Community assembly of glass frogs (Centrolenidae) in a Neotropical wet forest: a test of the river zonation hypothesis

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Abstract: The river zonation hypothesis predicts that abiotic and biotic conditions along riparian gradients drive variation in animal communities. Glass frogs are a diverse group of Neotropical anurans that use riparian habitats exclusively for oviposition and larval development, but little is known about how glass frog communities are distributed across riparian gradients. Here, we measured glass frog community assembly across a gradient of riparian habitats from first- to fifth-order streams at La Selva Biological Station, Costa Rica. We performed repeated nocturnal frog calling surveys and built occupancy and *N*-mixture abundance models to test for varying patterns of species occupancy, community assembly, species richness (α-diversity) and species turnover (ß-diversity). We observed significant differences in patterns of species occupancy and community assembly across a stream-order gradient: occupancy of two species increased with stream order (*Teratohyla pulverata*, *Hyalinobatrachium fleischmanni*), one species decreased (*Teratohyla spinosa*), and one species did not vary (*Espadarana prosoblepon*). We evaluated four a priori hypotheses describing how α- and ß-diversity of centrolenids are shaped across the riparian gradient; our data were most consistent with a pattern of nested assemblages and increasing species richness along the riparian gradient. Species-specific patterns of occupancy and abundance resulted in assemblage-level differences consistent with theoretical predictions for highly aquatic organisms along riparian gradients.

Key Words: alpha diversity, beta diversity, Centrolenidae, community structure, Costa Rica, La Selva Biological Station, Neotropics, occupancy models

INTRODUCTION

Two theoretical frameworks have been proposed to explain how abiotic changes along stream gradients interact to drive changes in riparian communities. First, the river continuum concept suggests that animal assemblages shift along stream gradients adaptively in response to concurrent changes in physical and chemical parameters (Vannote *et al.* 1980). Second, the river zonation concept suggests that changes in stream geomorphology along gradients drive changes in biotic communities (Covich 1988, Hynes 1971, Ramírez & Pringle 2001). Together, these two broad concepts in stream ecology predict biological communities to change along riparian gradients.

In Neotropical forests of Central and South America, glass frogs (Centrolenidae) are a species-rich family of anurans that are specialized to riparian habitats. Centrolenids reproduce almost exclusively by ovipositing on riparian vegetation or structures above lotic water (Guayasamin *et al.* 2009, Kubicki 2007, Savage 2002). Egg survival has been linked to oviposition in wet microhabitats and parental care that maintains moisture (Delia *et al.* 2013, Jacobson 1985). After the developmental period, larvae hatch and drop into the stream below to grow aquatically as tadpoles until metamorphosis. Breeding phenology is thought to be seasonally linked to rainfall (Rios-Soto *et al.* 2017, Savage 2002), although this hypothesis has not been rigorously tested. Most studies of glass frogs to date have examined aspects of reproduction (Delia *et al.* 2013, Greer & Wells 1980, Hayes 1991, Jacobson 1985, Mangold *et al.* 2015, McDiarmid 1983) or population demography

Table 1. Hypothetical models for how community assembly of riparian frogs could be shaped along a riparian gradient at La Selva Biological Station, Costa Rica. Alpha (α) diversity describes species richness; beta (ß) diversity describes changes in community assembly, as measured by the Sørensen dissimilarity index. See Figure 2 for graphed illustrations of the model predictions.

Model	Hypothesis	α diversity Slope	ß diversity Slope	ß diversity Y-intercept
Null	Community assembly does not vary	0	0	1
Nested assemblages	Species richness increases, assemblages are nested sets along a stream-order gradient	x	−x	1
Intermediate richness	Species richness is highest and assemblage structure overlaps at intermediate stream order	$-x^2$	-2^x	1
Species replacement	Species replace each other across the gradient; assemblages are unique to different stream orders	0	0	<1

(McCaffery & Lips 2013). However, no studies have described patterns of centrolenid community structure, and, in general, glass frogs have been a historically understudied group among Neotropical anurans (Donnelly 1994).

In an attempt to better understand variation in glass frog community structure, preliminary survey efforts were performed in first-order streams at La Selva Biological Station, Costa Rica during 2014. These data suggested that two species of glass frog, *Teratohyla spinosa* and *Espadarana prosoblepon*, have consistent occupancy and apparently high abundance in first-order streams, while the other five species known to occur at the site (Guyer & Donnelly 2005) are absent in those habitats (B.F. unpubl. data). Given the strong association between glass frogs and riparian habitats, the river zonation hypothesis predicts centrolenid community assembly to vary along riparian gradients, such that other species present in the regional species pool may be occupying different sites along the stream gradients at La Selva. Thus, the river zonation hypothesis predicts that patterns of glass frog diversity and community assembly should vary along the riparian gradient at La Selva Biological Station and at other comparable sites.

In this study, we tested whether the glass frog assemblage at La Selva, Costa Rica, conforms to predictions of the river zonation hypothesis. We performed calling frog surveys to evaluate whether patterns of species occupancy, community assembly and diversity vary across a large riparian gradient. Because arboreal amphibians can be difficult to detect, we used analyses that modelled detection probability to estimate site occupancy and abundance. We developed four a priori hypotheses for how α-diversity and β-diversity could vary across the stream-order gradient (nested assemblages, intermediate richness, species replacement and null; Table 1), and we evaluated support for these hypotheses using information-theoretic methods.

MATERIALS AND METHODS

Study area and focal taxa

La Selva is a private reserve in the Caribbean lowlands of north-eastern Costa Rica (10.42°N, 84.02°W) that is owned and operated by the Organization for Tropical Studies. La Selva is characterized by an average temperature of 25.8°C, receives *c*. 4 m y^{-1} of precipitation (Sanford *et al.* 1994), and is characterized as within Holdridge's Tropical Wet Forest life zone (McDade & Hartshorn 1994). Rainfall is seasonal, with the vast majority of rain occurring during a wet season from May–December relative to a dry season from January–April. Altitude ranges from 30–130 m asl.

We selected 25 study sites to survey for breeding anuran activity (i.e. calling frogs). Study sites were selected to encompass the variation of riparian habitats existing at La Selva, by spanning a gradient from small to large stream sizes. We visually examined maps of La Selva's streams and used Strahler's stream-order system (Strahler 1957) to identify and select study-site localities in five categories – first, second, third, fourth and fifth-order streams. Sites were then visited to confirm the occurrence of flowing water prior to inclusion in the study. Five sites were selected for each category, except for first (N = 6) and second-order (N = 4) categories. When possible, sites were selected to be interspersed among different drainages across the alluvial landscape and within La Selva, to account for any effects of drainage identity, soil type, and/or flooding frequency on community structure. No preliminary calling frog surveys were performed when selecting sites. Locations of study sites are listed in Appendix 1.

Seven species of centrolenid frog have been reported from La Selva (Guyer & Donnelly 2005, Savage 2002) and were surveyed for site occupancy and abundance: *Cochranella granulosa* (granulated glass frog), *Espadarana prosoblepon* (emerald glass frog), *Hyalinobatrachium fleischmanni* (Fleischmann's glass frog),
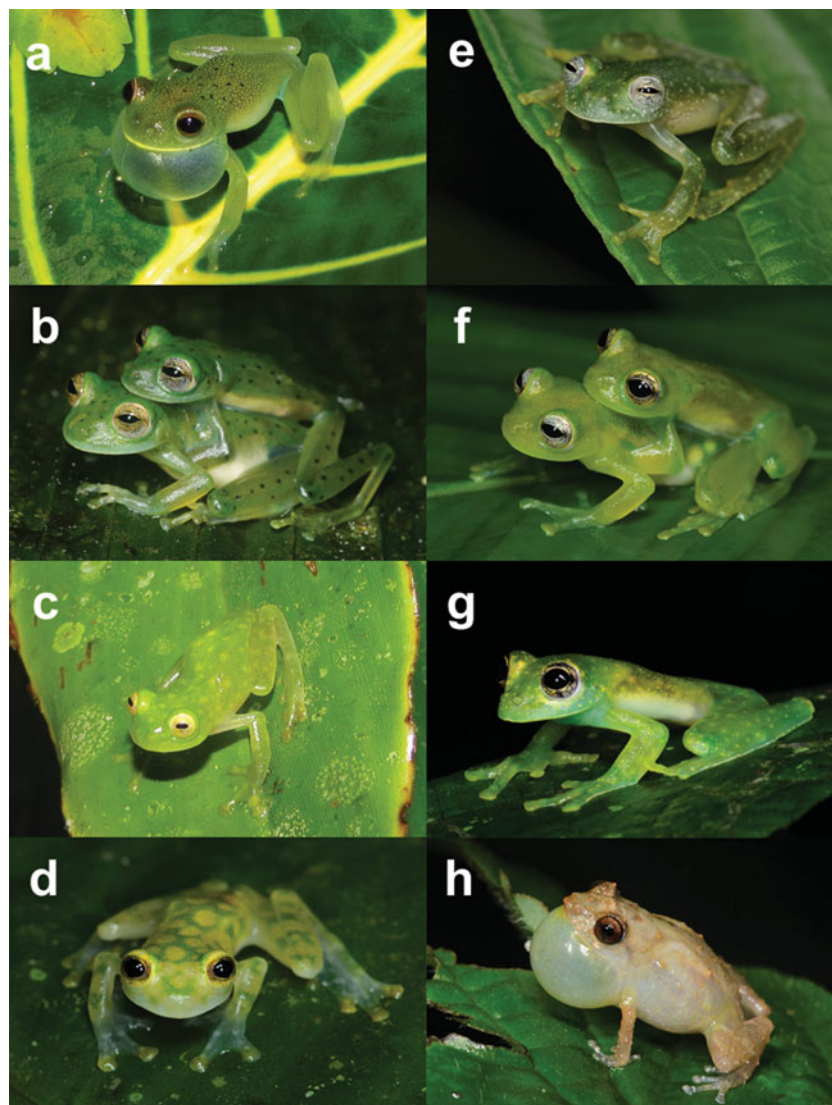
Figure 1. Eight species of riparian anuran that were sampled for site occupancy at La Selva Biological Station, Costa Rica: *Cochranella granulosa* (granulated glass frog) (a), *Espadarana prosoblepon* (emerald glass frog) (b), *Hyalinobatrachium fleischmanni* (Fleischmann's glass frog) (c), *Hyalinobatrachium valerioi* (reticulated glass frog) (d), *Teratohyla pulverata* (powdered glass frog) (e), *Teratohyla spinosa* (spined glass frog) (f), *Sachatamia albomaculata* (yellow-flecked glass frog) (g), *Diasporus diastema* (tink frog) (h).

Hyalinobatrachium valerioi (reticulated glass frog), *Teratohyla pulverata* (powdered glass frog), *Teratohyla spinosa* (spined glass frog) and *Sachatamia albomaculata* (yellow-flecked glass frog) (Figure 1). *Diasporus diastema* (tink frog; Eleutherodactylidae) is another common species of arboreal anuran that is frequently encountered in riparian habitats and was included in our survey efforts. Nomenclature follows Guayasamin *et al.* (2009), with the common English names from Guyer & Donnelly (2005).

Sampling methods

We sampled anuran site occupancy by monitoring for calling frogs. Auditory surveys to detect calling frogs

are a common standard method for determining species presence/absence in aquatic systems (Dorcas *et al.* 2009, Heyer *et al.* 1994) that provide an easy method to collect occupancy and abundance data without physically capturing animals. We sampled sites using a stratified random sampling design from 17 June–1 August 2015. Surveys (N = 8) were divided into three sampling blocks comprising three, three and two surveys. Within each block, sites were sampled on consecutive nights, in an attempt to control for environmental variables which may vary between nights within sampling blocks and seasonal variables that may vary across sampling blocks during the study. Each survey was performed for 4 min, during which we recorded the presence and abundance of each species detected. Abundance was estimated based

Table 2. Models used to evaluate anuran site occupancy (ψ) and detection probability (p) at 25 stream localities in La Selva Biological Station, Costa Rica. The same 16 models were also used to estimate abundance in the *N*-mixture models (Appendix 2). Stream – stream order (Strahler 1957); precip – precipitation; temp – temperature (°C).

Models	Hypothesis
$\psi(.)\,p(.)$	Occupancy and detection do not vary
$\psi(\text{stream})\,p(.)$	Occupancy varies by stream order
$\psi(.)\,p(\text{prec})$	Detection probability varies by precipitation
$\psi(\text{stream})\,p(\text{precip})$	Occupancy varies by stream order, detection varies by precipitation
$\psi(.)\,p(\text{temp})$	Detection varies by temperature
$\psi(\text{stream})\,p(\text{temp})$	Occupancy varies by stream order, detection varies by temperature
$\psi(.)\,p(\text{precip+temp})$	Detection varies by both temperature and precipitation
$\psi(\text{stream})\,p(\text{precip+temp})$	Occupancy varies by stream order, detection varies by both temperature and precipitation
$\psi(.)\,p(\text{stream})$	Detection varies by stream order
$\psi(\text{stream})\,p(\text{stream})$	Occupancy and detection vary by stream order
$\psi(.)\,p(\text{prec+stream})$	Detection probability varies by precipitation and stream order
$\psi(\text{stream})\,p(\text{precip+stream})$	Occupancy varies by stream order, detection varies by precipitation and stream order
$\psi(.)\,p(\text{temp+stream})$	Detection varies by temperature and stream order
$\psi(\text{stream})\,p(\text{temp+stream})$	Occupancy varies by stream order, detection varies by temperature and stream order
$\psi(.)\,p(\text{precip+temp+stream})$	Detection varies by temperature, precipitation and stream order
$\psi(\text{stream})\,p(\text{precip+temp+stream})$	Occupancy varies by stream order, detection varies by temperature, precipitation and stream order

on the number of distinct vocalizing males heard and was recorded as an integer from 0–10; for situations when more than 10 males were calling and it was difficult to assign an exact number, we recorded these situations as >10. Because breeding activity and vocalization can vary by both season and environmental conditions within seasons (e.g. precipitation, temperature; Bridges & Dorcas 2000, Smith *et al.* 2006, Steen *et al.* 2013), we sought to measure how well the survey method can detect species when they are present at sites (detection probability), and the environmental variables that influence the detection process. Thus, we measured the ambient temperature using a thermometer, and recorded qualitative assessments of precipitation (none, light, moderate, heavy, or torrential) and cloud cover (clear, partly cloudy, or overcast).

We used calling surveys because our previous experiences at La Selva indicated that other standard methods for sampling tropical anurans (e.g. visual-encounter surveys, mark-recapture surveys) have extremely low detection probability in higher-order sites where species most frequently occupy habitat 5–20 m above the ground and are not readily sampled by hand. However, calling surveys can provide an alternative and effective method for surveying the occupancy of frogs by increasing detection probability for species that are otherwise difficult to survey with standard methods, allowing us to more accurately determine site occupancy of species.

Statistical analyses

Occupancy and abundance models We developed 16 models explaining how different covariates (temperature, precip-

itation, stream order) may influence detection probability and site occupancy (stream order) for species (Table 2). We included stream order as a hypothesis influencing detection probability in our model set because our ability to detect frog vocalizations may be a function of habitat structure varying across the riparian gradient (sensu Darras *et al.* 2016).

To evaluate these hypotheses, we sought to understand abundance across the landscape using analytical methods that account for imperfect detection (Mazerolle *et al.* 2007). We built single-season occupancy models (MacKenzie *et al.* 2002) which estimated detection probability and occupancy abundance with eight different combinations of covariates influencing occupancy and detection for each species. We built models for a subset of species in the study (N = 5), which were detected at enough sites to build relatively robust models describing occupancy and detection. Models were ranked using Akaike's Information Criterion (AIC); we used the most well supported model for inference, but also considered covariates included within the top-model set (ΔAIC $\leq$ 2; Burnham & Anderson 2002) as being useful for inference. Because *D. diastema* occupied all sites surveyed, we restricted the model selection process to only evaluate hypotheses relating to detection for this species.

To estimate abundance of species at each site, we used the latent *N*-mixture model for point count data from Royle (2004). We set the latent abundance distribution as a zero-inflated Poisson variable, because these models performed better than when it was modelled as a Poisson and negative-binomial distribution. We built and ranked models describing each hypothesis (Table 2), and we then estimated abundance with the top model using empirical Bayes methods.

Community assembly To visualize how patterns of community assembly varied across stream order at La Selva, we used the abundance estimates from the top N-mixture model for each species to generate a matrix of species presence and abundance among sites. We first visualized assembly by plotting the relative proportion of species among pooled sites within each stream order and tested for different species proportions using a Pearson's Chi-squared test. We then constructed non-metric multidimensional scaling (NMDS) plots with Jaccard's shortest dissimilarity measure to represent community assembly on a non-metric scale. NMDS is an ordination technique that describes the dissimilarity local assemblages as points in low-dimensional space. We evaluated whether the ordination accurately described variation with a stress function ranging from 0–1, where values <0.20 suggested that ordinations accurately represented dissimilarity among samples. We categorized samples based on stream order and plotted ordination ellipses around those groups based on the 95% confidence intervals of each treatment's centroid. We used permutation multivariate analysis of variance (PERMANOVA) to (1) test for assemblage-level differences across all stream categories, and (2) to test for pairwise differences between stream categories, with Bonferroni correction of P values (Rice 1989). We identified species that were significantly characteristic of different stream categories using an indicator species analysis (Dufrêne & Legendre 1997); statistical significance was determined with 1000 Monte Carlo simulations. Because *D. diastema* was the most dominant species at all sites, we also performed a second ordination analysis without *D. diastema* to better understand centrolenid assembly across the landscape. Last, Donnelly (1994) predicted that population density of riparian anurans at La Selva may be greater in smaller-order streams. We tested this prediction using a linear mixed-effects model (LME) with a Poisson distribution and species as a random effect.

Model-testing landscape diversity To understand how community composition of riparian frogs could vary across the landscape at La Selva, we developed multiple hypothetical models with distinct predictions for how species richness (α-diversity) and species turnover (β-diversity) could vary among sites. To test predictions from these models, we used the survey data to calculate the Chao's species richness estimate at each site (α-diversity; Chao 1987) and used the species abundance matrix from the N-mixture models to measure change in community composition across sites (β-diversity) using the Sørensen index. The Sørensen index uses species presence-absence data to estimate species turnover among sites where values near or equal to one indicate similar or identical assemblages, while more dissimilar assemblages have lower
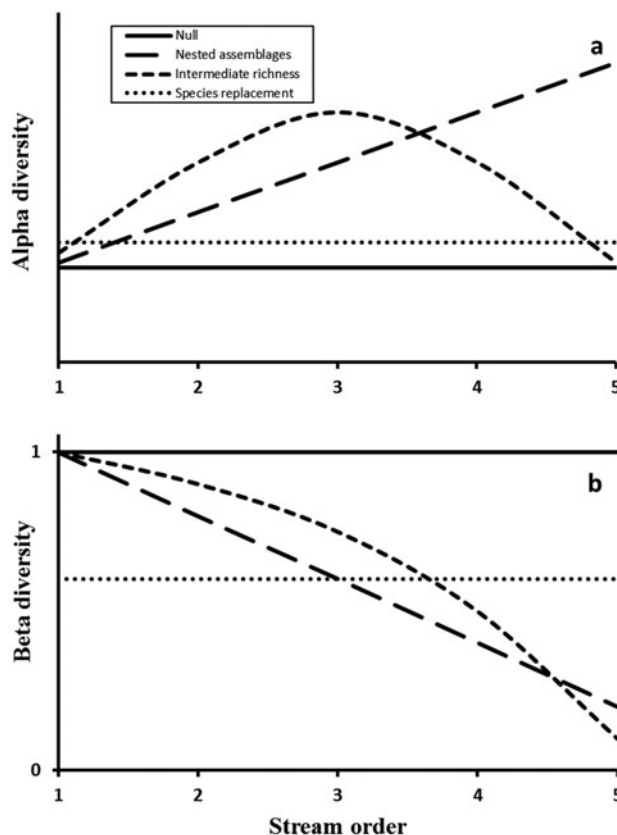

Figure 2. Hypothetical patterns for how species richness (α-diversity; a) and species turnover (β-diversity; b) may vary for riparian anurans along a stream-order gradient at La Selva Biological Station, Costa Rica. Each model (null model, nested assemblages, intermediate richness, unique assemblages) generates distinct linear predictions for how α- and β-diversity should be characterized along the gradient (Table 1).

values. We used the Sørensen index because our primary hypotheses and predictions involved understanding how communities structure differed given the presence or absence of species, rather than other β-diversity indices that describe changes in assemblage structure based on differences in abundance.

We conceived four hypothetical models explaining how α- and β-diversity could vary distinctly: a null model, a nested assemblages model, an intermediate richness model and a species replacement model (Table 1). Each of these models generates distinct predictions for how α- and β-diversity are characterized along the stream-order gradient (Table 1, Figure 2). The null model predicts that community assembly does not vary with stream order, such that α- and β-diversity have slopes $\sim$0 and a y-intercept of $\sim$1. The nested assemblage model describes increasing species richness with stream order (α-diversity has a positive slope) and low-order assemblages to be nested subsets within those at higher orders (β-diversity has a negative slope; i.e. similar to a nestedness model, sensu Baselga 2013). The intermediate richness model

predicts that species richness is highest and assemblage structure overlaps at streams of intermediate size; this model describes α-diversity as $-x^2$ function, and for β-diversity to decrease at an accelerating rate with stream order (-2^x). Last, the species replacement model predicts that species replace each other along the gradient, such that assemblages are unique to different stream orders; this model predicts that α- and β-diversity have slopes $\sim$0, but that β-diversity has y-intercept for $<$1. These models are similar to β-diversity models of nestedness (nested assemblages model) and turnover (intermediate-richness and species-replacement models) from Baselga (2013). Given the predictive model structure of each candidate hypothesis (Table 1), we built generalized linear models describing α- and β-diversity and assessed which model best fitted the data using Akaike's Information Criterion adjusted for small sample sizes (AIC$_c$; Burnham & Anderson 2002).

We performed all analyses using the statistical program R version 3.2.1. We used the package 'unmarked' (Fiske & Chandler 2011) and the functions 'occu()' and 'pcount()' to build the occupancy and point-count models. We used the packages 'vegan' to perform NMDS and PERMANOVA, 'labdsv' for the indicator species analysis and 'lme4' for the linear mixed-effects models. Our data and R code are available upon request.

RESULTS

We recorded calling activity of seven frog species during 200 surveys across the 25 sites. *Diasporus diastema* was the most frequently recorded species, as it was detected during almost all surveys (N = 199). *Teratohyla spinosa* was recorded during 118 surveys, while *T. pulverata* and *H. fleischmanni* were recorded during 55 and 53 surveys, respectively. *Espadarana prosoblepon* was detected during 40 surveys. Of the species detected, *Hyalinobatrachium valerioi* and *Sachatamia albomaculata* were recorded the least: *H. valerioi* was recorded only seven times across two sites, and *S. albomaculata* was only detected once at a single site. *Cochranella granulosa* was not detected during the study period. Because *H. valerioi* and *S. albomaculata* were recorded so infrequently, we focused the analyses on the five species most frequently encountered: *E. prosoblepon*, *H. fleischmanni*, *T. pulverata*, *T. spinosa* and *D. diastema*.

Occupancy and abundance models

The model-selection process for the occupancy models identified top models explaining site occupancy and detection probability which varied among species (Table 3). *Espadarana prosoblepon* and *D. diastema* did not vary in occupancy across the landscape, as those

species were respectively estimated to have occurred at half and all of sites, irrespective of stream order (Figure 3). Occupancy of *H. fleischmanni*, *T. pulverata* and *T. spinosa* varied across the stream-order gradient. *Hyalinobatrachium fleischmanni* and *T. pulverata* occupied $>$0.90 of fourth and fifth-order sites, but predicted occupancy of first and second-order streams was 0–0.15. Conversely, *T. spinosa* was characterized by the opposite pattern; occupancy was 0.83 at first-order streams but decreased slightly to 0.78 in fifth-order streams.

Detection probability of *E. prosoblepon*, *H. fleischmanni*, *T. pulverata* and *T. spinosa* decreased with temperature and increased with precipitation (Figure 3). *Espadarana prosoblepon*, *H. fleischmanni* and *T. pulverata* had high detection (0.78–0.90) during cooler temperatures (20°C); detection decreased to as little as 0.22–0.35 as temperature increased to 29°C. Detection of *T. spinosa* also decreased during warmer temperatures, but the relationship was not as strong for this species. During surveys with no precipitation, detection was low for *E. prosoblepon*, *H. fleischmanni*, *T. pulverata* (less than 0.50 each) and *T. spinosa* (0.65); however, light to moderate rainfall increased detection to $>$0.90 for most species. Detection of *D. diastema* was not found to vary by ambient temperature or precipitation: this species was always vocalizing and highly detectable, irrespective of weather conditions (Figure 3).

Model-selection for the *N*-mixture abundance models identified top-models explaining abundance and detection probability as varying among species (Appendix 2), but generally included the same covariates in the top-model set as the occupancy models (Table 3).

Community assembly

The relative proportion of species estimated by *N*-mixture abundance models varied significantly by stream order ($\chi^2 = 266$, df = 16, P $<$ 0.0001; Figure 4). Non-metric MDS plots also found distinct assemblage structure by stream order, as 95% confidence ellipses of cluster centroids generally showed separation between fifth-order streams and all other categories (Figure 5). Ordinations recorded stresses of 0.084 and 0.038, respectively, after two iterations at two dimensions. PERMANOVA found assemblages to vary significantly across stream order, both with and without *D. diastema* in the analysis (Table 4). Pairwise comparisons could not distinguish between two assemblage groups: first and second-order assemblages, and those at second, third and fourth-order sites. The assemblage at fifth-order sites was found to be distinct from all others. Three species were found to be characteristic of stream categories: *E. prosoblepon* was significantly characteristic of fourth-order streams (P = 0.020), while *T. pulverata* and *H. fleischmanni*
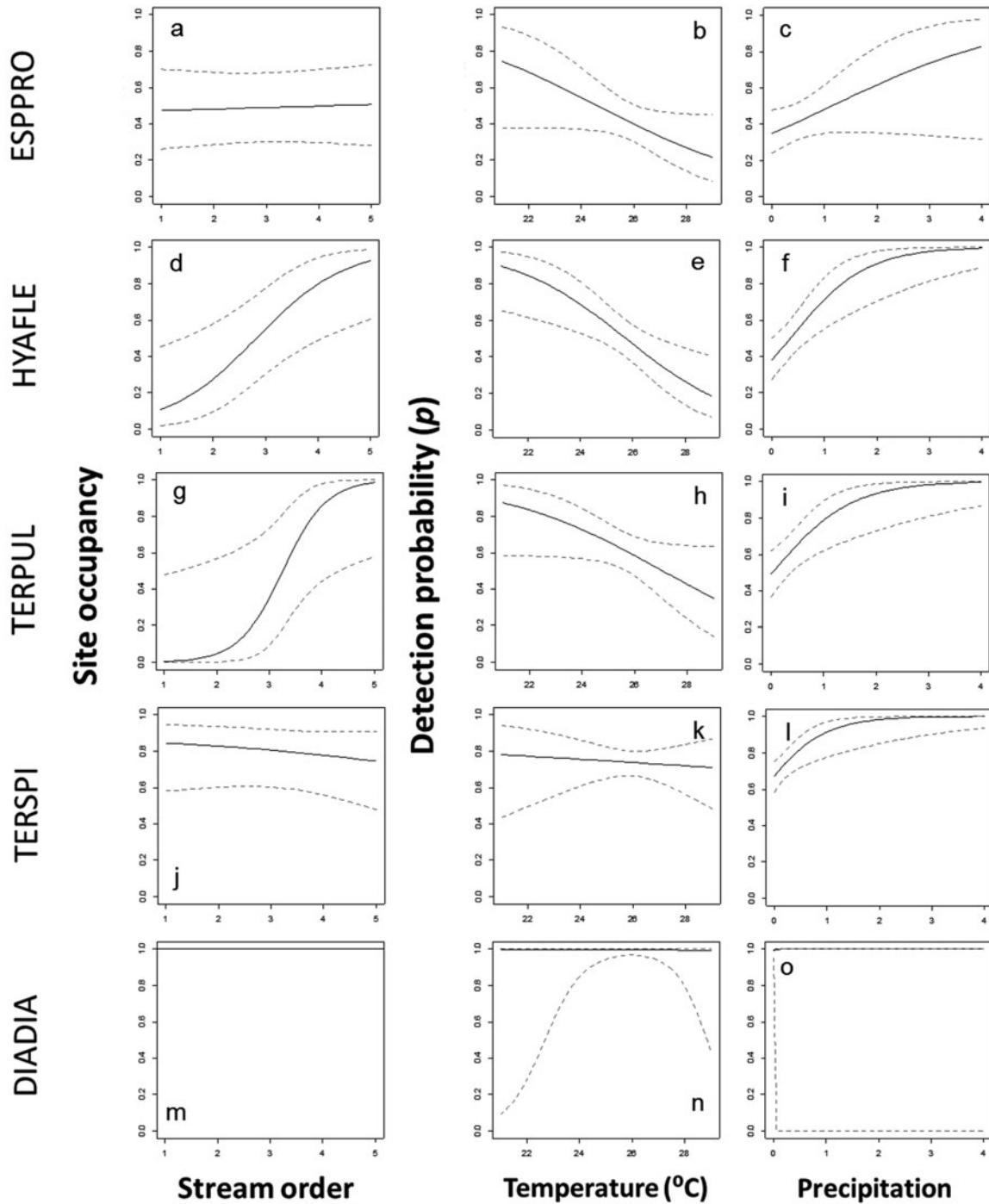
Figure 3. Estimated relationships of how the proportion of sites occupied (occupancy; *psi*) varied by stream order and the probability of detecting species at sites where they occur (detection probability; *p*) varied by temperature and precipitation for five riparian anurans at La Selva Biological Station, Costa Rica: *Espadarana prosoblepon* (a–c), *Hyalinobatrachium fleischmanni* (d–f), *Teratohyla pulverata* (g–i), *Teratohyla spinosa* (j–l), and *Diasporus diastema* (m–o). Ambient precipitation during surveys was scored as 0 = none, 1 = light, 2 = medium, 3 = heavy, 4 = torrential. Species differed in patterns of occupancy along the riparian gradient; however, detection probability decreased with ambient temperature and increased with ambient precipitation for all four centrolenid frogs. Occupancy and detection estimates were generated after model-averaging across a candidate model set (Table 1).

Table 3. Number of parameters, Akaike's Information Criterion (AIC), ΔAIC, and model weight of models built to describe site occupancy (ψ) and detection probability (p) from single-season occupancy models (MacKenzie *et al.* 2002) of four species of glass frog (*Espadarana prosoblepon, Hyalinobatrachium fleischmanni, Teratohyla spinosa, Teratohyla pulverata*) and *Diasporus diastema* at La Selva Biological Station, Costa Rica. Modelled covariates are stream-order (stream), precipitation (precip) and temperature (temp). Only the top-model set (ΔAIC$\leq$2) is shown for each species.

| Model	No. of parameters	AIC	ΔAIC	Weight
Espadarana prosoblepon				
ψ(.) p(temp)	3	167.15	0.00	0.25
ψ(.) p(precip + temp)	4	167.80	0.65	0.18
ψ(.) p(precip)	3	167.88	0.73	0.17
ψ(.) p(.)	2	168.68	1.53	0.12
ψ(stream) p(temp)	4	169.01	1.86	0.10
Hyalinobatrachium fleischmanni				
ψ(stream) p(precip + temp)	5	158.28	0.00	0.36
ψ(stream) p(precip + temp + stream)	6	158.89	0.61	0.26
ψ(stream) p(precip)	4	159.73	1.44	0.17
ψ(stream) p(precip + stream)	5	159.85	1.57	0.16
Teratohyla pulverata				
ψ(stream) p(precip + stream)	5	131.83	0.00	0.48
ψ(.) p(precip + temp)	4	133.51	1.69	0.21
ψ(stream) p(precip + temp + stream)	6	133.82	1.99	0.18
Teratohyla spinosa				
ψ(.) p(precip + stream)	4	200.17	0.00	0.32
ψ(stream) p(precip + stream)	5	200.93	0.76	0.22
ψ(.) p(precip + temp + stream)	5	201.88	1.71	0.14
Diasporus diastema				
ψ(.) p(.)	2	16.59	0.00	0.35
ψ(.) p(precip)	3	17.88	1.28	0.18
ψ(.) p(temp)	3	18.55	1.95	0.13
ψ(.) p(stream)	3	18.59	2.00	0.13
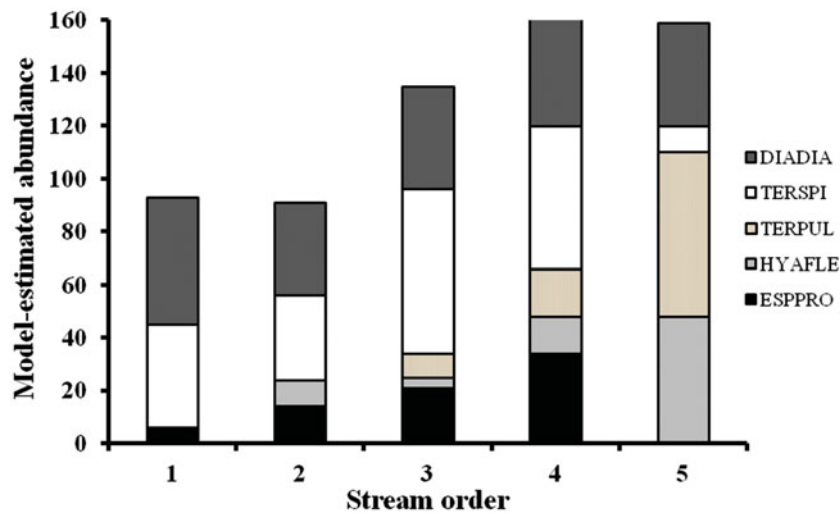
Figure 4. Abundance estimates of five riparian frogs along a riparian gradient at La Selva Biological Station, Costa Rica. Abundance estimates were derived from *N*-mixture abundance models (see Methods); sites were pooled by stream size using the Strahler (1957) stream-order system. Asterisks indicate species significantly characteristic of a stream order, as determined by indicator species analysis. DIADIA = *Diasporus diastema*; TERSPI = *Teratohyla spinosa*; TERPUL = *Teratohyla pulverata*; HYAFLE = *Hyalinobatrachium fleischmanni*; ESPPRO = *Espadarana prosoblepon*.

were significantly characteristic of fifth-order streams (P = 0.001, P = 0.006, respectively). A linear mixed-effects model found frog abundance to increase along the stream-order gradient (β = 0.88; 95% CI = 0.39–1.39; P < 0.001).

Model-testing landscape diversity

Among the models considered, our model selection process most strongly supported the nested assemblages model (Table 5). For α-diversity, this model received model
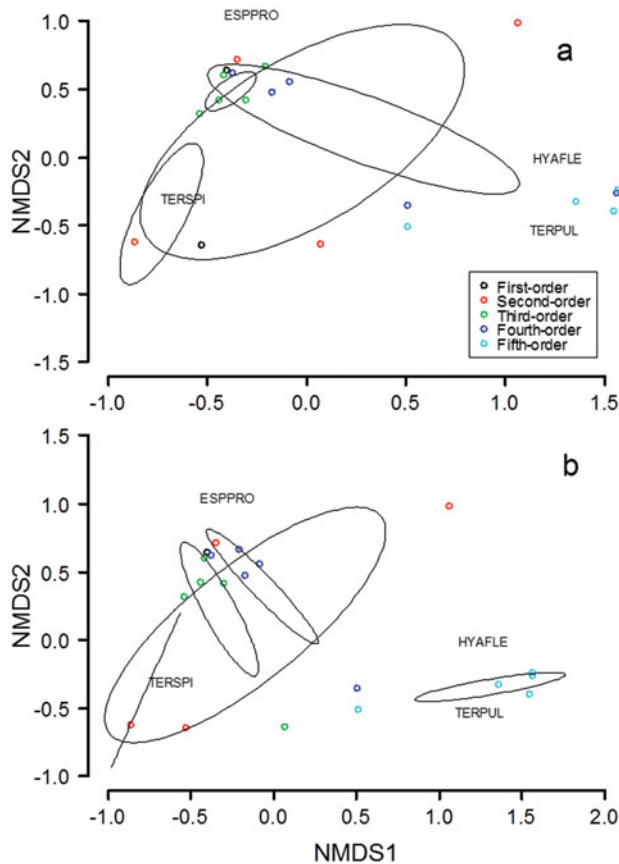
Figure 5. Non-metric multidimensional scaling (NMDS) plots describing assemblage-level variation of riparian anurans along a riparian gradient at La Selva Biological Station, Costa Rica. Points more closely situated are more similar in local community composition than more distant points. Black ellipses indicate 95% confidence intervals for clusters of each stream category. Stress values indicate a measure of distortion as a result of the ordinations being described in two-dimensional space. Species abbreviations are as in Figure 4. Dataset of five species (four centrolenids and *Diasporus diastema*) (a); centrolenid species only, with one first-order site removed because *D. diastema* was the only species recorded there during the study (b).

Table 4. P values associated with permutational analysis of variance (PERMANOVA) tests of variation in riparian anuran community structure at La Selva Biological Station, Costa Rica. Columns and rows indicates stream categories based on the Strahler (1957) stream-order system, from first to fifth order (1–5). Analyses of four centrolenids + *Diasporus diastema*; analyses of a dataset restricted to centrolenid species only.

	1	2	3	4	5
All species: $F = 5.37$, df $= 4$, $P = 0.001$					
1	–				
2	0.243	–			
3	0.028	0.600	–		
4	0.006	0.523	0.531	–	
5	0.003	0.006	0.001	0.001	–
Centrolenids only: $F = 5.00$, df $= 4$, $P = 0.001$					
1	–				
2	0.246	–			
3	0.034	0.782	–		
4	0.008	0.514	0.458	–	
5	0.001	0.022	0.001	0.001	–

weight of 0.85; relative to the second-best model, the top model was over five times more likely to be the true best model. For β-diversity, the nested assemblages model was most strongly supported (model weight = 0.78) and received *c.* three times more support than the next best model. The y-intercept of the nested assemblage model did not differ from 1 (0.96 ± 0.06 SE), but this metric did deviate from 1 for the other models constructed (Table 5).

DISCUSSION

We found patterns of glass frog occupancy and abundance that varied across a stream-order gradient at La Selva. Within the riparian assemblage, species exhibited trends in site occupancy that conformed to

three general patterns along the gradient from low- to high-order streams: (1) decreasing occupancy (*T. spinosa*), (2) increasing occupancy (*H. fleischmanni*, *T. pulverata*), and (3) occupancy independent of stream order (*E. prosoblepon*, *D. diastema*). Together, species-specific patterns in occupancy and abundance drove significant assemblage-level variation along the stream-order gradient. Variation in community structure largely separated (1) first- and second-order streams relative to all others, (2) fifth-order streams distinct from all others and (3) overlap in community structure at intermediate stream sizes. This is the first study to describe significant variance in centrolenid community assembly at any spatial scales. We suggest that this variance is consistent with predictions of the river continuum and zonation concepts, and we add centrolenid frogs to a growing list of Neotropical organisms that are characterized by patterns of community assembly that vary predictably along riparian gradients (Covich 1988, Hynes 1971, Ramírez & Pringle 2001). However, glass frogs provide an interesting case for the river zonation model, because they are largely arboreal but only use aquatic habitat as larvae.

The examination of variance in α- and β-diversity across the stream-order gradient found strongest support for a model where assemblages are nested subsets with increasing α-diversity along the stream-order gradient. Our results are consistent with previous studies that found community structure of riparian anurans to vary with stream size in tropical forests (Eterovick & Barata 2006, Inger & Voris 1993, Keller *et al.* 2009). However, our support for increasing α-diversity is contrary to previous studies that found stream size to be a poor predictor of anuran species richness (Eterovick 2003, Eterovick & Barata 2006). Our results from La Selva,

Table 5. Number of parameters, Akaike's Information Criterion adjusted for small sample size (AIC$_c$), ΔAIC$_c$, model weight, and log-likelihood of candidate models (Table 1) built to describe patterns of species richness (α-diversity) and species turnover (β-diversity) of riparian frogs at La Selva Biological Station, Costa Rica. Because the Null and Species replacement models had the same predictive structure for slope and varied only in the position of the Y-intercept for beta diversity, we only built one model to avoid redundancy. We only report Y-intercept values for β-diversity, because this was the only variable of the two for which we had a priori predictions for that metric. *indicates significantly different from 1.

Model	K	AIC$_c$	ΔAIC$_c$	Model weight	Log-likelihood	Y-intercept (± SE)
α-diversity						
Nested assemblages	3	82.05	0.00	0.85	− 37.45	−
Intermediate richness	3	85.68	3.64	0.14	− 39.27	−
Null model/species replacement	2	92.11	10.06	0.01	− 43.78	−
β-diversity						
Nested assemblages	3	− 21.28	0.00	0.78	14.24	0.96 ± 0.07
Intermediate richness	3	− 18.77	2.51	0.22	12.99	0.82 ± 0.05*
Null model/species replacement	2	− 8.14	13.14	0.00	6.35	0.69 ± 0.04*

Costa Rica generate predictions for how glass frog community assembly may vary across riparian gradients at other localities.

A diverse suite of abiotic or biotic factors (Wellborn *et al.* 1996) may be driving changes in the riparian frog assemblages along the stream gradients at La Selva. Ultimately, the abundance of each species within an assemblage is driven by population demography, such as female fecundity and the survival of eggs, larvae, metamorphs and/or adults. Future studies should seek to understand factors influencing population demography of species along the gradient; in particular, we point to centrolenid reproduction and larval life history as being promising for this pursuit (Hoffmann 2010), because those two features are intrinsically linked to the riparian habitat that was linked with variation in population and community structure here. Further, we did not observe turnover in occupancy or abundance of *D. diastema*, a species that was extremely abundant across all sites at La Selva. *Diasporus diastema* likely does not conform to predictions of the river zonation model because it is not restricted to aquatic habitat for reproduction. Rather, *D. diastema* lays eggs in arboreal habitats (Ovaska & Rand 1999) which develop directly into small frogs and lacks a larval stage (Savage 2002).

Previous natural history accounts for centrolenids have suggested that reproduction primarily occurs during the wet season (Savage 2002), and a recent study of *Centrolene quindianum* found calling activity and oviposition to be greatest during wetter and cooler months during the wet season in Colombia (Rios-Soto *et al.* 2017). We observed strong effects of temperature and precipitation on breeding activity of four centrolenid species at La Selva, where calling behaviour was greatest on cool and wet nights. While our results are similar and consistent to previous studies describing seasonal reproduction linked to precipitation, our results suggest that reproduction is also influenced by environmental variables at shorter temporal scales within seasons. Reproduction may be highest during wet nights with cooler

temperature because wet, humid and cool conditions during and after oviposition may increase hydration of egg masses and reduce mortality due to desiccation (Delia *et al.* 2013). Thus, our results support the generality of the hypothesis that glass frogs breed during wet conditions to aid in egg hydration, which may drive variance in reproductive effort that varies seasonally with rainfall.

Riparian frogs remain the least studied assemblage of frogs at La Selva. Donnelly (1994) hypothesized that this research bias results from riparian frogs reproducing less frequently and occurring in lower densities in the large stream habitats most proximate to the station's facilities, because those streams support a conspicuous assemblage of fish. She then hypothesized that areas with greater relief, smaller streams and fewer fish located more remotely in the reserve may support more abundant assemblages of riparian frogs. Contrary to this prediction, we found that riparian frog abundance increased along the stream-order gradient; glass frogs were most abundant at larger-order streams and rivers at La Selva. Rather, we suggest that the historical paucity of riparian frog research at La Selva may be more simply explained by detectability: in larger streams near the station's facilities, glass frogs are using habitat in trees at great heights over the water (>20 m), where they are undetectable by visual survey methods. For example, we observed glass frogs calling, amplexing and ovipositing in canopy trees over large rivers (e.g. Río Puerto Viejo). Contrary to previous accounts describing glass frog habitats as being restricted to small- to middle-order streams, we observed the greatest glass frog abundance at La Selva in the largest riparian habitat available, fourth- and fifth-order streams.

In conclusion, we found assemblages of riparian glass frogs to vary across the landscape in ways consistent with the river zonation hypothesis. Patterns of community assembly were best characterized by models where assemblages are nested subsets with increasing α-diversity along the stream-order gradient. Future studies should seek to understand how demographic variation within

species contributes to patterns of community assembly along the riparian gradient at La Selva. Because our study was simple and relatively easy to implement, our methods provide an easy opportunity for replication at other sites and for long-term monitoring of the riparian frog assemblage at La Selva.

ACKNOWLEDGEMENTS

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Appendix 1. Study sites monitored for nocturnal calling frog activity during June–August 2015 at La Selva Biological Station, Costa Rica. Sites were selected to encompass the variation of riparian habitat at La Selva and to span a gradient from first- to fifth-order streams (Strahler 1957). *coordinates estimated from map.

Site number	Locality	Stream order	Latitude	Longitude
1	Sendero Tres Rios (STR), 400 m; unnamed stream	1	10.4343	−84.00813
2	Sendero Suroeste (SSO), 250 m; unnamed stream ca. 150 m north of trail sign	1	10.42746	−84.01107
3	Sendero Suroeste (SSO), 250 m; unnamed stream ca. 100 m south of trail sign	1	10.42566	−84.01159
4	Lindero Occidental (LOC), 900 m; Taconazo	1	10.42717	−84.01831
5	Sendero Hartshorn (SHA), 425 m; unnamed stream	1	10.42332	−84.01382
6	Suampo (SUA), 450 m; unnamed stream	1	10.41965	−84.0072
7	Sendero Tres Rios (STR), 750 m; unnamed stream	2	10.4368	−84.00993
8	Sendero Sura (SURA), 1000 m; Taconazo	2	10.43212	−84.02388
9	Bridge in road into La Selva	2	10.43518	−84.00288
10	125 m. west of SSO and SHA intersection; Arboleda	2*	10.42366	−84.01434
11	Sendero Tres Ríos (STR), 3350 m; El Piper	3	10.43252	−84.02901
12	Camino Circular Centrano (CCC), 1050 m; La Selva	3	10.42471	−84.00972
13	Arboleda; adjacent to southern-most point of Arboretum	3	10.42956	−84.0111
14	Suampo (SUA), 300 m; unnamed stream	3	10.41889	−84.0063
15	Sendero Hartshorn (SHA), 250 m; La Selva	3	10.42235	−84.01247
16	Lindero Occidental (LOC), 100 m; El Surá	4	10.43336	−84.01395
17	Sendero Oriental (SOR), 400 m; El Salto	4	10.42737	−84.00267
18	Camino Circular Lejano (CCL), 575 m; El Salto	4	10.42459	−84.00564
19	Bridge to arboretum; El Surá	4	10.43016	−84.00941
20	Sendero Jaguar (SJ), 250 m; El Surá	4	10.42945	−84.02554
21	Stone Bridge, southern side; Río Puerto Viejo	5	10.43136	−84.00522
22	River Station Stairs; Río Puerto Viejo	5	10.43016	−84.00454
23	Casa Rafael; Río Puerto Viejo	5	10.42753	−84.00161
24	Sendero Río (SR), 275 m; Río Sarapiquí	5	10.4324	−84.02738
25	Sendero Tres Ríos (STR), 2600 m; Río Sarapiquí	5	10.43246	−84.02388

Appendix 2. Number of parameters, Akaike's Information Criterion (AIC), ΔAIC, and model weight of models built to describe site abundance (*N*) and detection probability (*p*) using *N*-mixture models with latent abundance as a zero-inflated Poisson variable for *Espadarana prosoblepon*, *Hyalinobatrachium fleischmanni*, *Teratohyla spinosa*, *Teratohyla pulverata* and *Diasporus diastema* at La Selva Biological Station, Costa Rica. Modeled covariates are precipitation (precip), temperature (temp), and stream order (stream). Only the top-model set (ΔAIC < 2) is shown for each species.

Model	No. of parameters	AIC	ΔAIC	Weight
Espadarana prosoblepon				
N(.) p(precip+temp)	5	318.36	0.00	0.38
N(.) p(precip)	4	318.78	0.41	0.31
N(stream) p(precip + temp)	6	320.07	1.71	0.16
Hyalinobatrachium fleischmanni				
N(stream) p(precip + stream)	6	371.59	0.00	0.17
N(stream) p(precip + temp + stream)	7	371.97	0.38	0.14
N(stream) p(precip)	5	372.35	0.76	0.12
N(.) p(precip)	4	372.64	1.05	0.10
N(.) p(precip + temp)	5	373.04	1.45	0.08
N(stream) p(precip + temp)	6	373.09	1.50	0.08
N(stream) p(temp + stream)	6	373.40	1.81	0.07
Teratohyla pulverata				
N(stream) p(precip)	5	373.10	0.00	0.39
N(stream) p(precip + temp)	6	373.43	0.33	0.33
N(stream) p(precip + stream)	6	375.06	1.97	0.14
Teratohyla spinosa				
N(.) p(precip)	4	618.92	0.00	0.31
N(.) p(precip + temp)	5	619.82	0.90	0.20
N(.) p(precip + stream)	5	620.84	1.91	0.12
N(stream) p(precip)	5	620.89	1.96	0.12
Diasporus diastema				
N(.) p(.)	3	728.25	0.00	0.26
N(.) p(stream)	4	729.89	1.64	0.11
N(.) p(temp)	4	729.99	1.75	0.11
N(.) p(precip)	4	730.1	1.85	0.10
N(stream) p(.)	4	730.24	1.99	0.09