solid line) and to the right of the dot is extrapolated data (short dash; barely visible for Samp10, not visible for other samples).

The difference between observed diversities and diversities compared at a common base coverage is highlighted in Figure A3.2, which extracts the pertinent information from Figure A3.1.

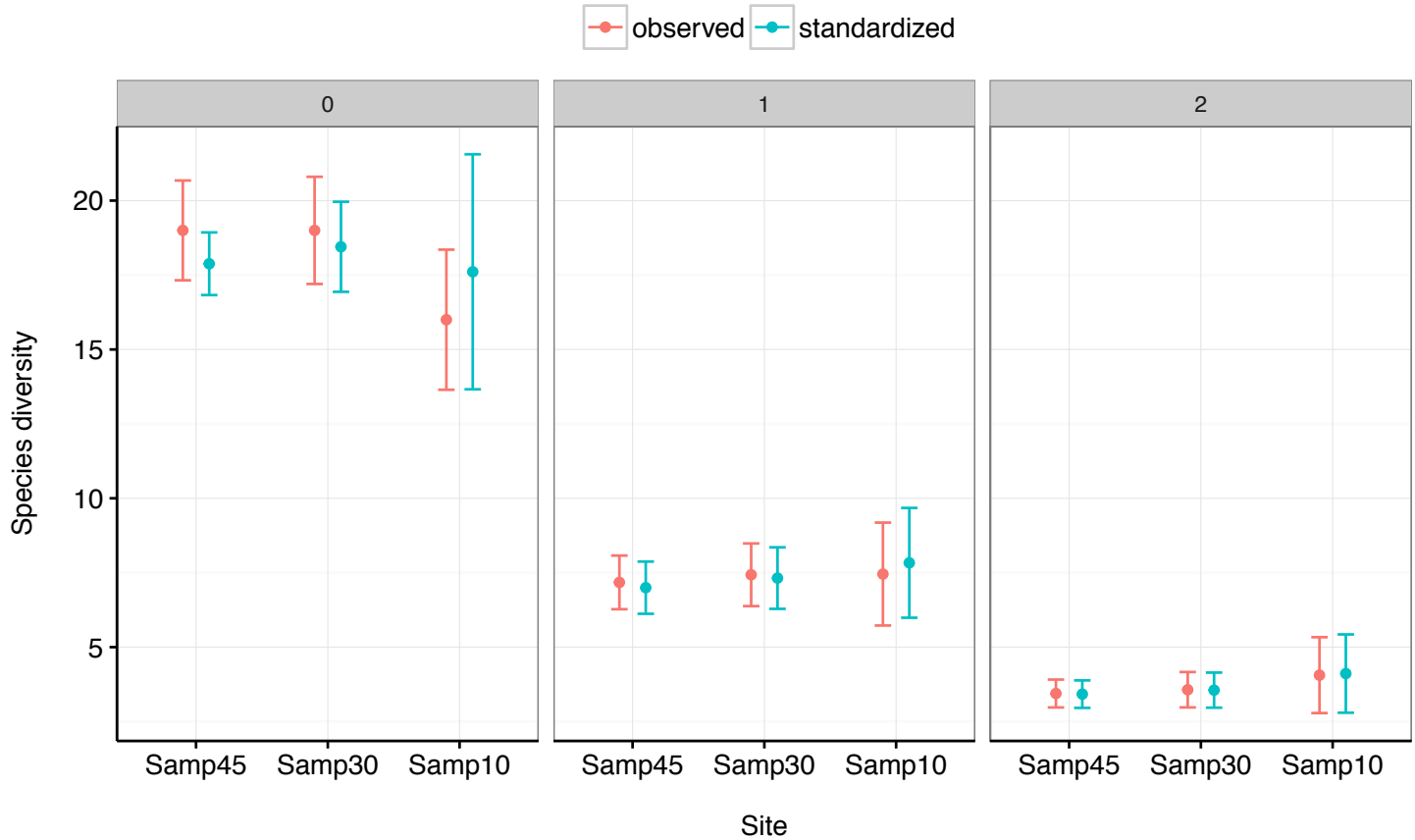


Figure A3.2. Estimated diversities and bootstrapped 95% confidence intervals for three samples with moderate proportion of population observed. Observed diversities (red) estimated from sample data at three orders (0–2; vertical panels), where Samp45 = 45% of the population observed, Samp30 = 30% observed, and Samp10 = 10% observed. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage of 100%.

With the observed sample data in Figure A3.2, species richness (left panel; diversity order = 0) is lower for Samp10 but the 95% confidence interval partially overlaps other samples. When data are standardized to base coverage, the 95% confidence intervals for the three samples overlap extensively, more clearly demonstrating that species richness does not differ among the three samples. At higher orders (center and right panels), diversities are less sensitive to sample coverage, so differences between observed and standardized diversities are minimal.

The main steps involved in generating the above results are: 1. Determine the base coverage at which to compare sample diversities. 1.a. For each reference sample (the observed data), compute the sample coverage when the sample is extrapolated to twice its sample size. For example, 118 individuals were recorded for Samp10. Extrapolate the data to a sample size of 236 individuals. Calculate the sample coverage at that extrapolated sample size. This is the “maximum reliable coverage” for that sample. For estimates of species richness, extrapolating beyond the maximum reliable coverage is not recommended (Chao et al. 2014). However, estimates of diversity at orders of 1 or higher can be extrapolated to the asymptote. Sample extrapolation and estimation of coverage are automated functions in ‘iNEXT’. The sample coverage for any sample (or interpolation/ extrapolation of a sample) is calculated in ‘iNEXT’ using a modification of the original Good (1953) formulation for coverage (Chao and Jost 2012). 1.b. Select the smallest among the maximum reliable coverages calculated in 1.a. (one calculated per sample). Chao and Jost (2012) recommend using this value, or the maximum coverage for reference samples, whichever is larger, as the base coverage. Note that ‘iNEXT’ will generate warning messages when the maximum size of the

extrapolation exceeds double reference sample size. Therefore, in our examples we use a base coverage set at the smallest among the maximum reliable coverages. 2. Generate diversity accumulation curves for each sample at (typically) diversity orders 0, 1, and 2. Diversity accumulation curves plot diversity against sample coverage for a range of coverages that include the base coverage. The bootstrapped confidence intervals generated in ‘iNEXT’ measure *unconditional* variance. They are not generated from resampling the original sample, but instead resample from a predicted sample that includes the original sample plus an estimated number of undetected species. 3. For each order of diversity, examine 95% confidence intervals at base coverage. If the confidence intervals do not overlap, diversity is significantly different (at $\alpha = 0.05$, for 95% confidence intervals) among samples. If confidence intervals slightly overlap, diversity may still be significantly different among samples—the means and standard errors of the bootstrap samples can be used to compute a t-statistic for determining significance of differences among pairs of samples.

Example A3.2

In this example we compare diversities from samples with much lower coverage than in the previous example. Starting from the same known community (Comm), we sample 10% of the individuals (Samp10), 7% (Samp07), and 3% (Samp03):

```
Comm <- c(600, 80, 70, 50, 50, 40, 40, 30, 30, 30, 30, 30, 30, 20, 20, 20, 20, 5,
5, 5)
Samp10 <- c(56, 12, 7, 6, 6, 5, 4, 4, 4, 4, 2, 2, 2, 2, 1, 1)
Samp07 <- c(40, 11, 6, 6, 6, 4, 1, 1, 1, 1, 1, 1, 1, 1, 1)
Samp03 <- c(14, 3, 3, 2, 2, 2, 2, 1, 1, 1, 1, 1, 1, 1)
### KEEP IN MIND that this example represents one iteration of random
### sampling---if sampled again, results could be somewhat different.###
```

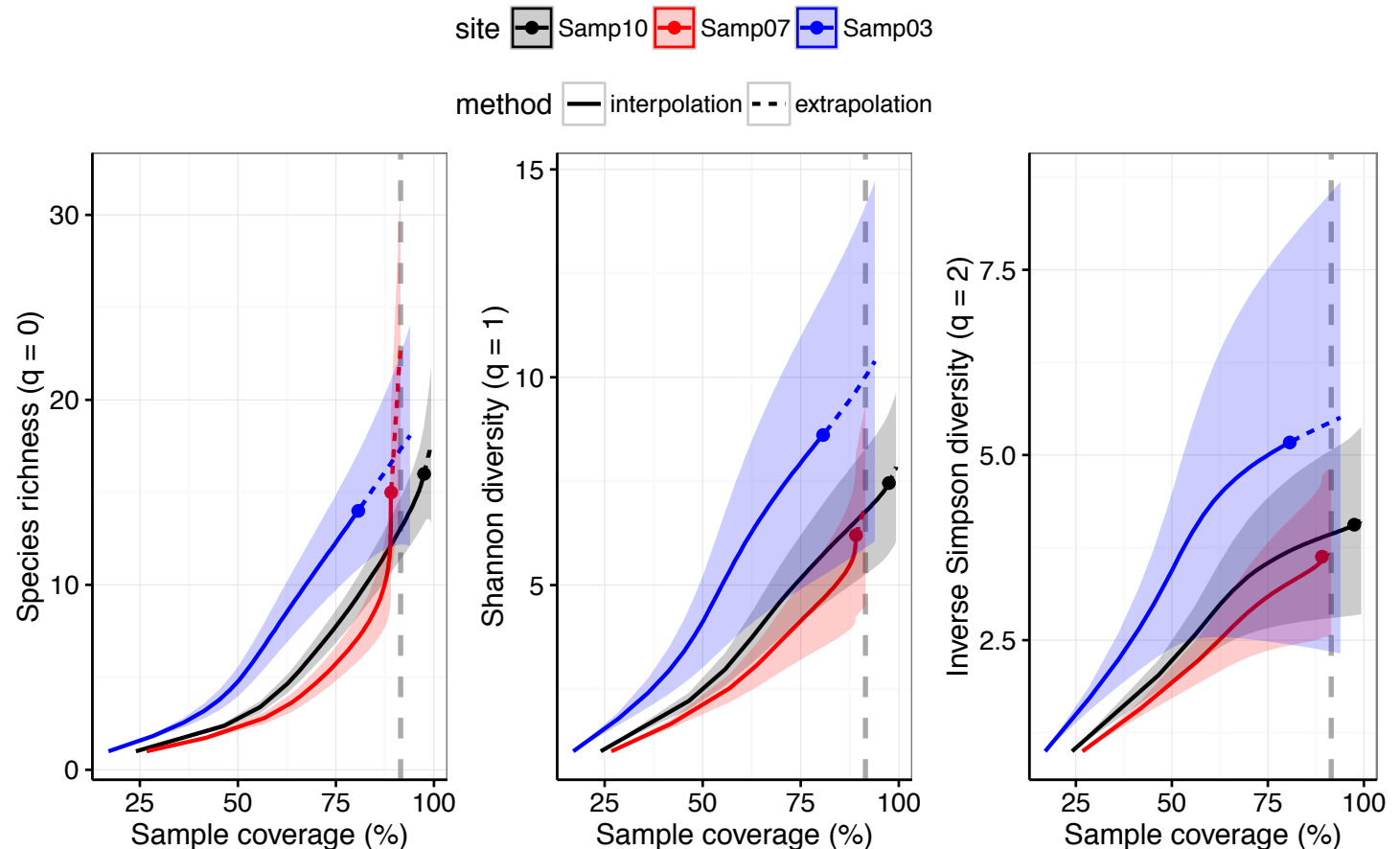


Figure A3.3. Estimated diversity as a function of sample coverage for three samples with low proportion of population observed. Samp10 (black) = 10% of the population observed, Samp07 (red) = 7% observed, and Samp03 (blue)

= 3% observed. Each of the vertical panels shows the diversity accumulation curves at a specified diversity order: 0 = species richness, 1 = Shannon diversity, and 2 = inverse Simpson diversity (note differences in y-axes and see Appendix A2 for an explanation of diversity orders). The vertical gray dashed lines identify the base coverage (92%, in this case) at which to compare diversities for the three samples. The shaded polygons represent 95% bootstrapped confidence intervals. For each sample, the colored dot represents the sample coverage and estimated diversity for the observed data. The line extended to the left of the dot is interpolated data (solid line) and to the right of the dot is extrapolated data (short dash).

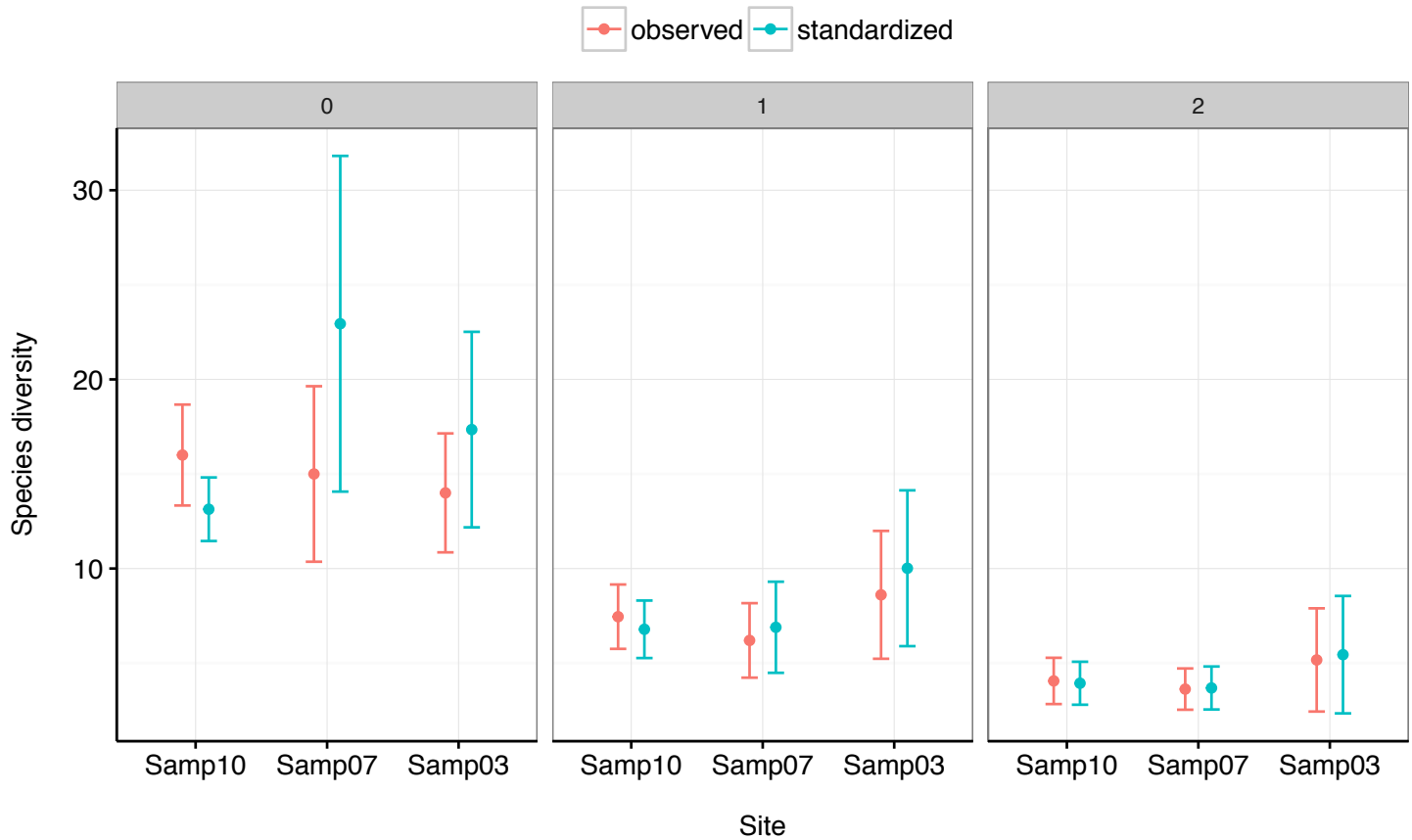


Figure A3.4. Estimated diversities and bootstrapped 95% confidence intervals for three samples with low proportion of population observed. Observed diversities (red) estimated from sample data at three orders (0–2; vertical panels), where Samp10 = 10% of the population observed, Samp07 = 7% observed, and Samp03 = 3% observed. Standardized diversities (blue) are based on interpolation or extrapolation from the sample data, to a common sample coverage of 92%.

In this example, comparing standardized species richness instead of simply using the observed data does not bring us closer to the “truth”, which is that these three samples are derived from the same population. This example is a good reminder that statistical methods to “correct” diversity estimates can reduce erroneous conclusions in some cases, but they are not magic bullets. Ultimately, accuracy and precision in diversity estimates is best achieved through unbiased and good sample coverage in the field. Especially when diversity estimates are standardized to a base coverage less than 100% (in this example, base coverage is 92%), comparisons may not be reliable, whether using observed or standardized data. Standardizing data to a higher base coverage is not an appropriate solution because extrapolating samples beyond maximum reliable coverage (i.e., beyond base coverage) can yield poor estimates of species richness (Chao et al. 2014).

In addition to their limited application, methods for mathematically interpolating, extrapolating, and standardizing diversity estimates are based on assumptions about sampling and species abundance distributions that may not always be true. One assumption is that if sample counts include many singletons and doubletons (species represented by one or two individuals in the sample), then sample coverage is low and the actual number of species may be much higher than the observed number. As always with statistics, understanding the assumptions and mathematics behind these approaches is important for determining when they may be appropriate.

Literature cited

- Chao, A., N.J. Gotelli, T.C. Hsieh, E.L. Sander, K.H. Ma, R.K. Colwell, and A.M. Ellison. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* 84:45–67.
- Chao, A. and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93: 2533–2547.
- Colwell, R.K. and J.A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society of London B* 345:101–118.
- Good, I.J. 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40:237–264.
- Gotelli, N.J. and R.K. Colwell. 2010. Estimating species richness. pp. 39-54 in: *Biological Diversity: Frontiers In Measurement And Assessment*. A.E. Magurran and B.J. McGill (eds.). Oxford University Press, Oxford. 345 pp.
- Hsieh, T.C., K.H. Ma, and A. Chao. 2016. iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 2.0.8, URL: <http://chao.stat.nthu.edu.tw/blog/software-download>
- Sanders, H.L. 1968. Marine benthic diversity: a comparative study. *American Naturalist* 102:243–282.