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Mechanisms Underlying Frog Occupancy Patterns in a Landscape Mosaic of Tropical Forest and Pasture

Tanya J. Hawley

University of Miami, thawley@bio.miami.edu

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UNIVERSITY OF MIAMI

MECHANISMS UNDERLYING FROG OCCUPANCY PATTERNS IN A
LANDSCAPE MOSAIC OF TROPICAL FOREST AND PASTURE

By

Tanya J. Hawley

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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MECHANISMS UNDERLYING FROG OCCUPANCY PATTERNS IN A
LANDSCAPE MOSAIC OF TROPICAL FOREST AND PASTURE

Tanya J. Hawley

Approved:

Dr. Julian C. Lee
Professor Emeritus of Biology

Dr. Terri A. Scandura
Dean of the Graduate School

Dr. Don DeAngelis
Research Professor of Biology

Dr. Matthew D. Potts
Assistant Professor of Biology

Dr. Raymond Semlitsch
Curators' Professor of Biology
University of Missouri - Columbia

HAWLEY, TANYA J.

(Ph.D., Biology)

Mechanisms Underlying Frog Occupancy Patterns in
a Landscape Mosaic of Tropical Forest and Pasture

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Habitat modification is the primary cause of amphibian population declines worldwide. Some species survive in modified habitats whereas others become restricted to small, isolated forest patches. Although many studies compare species richness and composition between modified and intact habitats, the factors and mechanisms that maintain biodiversity in these landscapes are poorly understood. I asked how life history traits and habitat features influence interspecific variation in frog occupancy patterns in tropical pasture and forest. To identify mechanisms underlying occupancy patterns, I used experiments to examine how abiotic conditions in different habitats influence the vital rates of tadpoles. I also explored whether tadpoles use a carnivorous foraging strategy to improve performance in nutrient-poor, ephemeral pools in pasture. Although modified and intact habitats offer abiotic environments that differ in quality for frogs, pastures contribute to the maintenance of biodiversity in fragmented landscapes. I detected an equal number of species but substantially different assemblage composition in forest and pasture. Species that occurred in pasture had different traits than those in forest, including larger body sizes, larger clutch sizes, larger geographic ranges, and reproductive modes that depend on water. The occurrence of pasture-specialists was associated with habitat features at small spatial scales, whereas the occurrence of forest-specialists was associated with habitat features at small and large spatial scales. An

experiment indicated that abiotic conditions in pastures may deter or facilitate adult movements to breeding sites. Behavioral selection of sites by two model species was consistent with tadpole performance. Tadpoles of a pasture-specialist performed well across the pasture-forest gradient, but abiotic conditions in pasture facilitated faster growth and development than in edge or forest. In contrast, tadpoles of a forest-specialist performed well only in edge and forest. Most tadpoles occupying ephemeral pasture pools were facultative carnivores or cannibals of eggs and hatchlings. Tadpoles may contribute to the regulation of assemblages in pasture pools, influencing the relative abundance and composition of species through differential predation on eggs and hatchlings. The study of factors and mechanisms that contribute to population growth or decline of species can facilitate understanding of assemblage-level patterns of amphibian diversity in modified landscapes.

To my grandma, Retha Margaret Bartusch

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CHAPTER 1

INTRODUCTION

Habitat loss and modification remain the primary causes of worldwide amphibian population declines and extirpations (Young et al. 2001). In the neotropics, 37% of amphibian species are threatened by habitat loss and 25% are considered data deficient (Red List criteria; IUCN 2001, IUCN et al. 2006). These statistics demand that we focus more effort on understanding the basic ecology, occupancy patterns, and population dynamics of species residing in modified landscapes. Previous studies recognize that some frog species persist in modified habitats whereas others do not, often resulting in lower species richness and different species composition in modified compared to intact habitats (reviewed by Gardner et al. 2007). However, few studies have examined the mechanisms underlying frog occupancy patterns in modified landscapes. With an understanding of how abiotic conditions influence vital rates of particular life stages of frogs, we can begin to identify the factors that contribute to either population growth or decline in modified landscapes. Then land managers will be able to concentrate their efforts on the factors that lead to population decline of species. My study identifies patterns of species occupancy in pasture and forest and mechanisms that underlie these patterns in a modified landscape in the Osa Peninsula, Costa Rica.

Patterns and mechanisms

The landscape that remains after deforestation often consists of forest fragments surrounded by modified matrix habitats, both differing in a suite of abiotic and biotic characteristics compared to the former intact forest (Saunders et al. 1991; Fig. 1.1). These new abiotic environments often have increased solar input, higher air and water

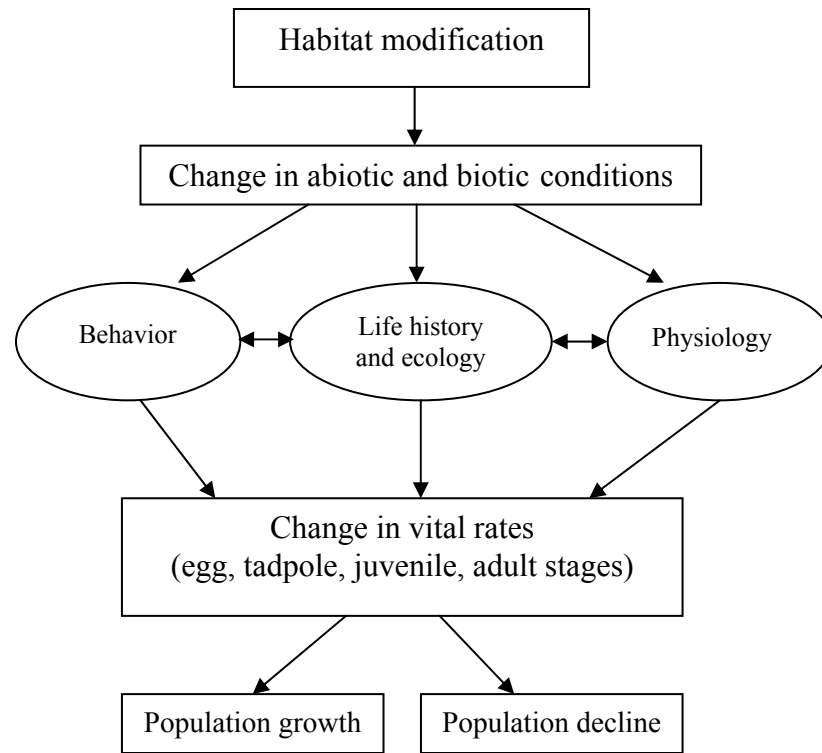


Figure 1.1. Flow chart illustrating ecological consequences of anthropogenic habitat modification for frog populations. Patterns (in squares) include changes in abiotic and biotic conditions and vital rates of different life stages of species. Mechanisms (mediated by characteristics of species in circles) are the link between patterns and population-level outcomes.

temperatures, greater wind speeds, and reduced humidity and soil moisture compared to intact forest (Holl 1999, Saunders et al. 1991). Therefore, modified and intact habitats offer environments that differ in quality for amphibians. Species that persist in fragments or in matrix habitats may have populations that differ in abundance compared to those in the former intact habitat, and the populations often exist in isolation (Laurance et al. 2002). New species may invade fragments and matrix habitats (Tocher et al. 1997), creating assemblages that are equal in richness but different in composition compared to intact forest (Murphy 1989). The role of matrix habitats in maintaining regional amphibian biodiversity is not well understood.

Research examining the effects of habitat modification on amphibians has started to shift from a focus on comparing species' occupancy patterns to a focus on elucidating the mechanisms underlying these patterns (Fig. 1.1). To understand mechanisms underlying occupancy patterns, we must identify changes in abiotic conditions in the modified habitats that lead to changes in vital rates of particular life stages of species (Funk and Mills 2003). Because amphibians typically undergo three distinct life stages, egg, tadpole, and juvenile-adult, often in different habitats, it is necessary to study mechanisms acting on each life stage. For instance, adult frogs may select particular breeding sites across a forest-modified habitat continuum (Gascon 1993, Binckley and Resetarits 2007, Hocking and Semlitsch 2007). The hatching success of eggs can be lower in pasture compared to forest habitats (Neckel-Oliveira 2004). Tadpole performance (survival, growth, and development) differs in forest compared to modified habitats depending on the species (Werner and Glennemeier 1999, Skelly et al. 2002, Halverson et al. 2003, Schiesari 2006). Juveniles may avoid modified habitats when

dispersing from ponds (deMaynadier and Hunter 1999, Rothermel and Semlitsch 2002), and may have improved performance in forest compared to modified habitats (Todd and Rothermel 2006, Harper 2007). During each life stage, life history and ecological, behavioral, and physiological characteristics of species contribute to the mechanism and eventual outcome for population growth and persistence in different habitats (Fig. 1.1). Detailed knowledge of mechanisms underlying occupancy patterns may explain why some species persist whereas others undergo population declines in modified habitats. This understanding can be used by land managers to implement conservation plans designed to reduce population declines and extirpations within fragmented landscapes.

Study synopsis

My research contributes to improving our understanding of species' occupancy patterns and underlying mechanisms within modified landscapes. In my second chapter, I asked whether species richness and composition differ between forest and pasture and I examined factors that influence occupancy patterns. I used visual encounter and vocalization survey methods to detect frogs in 82 aquatic and terrestrial sites in pasture and forest. Using logistic regression, I examined how occupancy patterns of 12 species are related to local and landscape characteristics, generated using a Geographic Information System. Using linear regression, I examined whether life history and ecological traits differ among species that occupy forest versus those that occupy pasture. Although species richness was the same, species composition was substantially different between forest and pasture habitats. Species occupying pastures were negatively associated with forest cover within 100 m of sites, whereas species occupying forest were positively associated with forest cover at small and large spatial scales. Species in

pastures had larger body sizes, mean clutch sizes, and geographic distributions than species in forest. Clutch size explained over 66% of variation in pasture occurrence among species. Spatial habitat characteristics and species-specific life history traits influenced patterns of frog occupancy in the pasture-forest landscape mosaic.

In Chapter 3, I investigated two mechanisms that may contribute to the occupancy patterns I detected in Chapter 2. I asked whether adult breeding site selection and tadpole performance differs among pasture, edge and forest habitats. I allowed free-ranging adult frogs to select pools along pasture-forest transects and recorded egg and tadpole depositions over five months. Then I determined whether patterns of breeding site selection were consistent with tadpole performance among habitats for two model species, *Engystomops pustulosus*, a species associated with disturbed habitats, and *Dendrobates auratus*, a species associated with intact forests. I introduced hatchlings into artificial pools in pasture, edge, and forest and quantified survival, growth, and development through metamorphosis. I quantified incident radiation in each habitat and water temperature in pools to identify how abiotic conditions differ among habitats. Breeding site selection was nonrandom across the pasture-forest gradient. *Engystomops pustulosus* adults selected sites in pasture and near the pasture-forest edge, where their tadpoles had high performance. In contrast, *D. auratus*, and species with life histories similar to *D. auratus*, selected sites in forest and *D. auratus* tadpoles had high performance in edge and forest habitats. Abiotic conditions in pasture and forest may inhibit or facilitate frog movements to breeding sites and influence tadpole vital rates, ultimately contributing to occupancy patterns of species across the landscape.

In Chapter 4, I asked whether two species with similar life histories, *Agalychnis callidryas* and *Dendropsophus ebraccata*, differ in tadpole performance across a forest-pasture gradient. Adults of these species often breed in the same ponds. Using the same design as the tadpole performance experiment in Chapter 3, I quantified tadpole growth and survival over time in pasture, edge, and forest habitats. Survival of *D. ebraccata* tadpoles was high in all habitats; however, *A. callidryas* survival was lower in pasture than in edge or forest. Both species grew larger and developed faster in pasture than in edge or forest. My results indicate that co-occurring species may differ in tolerance to abiotic conditions. Additionally, pastures can contribute to the maintenance of hydrid diversity in fragmented landscapes.

In Chapter 5, I investigated whether species are utilizing an alternative foraging mode as a strategy to survive in nutrient-poor, ephemeral pasture pools. First, I documented the incidence of carnivory and cannibalism among tadpole species that utilize pasture pools. I tested whether three of these species consume conspecific and heterospecific eggs and hatchlings. Then I asked whether the incidence of carnivory is dependent on predator and prey size and developmental stage, tadpole density and relatedness, and food quantity using a model species, *Smilisca phaeota*. I found a high incidence (8 of 11 species) of egg and tadpole carnivory among species occupying ephemeral pools in pasture. Cannibalism by *S. phaeota* tadpoles was dependent on the size and stage of prey and predators and the quantity of plant food. Tadpoles that act as predators of hetero- and conspecific eggs and tadpoles may gain fitness advantages and contribute to regulation of assemblage composition in ephemeral pools in pasture.

In Chapter 6, I conclude my study by briefly summarizing the important findings contained in each chapter. I then consider the implications my findings for amphibian conservation, and I offer suggestions for future investigation.

CHAPTER 2

PATTERNS OF SPECIES OCCUPANCY IN RELATION TO LIFE HISTORY TRAITS IN A LANDSCAPE MOSAIC OF TROPICAL FOREST AND PASTURE

Habitat loss and modification are the primary causes of amphibian population declines and extirpations worldwide (Blaustein and Wake 1995, Houlahan et al. 2000, Young et al. 2001). It is expected that eventually most tropical forests will be fragmented and surrounded by an agricultural matrix (Bierregaard et al. 1997); therefore, threats to amphibian diversity are likely to intensify in the future. Research indicates that amphibian species richness, abundance, and composition are often impoverished in human-modified habitats compared to intact habitats. However, we have a poor understanding of how landscape characteristics influence species-specific responses on different spatial scales (Cushman 2006) and how matrix habitats contribute to the maintenance of regional diversity (Pimentel et al. 1992). Here, I examine how local and landscape characteristics, as well as life history and ecological traits, influence frog occupancy patterns in a landscape mosaic of tropical forest and pasture.

Amphibian species richness, diversity, and distributions are influenced by processes at local and landscape scales. Landscape-scale characteristics, such as the abundance of fragments, matrix habitat composition, and connectivity influence amphibian dispersal (Gascon and Lovejoy 1998) and therefore may be important determinants of species diversity and distributions. Many studies have used the relationships between species richness or abundance and landscape characteristics to examine occupancy patterns of amphibians in modified landscapes. Habitat configuration and area are predictors of amphibian abundance and species richness in

temperate regions (Kolozsvary and Swihart 1999, Mazerolle and Villard 1999, Ray et al. 2002). Distance to pasture is a significant predictor of *Eleutherodactylus* species richness in tropical wet forest (Pearman 1997). Modified habitats may not provide all of the requirements of a species; therefore, the presence of some species there may reflect proximity to a suitable habitat with a source population rather than habitat quality (Pullinam 1988). Adult amphibians may be able to temporarily occupy a matrix, but persistence in the matrix will be determined by the demographic performance of sub-adult life stages.

At the local scale, habitat quality influences species richness and abundance. During the complex life cycle of an amphibian, most species require aquatic habitat for breeding and terrestrial habitat for foraging and retreat; each habitat is critical for population persistence (Semlitsch 2000). The primary factor limiting amphibian persistence in fragments may be the availability of suitable breeding habitat (Zimmerman and Bierregaard 1986). Breeding site availability is likely to have a large influence on species occupancy and persistence in matrix habitats as well. Additionally, the extent to which species can tolerate abiotic conditions in the matrix will influence occupancy patterns. Abiotic environments in pastures differ from those in forests by having higher air temperature and photon flux density, and lower humidity and soil moisture (Holl 1999). Differences in these variables lead to edge effects, altering abiotic conditions in forest up to 50 m from the edge (Laurance et al. 2002).

Some generalizations about how amphibian species richness and diversity change in response to habitat modification have been suggested. Species richness tends to decrease with increasing levels of disturbance (Pearman 1997, Tocher et al. 2001), but is

dependent on the size and quality of the remaining modified habitat. In Brazil, species richness in forest fragments is increased relative to intact forest of equal size, in part because of the invasion of disturbance-tolerant species (Tocher et al. 1997). Species composition consistently differs between modified and intact habitats but some species are encountered in both habitats. Generally, species tolerant of the matrix are more likely to persist in fragments than those that avoid the matrix (Gascon et al. 1999). Studies that quantify amphibian abundance suggest that the magnitude and direction of changes (positive or negative) after habitat modification are species-specific. Even closely related species of anurans are not consistent in their response to habitat alteration and sometimes show opposite trends (Pearman 1997, Toral et al. 2002). It is important to understand the mechanisms that underlie these species-specific responses in order to design effective conservation strategies.

Insight about causes for interspecific variation in frog response to habitat modification can be gained by examining similarities in life history and ecological traits among vulnerable versus tolerant species. Studies using this approach have been helpful in understanding bird (Bierregaard and Stouffer 1997, Rodewalk and Yahner 2001) and butterfly (Hamer et al. 2003) response to habitat modification. Generally, traits that increase extinction risk in vertebrates include specialized habitat requirements, small population sizes, small geographic ranges, low fecundity, regular migration, and large body sizes (Pimm et al. 1988). If there are consistent trends in traits distinguishing tolerant from vulnerable species, we can develop predictive models of species response to future habitat loss.

Here I examine the spatial patterns of amphibian response to habitat modification within a landscape mosaic of pasture and forest. First, I identify patterns of species richness and composition in forest and pasture using two survey methods. Second, I relate patterns of diversity to local and landscape-scale characteristics. Lastly, I examine whether species occupying each habitat type differ in ecological and life history traits.

METHODS

Study region

My study region is located in the southeastern portion of the Osa Peninsula, Costa Rica (N 8.5°, W 83.5°; Fig. 2.1). Forest area declined in the peninsula from 977 km² in 1979 to 896 km² in 1997 (Sanchez-Azofeifa et al. 2002). The remaining mature forest occurs primarily within Corcovado National Park. Most forest outside the park has been modified and exists in fragments surrounded by a matrix of pasture (Sanchez-Azofeifa et al. 2002). Mean annual precipitation is 5500 mm per year, with the wet season occurring May to November (Sanchez-Azofeifa et al. 2002).

Site selection

Study site selection was restricted to properties where I was able to gain permission to work, because the majority of land in the region is privately owned. I used a stratified random approach to select sites along trail, dirt road, and stream transects. I characterized transects as forest or pasture and as terrestrial (lacking permanent water) or aquatic (along a stream or river). Pasture aquatic transects included some streams with remnant forest in linear buffers extending from the stream edge up to 15 m. In all there were four habitat types (Fig. 2.1): forest aquatic (FA), forest terrestrial (FT), pasture

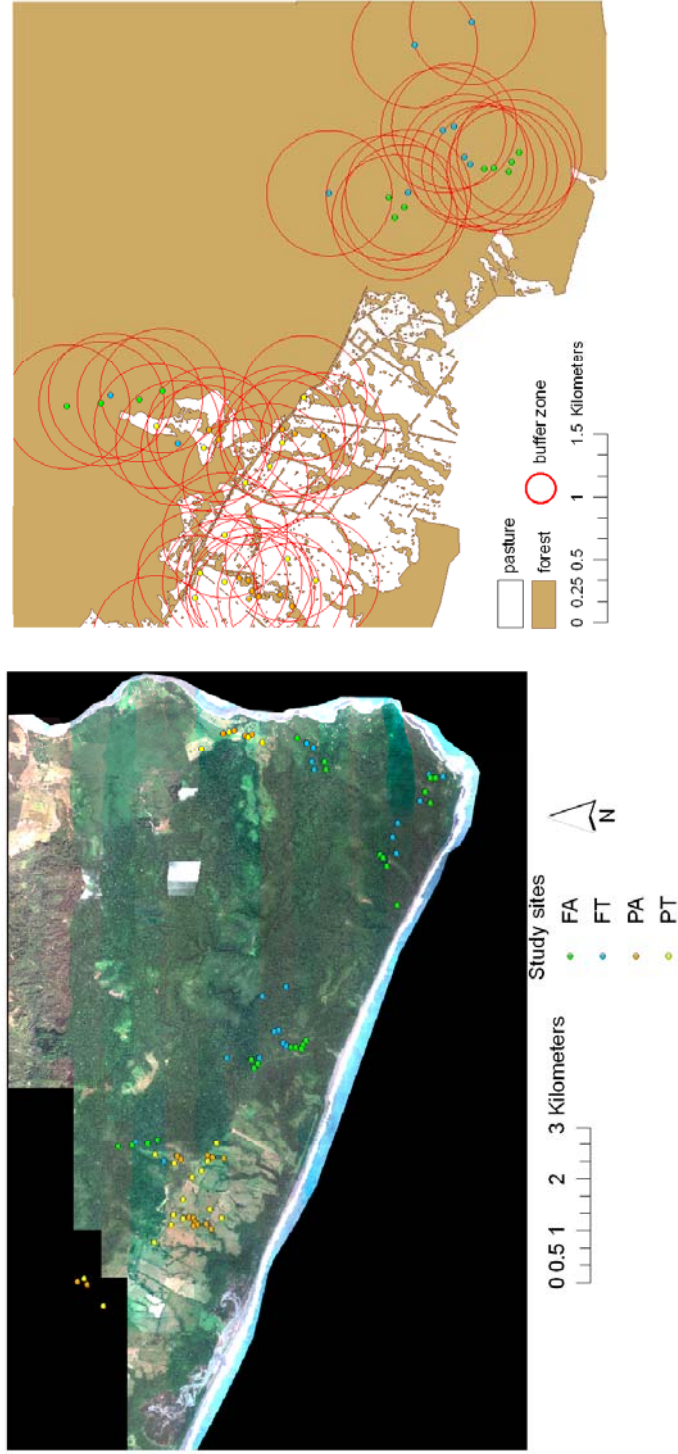


Figure 2.1. Study sites in the Osa Peninsula, Costa Rica; on left are aerial photos of the study region, including the four habitat types (FA = forest aquatic, FT = forest terrestrial, PA = pasture aquatic, and PT = pasture terrestrial), on right is the western subset of the study region with the habitat digitized into forest and pasture and 500 m buffer zones around each site.

aquatic (PA), and pasture terrestrial (PT). I divided transects into 100 m segments and randomly selected segments to visit. The number of segments selected on each transect was roughly dependent on the transect length. To determine the location of the site along each segment, I randomly selected a distance (0 to 100 m) to begin the survey (site origin). Sites were a minimum of 100 m from the next nearest site. I formed groups of sites (four/group), based on their proximities, and completed all surveys for a group during one or two consecutive nights. The date a group was surveyed was assigned randomly.

Survey protocol

I used visual encounter (VES; Crump and Scott 1994) and vocalization survey methods to estimate frog richness and composition at each site. The use of two methods allowed for a more robust estimate compared to a single method. I constrained surveys to 30 minutes and conducted two surveys at each site, one at night and one the following day. From the site origin, I surveyed frogs by walking the exterior of a plot, approximately 20 m x 10 m in terrestrial sites and 20 m x stream width in aquatic sites. Surveys in terrestrial sites began perpendicular to the main transect in a randomly selected direction (right or left). In aquatic sites, I surveyed the stream or river banks for 15 minutes in a downstream direction from the origin and returned to the origin on the opposite stream bank. The specific microhabitats surveyed during terrestrial visual encounter surveys included understory cover, tree trunk surfaces, and buttress roots (all to 3 m in height). In aquatic sites I surveyed the stream bank (within 3 m of water's edge) and overhanging vegetation. I noted the identity, activity, substrate location, and time of encounter for each frog. I completed nocturnal surveys between 18:30 and 24:00 and

diurnal surveys from 8:00 to 13:00. Simultaneous to the visual encounter surveys, I conducted a vocalization survey. I identified and estimated the number of vocalizing individuals of all species within an approximate 50-m diameter of the site.

Site characterization

I quantified several local and landscape characteristics surrounding each site. I assessed local canopy cover at each site using the canopy scope method (Brown et al. 2000). I estimated canopy cover every 2 m of the first 20 m (10 measurements) in each site. I noted the presence or absence of trees (> 30 cm diameter at breast height) within 20 m of the site origin and standing water in each site. When water was present, I measured depth, width (for streams), and temperature. I measured air temperature and relative humidity with an electronic thermohygrometer at the end of each survey. Finally, I recorded the latitude and longitude of each survey origin using a Global Positioning System (Garmin etrex legend).

I calculated landscape characteristics from aerial photos (taken in March 2005 by Winrock International) in a Geographical Information System (GIS) using ArcInfo software (ERSI Inc. 1999). I digitized individual trees and forest fragments to classify habitat into forest (including primary, secondary, tree plantations, and isolated trees in pasture) or pasture (included pasture and all non-forest habitats; Fig. 2.1). I calculated distance to pasture-forest edge, and the proportion of forest cover in overlapping buffer zones of increasing radii (100, 250, 500, and 1000 m) around each site. Distance to edge in pasture sites was defined as the distance to the nearest forest that extends at least 1000 m in length (i.e., not a fragment). Distance to edge in forest sites was defined as the distance to the nearest pasture that continues at least 200 m in length (i.e., not a small clearing).

Emigration distances are not known for species in the study region; therefore, 1 km was estimated to be a conservative maximum distance that the large-sized species might disperse. Similar landscape variables have been measured to describe the configuration and cover of habitats in other studies and have been successful predictors of amphibian species richness and presence (reviewed by Mazerolle and Villard 1999). Four sites were located outside of the aerial photograph coverage region and therefore landscape-scale characteristics for them could not be calculated.

Ecological and life history traits

To examine how ecological and life history traits of anurans relate to occupancy patterns in forest and pasture, I compiled data from the literature (Appendix). Traits included total size of each species' geographical distribution, activity period (diurnal or nocturnal), adult body size, reproductive mode, and clutch size. Total geographic distribution size was found on the Global Amphibian Assessment website (IUCN et al. 2006) and represents the total area of polygons that join known locations of where species have been reported. Adult body size was the maximum snout to vent length reported in Savage (2002). Clutch size was the mean of the values reported in the literature (Appendix). No data were available for two species; therefore I substituted values reported for similar-sized congeners (Appendix). Reproductive mode was ordered; high numbers were modes with decreased dependency on water, 1 = eggs and tadpoles develop in water, 2 = arboreal eggs, tadpoles develop in water, 3 = eggs deposited in a foam nest, tadpoles develop in water, 4 = terrestrial eggs, tadpoles develop in water, 5 = terrestrial eggs with direct development.

Analyses

I summarized the total number of species detected during my surveys in each habitat type. I assessed the ability of my surveys to detect the entire assemblage present in each habitat by calculating incidence-based coverage estimators (ICE) using EstimateS (Colwell 2006). ICE is considered a robust non-parametric approach to estimate species richness given presence-absence data (Chazdon et al. 1998). I also examined species accumulation curves. I used a chi-square test of homogeneity of variance to examine variation in species richness among habitats. I examined heterogeneity in assemblage composition among habitats using an analysis of similarity (ANOSIM) based on a Bray-Curtis dissimilarity matrix (PRIMER 5.0; Clarke and Warwick 2001). ANOSIM tests are non-parametric permutation procedures that analyze the null hypothesis that there is no difference in assemblage composition in different habitats (Clarke and Warwick 2001). Each site was treated as independent, with presence/absence of each species as a dependent variable. I conducted two-way crossed ANOSIMs comparing forest to pasture assemblages and aquatic to terrestrial assemblages. Then I compared each habitat to all others using a one-way ANOSIM. The output provides global and pairwise R test statistics, measures of how different assemblage composition is in different habitats, ranging from 0 (indistinguishable) to 1 (maximum difference).

I summarized all local and landscape variables by habitat type, then evaluated variation among habitats for a subset of variables (local canopy cover, distance to edge, forest cover 100 m, 250 m, 500 m, and 1000 m) using analysis of variance (ANOVA). Local canopy cover and all landscape-scale forest cover variables were arcsin-sqrt transformed to approximate normality and achieve homoscedascity. Levene's test for

equality of variance among groups was violated for local canopy cover and 100-m forest cover; therefore, I evaluated variation among habitats using a Kruskal-Wallis non-parametric test for these variables. Temperature, humidity, and stream width and depth are presented for qualitative examination only.

The importance of local and landscape characteristics in determining species occurrence was examined using logistic regression. I analyzed the occurrence of all species found in at least 10 sites. Additionally, I ran an analysis for *Engystomops pustulosus* because it is a model species in my experiments (see Chapters 3 and 5) and I pooled sites where *Phyllobates vittatus* and *Dendrobates auratus* occurred to examine factors that influence the occurrence of dendrobatids. I included local-scale canopy cover and stream characteristics, and landscape-scale distance to edge and forest cover characteristics. Stream characteristics were not included in analyses for species with fewer than 50% of site occurrences in aquatic sites. Initially all variables were included in the model and I removed variables using backward stepwise procedure when $p > 0.05$. I assessed model significance using a likelihood ratio test and model fit using a Homer-Lemeshow test (Homer and Lemeshow 1989).

I used nonmetric multidimensional scaling (MDS) to examine similarities in life history and ecological traits among species. I standardized variables (0 to 1) included in the analysis, including reproductive habitats, clutch size, geographic distribution size, body size, and activity period. Dissimilarities in traits among species were assessed using Euclidean distances. The result was a multidimensional plot with species scattered across the axes; species closer in space were more similar than those far away. I evaluated the first two dimensions, because together they explained the majority of

variation among species. I examined Spearman rank correlations between the axes produced by MDS with the original trait variables in order to identify which variables contribute the most to the separation of species on each axis. Species clustered into three groups based on similarity in traits. I identified the traits in common among members of each group and examined occupancy patterns of group members.

I calculated a pasture occurrence index by dividing the total number of pasture sites each species was encountered in by the total number of sites the species was encountered in either pasture or forest (0 indicates occurrence only in forest, whereas 1 indicates occurrence only in pasture). The number of sites in each habitat type was standardized prior to analysis by removing surveys (starting from the last) until survey effort equaled 18 sites per habitat. I used linear regression to determine whether species' ecological and life history traits explained variation in the pasture occurrence index, using the index as the dependent variable and traits as independent variables. First, I examined relationships between each independent variable with the dependent variable individually using simple linear regression. To approximate a normal distribution, I natural log-transformed geographic distribution, body size, and clutch size. Activity period was removed from the analysis because the assumption of linearity with the independent variable was violated. All variables explained a significant proportion of variation in the dataset; therefore, all were considered in fitting the full model. I ran two multiple regression models, one including all variables and one with the highly correlated variables removed. In the first, I entered all variables and used a backward stepwise procedure to remove variables one by one when $p > 0.10$. Collinearity diagnostics, including eigenvalues and condition indices, were checked to confirm that independent

variables included in the final model were not intercorrelated. In the second model, I first examined variables for collinearity and excluded one of each pair that produced highly correlated values. Reproductive mode was highly correlated with reproductive habitat (Spearman's $\rho = 0.771$) and body size was highly correlated with clutch size ($\rho = 0.730$). I removed reproductive habitat and body size from the analysis because reproductive mode and clutch size explained a larger amount of variation in the dependent variable in univariate analyses. All statistical analyses were run using SPSS 12.0 (SPSS Inc., Chicago, IL) unless indicated otherwise.

RESULTS

Frog richness and composition

I conducted amphibian surveys at 82 sites, 24 forest aquatic, 21 forest terrestrial, 18 pasture aquatic, and 19 pasture terrestrial (Fig. 2.1). Twenty-two species of eight families were encountered during surveys, 15 species in forest (13 in aquatic sites, 10 in terrestrial sites) and 15 species in pasture habitat (13 in aquatic sites, 9 in terrestrial sites) (Fig. 2.2). When survey effort was standardized across habitats, species richness remained the same. Species encountered opportunistically (before or after the 30-minute survey) increased richness in each habitat, with three additional species in forest (*Ollotis aucoinae* (Bufonidae), *Hyalinobatrachium valerioi* (Centrolenidae), and *Oophaga granuliferus* (Dendrobatidae)) and two additional species in pasture (*Smilisca phaeota* and *Scinax boulengeri* (Hylidae)) (Table 2.1). I detected 76 – 100% of the frog species in each habitat, according to ICE estimations (Table 2.1). Species accumulation curves

Table 2.1. Frog species richness in each habitat, detected during visual encounter and vocalization surveys (Richness), including opportunistic encounters (Richness +), and incidence-based coverage estimators (ICE; Colwell 2006).

Habitat	Richness	Richness +	ICE
All	22	27	22.24
FT	10	12	11.96
FA	13	15	14.85
PT	9	11	9.00
PA	13	13	17.00

reached asymptotes faster in terrestrial than in aquatic habitats, suggesting that additional species remain to be detected in aquatic habitats. Although species richness was lower in terrestrial than in aquatic sites, richness did not differ among habitats ($\chi^2 = 6.38$, $df = 3$, $P = 0.77$).

The contribution of different families to assemblage composition differed among habitats, with a single member of the Bufonidae and Leiuperidae found only in pasture and members of the Aromobatidae and Dendrobatidae detected only in forest (Fig. 2.2). Species of the families Leptodactylidae, Brachycephalidae, Hylidae, and Centrolenidae were encountered in both pasture and forest. Seven species were detected only in pasture and a different seven species were encountered only in forest (Fig. 2.3). Aquatic and terrestrial habitats differed in assemblage composition (global $R = 0.436$, $P < 0.001$) and forest and pasture habitats differed in composition (global $R = 0.561$, $P < 0.001$). Additionally, there were overall compositional differences among habitats (global $R = 0.574$, $P < 0.001$) and all pair-wise comparisons were also significant (forest aquatic – forest terrestrial (FA-FT), $R = 0.546$, $P < 0.001$; FA-pasture aquatic (PA), $R = 0.371$, $P < 0.001$; FA-pasture terrestrial (PT), $R = 0.804$, $P < 0.001$; FT-PA, $R = 0.666$, $P < 0.001$; FT-PT, $R = 0.791$, $P < 0.001$; PA-PT, $R = 0.236$, $P < 0.001$).

The species most frequently detected in pasture and forest were members of different families (Fig. 2.3). Species detected most often in forest (≥ 10 of 45 sites) included three leaf litter frogs, *Eleutherodactylus diastema*, *Craugastor fitzingeri*, and *C. stejnegerianus*, and two glassfrogs, *Cochranella granulosa* and *C. pulverata* (Fig. 2.3). In contrast, a toad, *Rhinella marina*, and a frog *Leptodactylus poecilochilus* were detected most often (≥ 10 of 37 sites) in pasture. The rarest species in forest (< 5 of 45 sites)

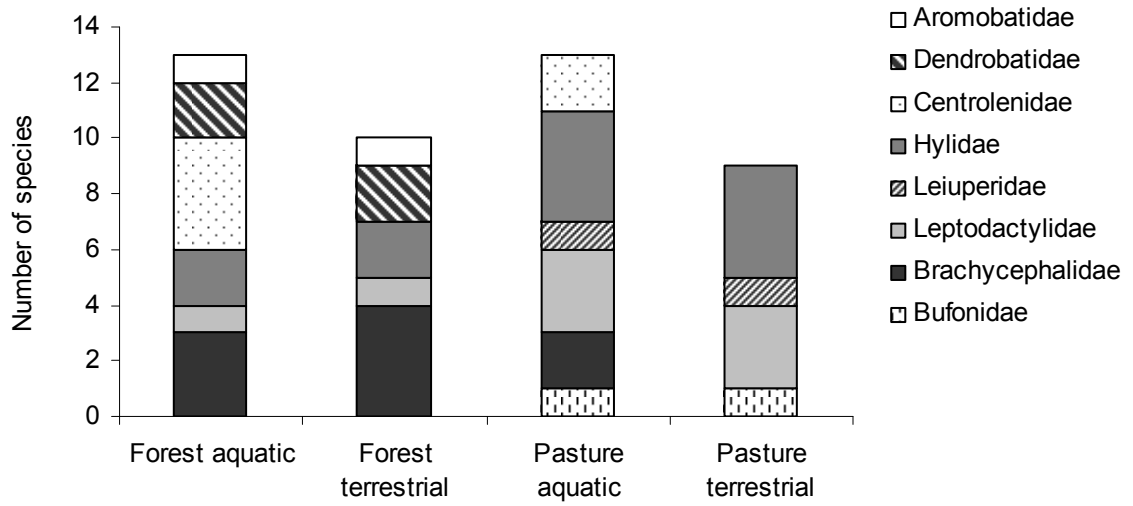


Figure 2.2. Frog species richness and family composition detected using visual encounter and vocalization survey methods in each habitat type.

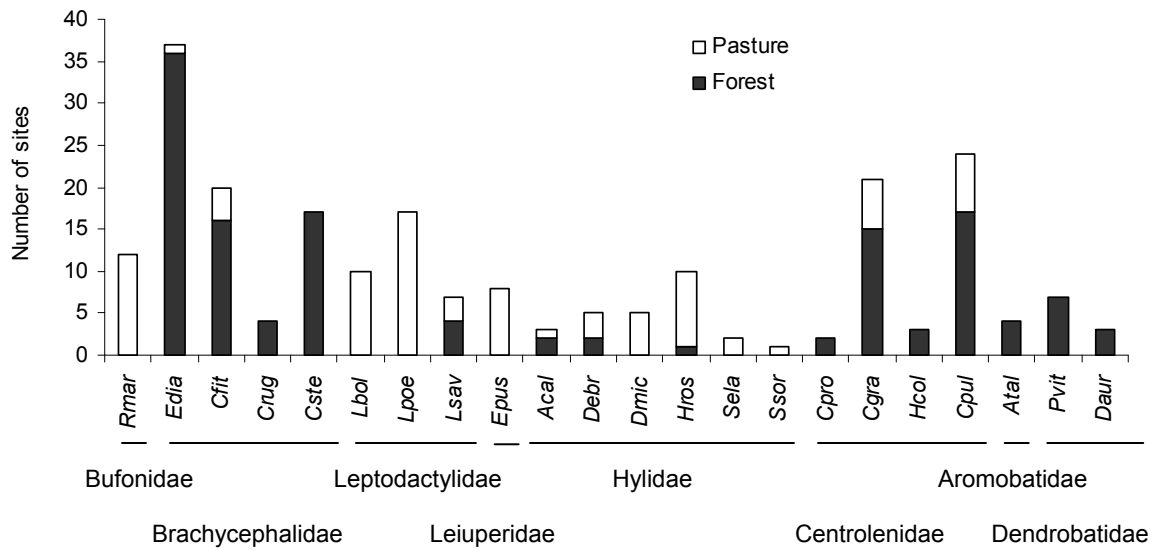


Figure 2.3. Species occurrence in pasture and forest; 82 sites were sampled, 45 in forest and 37 in pasture. Species are abbreviated by the first letter of the genus and first three letters of the species names (see Appendix for full names).

included a leaf litter frog, *Craugastor rugosus*, two glassfrogs, *Centrolene prosoblepon* and *Hyalinobatrachium colymbiphylum*, a rocket frog, *Allobates talamancae*, and a poison-dart frog, *Dendrobates auratus* (Fig. 2.3). In pasture, two treefrogs, *Scinax elanochrous* and *Smilisca sordida*, were rarely encountered (< 5 of 37 sites).

Site characterization and species occurrence models

The *a priori* habitat categories (FT, FA, PT, PA) differed in many local and landscape-scale characteristics (Table 2.2). Mean air temperature was 4 – 7° C warmer during the day and a maximum of 1° C warmer at night in pasture than in forest (Table 2.2). Mean relative humidity was 15 – 31% lower during the day and 7 – 15% lower at night in pasture compared to forest. Water was present in 100% of FA and PA, 0% of FT, and 47% of PT sites. Although terrestrial sites were controlled for permanent water presence by placing them at least 50 m from streams, ephemeral pools were omnipresent in pasture. Mean water temperature was approximately 1° C warmer in PA than in FA sites and 4 – 5° C warmer in PT than in PA or FA sites. Pasture streams were 17% wider and 50% deeper than those in forest (Table 2.2). Trees were present (within 20 m) in 100% of FA and FT, 53% of PT, and 83% of PA sites. Local canopy cover was 45 – 87% higher in FT and FA than in PT and PA sites ($X^2 = 58.721$, $df = 3$, $P < 0.001$).

Landscape-scale characteristics also differed among habitat types (Table 2.2). Distance to forest-pasture edge was 2.7 to 7 times further in forest than in pasture sites. In buffer zones around each site, the proportion of forest cover was always greater for forest than for pasture sites (Table 2.2). Forest cover surrounding forest sites decreased as buffer zone size increased. Cover surrounding pasture sites generally increased as buffer zone size increased, except for a decrease in cover between 100 m and 250 m

Table 2.2. Descriptive statistics for local and landscape characteristics in each habitat. All canopy and forest cover characteristics are proportions. For each characteristic, the maximum across all habitats is in bold and the minimum is italicized.

	Forest terrestrial mean \pm SE	range	Forest aquatic mean \pm SE	range	Pasture terrestrial mean \pm SE	range	Pasture aquatic mean \pm SE	range
Local characteristics								
Canopy cover	0.886 \pm 0.009	(0.78-0.94)	0.723 \pm 0.052	(0.03-0.94)	<i>0.016</i> \pm <i>0.016</i>	(0.00-0.31)	0.273 \pm 0.071	(0.00-0.76)
Stream width (m)			<i>4.49</i> \pm <i>0.58</i>	(1.85-11.04)			5.26 \pm 0.62	(2.12-11.50)
Stream depth (m)			<i>0.44</i> \pm <i>0.06</i>	(0.09-1.00)			0.81 \pm 0.10	(0.30-2.00)
Water temperature (°C)			25.27 \pm 0.22	(24.0-28.0)	30.33 \pm 0.67	(25.0-34.5)	26.12 \pm 0.33	(24.0-30.0)
Air temperature (D, °C)	26.52 \pm 0.19	(24.4-28.0)	27.47 \pm 0.425	(24.1-33.2)	33.56 \pm 0.61	(29.0-37.8)	30.87 \pm 0.83	(26.8-38.3)
Air temperature (N °C)	25.01 \pm 0.25	(23.0-27.7)	25.56 \pm 0.210	(24.2-28.0)	25.99 \pm 0.33	(23.2-29.2)	26.23 \pm 0.36	(24.0-28.8)
Relative humidity (D, %)	93.81 \pm 1.14	(77.0-99.0)	93.48 \pm 1.28	(81.0-99.0)	62.42 \pm 2.40	(44.0-83.0)	78.06 \pm 3.35	(50.0-96.0)
Relative humidity (N, %)	96.43 \pm 0.84	(88.0-99.0)	96.25 \pm 0.69	(88.0-99.0)	95.58 \pm 1.20	(81.0-99.0)	<i>94.82</i> \pm <i>0.74</i>	(89.0-99.0)
Trees present (% of sites)	100		100		53		83	
Water present (% of sites)	0		100		47		100	
Landscape characteristics								
Distance to edge (m)	1395.76	(50-3260)	1250.00	(96-3120)	206.24	(27-968)	340.81	(16-967)
	\pm 212.14		\pm 221.62		\pm 60.86		\pm 73.82	
Forest cover 100 m	0.997 \pm 0.003	(0.95-1.00)	0.997 \pm 0.002	(0.96-1.00)	<i>0.186</i> \pm <i>0.036</i>	(0.01-0.60)	0.337 \pm 0.031	(0.10-0.52)
Forest cover 250 m	0.969 \pm 0.015	(0.68-1.00)	0.971 \pm 0.007	(0.86-1.00)	0.310 \pm 0.048	(0.05-0.73)	0.278 \pm 0.029	(0.16-0.55)
Forest cover 500 m	0.961 \pm 0.017	(0.72-1.00)	0.956 \pm 0.012	(0.78-1.00)	0.413 \pm 0.044	(0.13-0.82)	0.330 \pm 0.047	(0.13-0.70)
Forest cover 1000 m	0.906 \pm 0.027	(0.54-1.00)	0.872 \pm 0.025	(0.63-0.99)	0.498 \pm 0.035	(0.30-0.86)	<i>0.432</i> \pm <i>0.032</i>	(0.31-0.73)

buffers in PA sites. Forest cover differed among habitats at 100 m ($X^2 = 65.925$, $df = 3$, $P < 0.001$), 250 m ($F_{3,77} = 207.751$, $P < 0.001$), 500 m ($F_{3,77} = 127.526$, $P < 0.001$), and 1000 m ($F_{3,77} = 58.152$, $P < 0.001$) buffer zones. Multiple comparisons tests revealed that, in each case, forest cover in FA did not differ from FT and PA did not differ from PT, but together forest sites differed from pasture sites.

The occurrence of each species was associated with local and landscape characteristics at different spatial scales (Table 2.3). Species detected only in pasture (*R. marina*, *L. bolivianus*, *L. poecilochilus*, and *E. pustulosus*) were negatively associated with the proportion of forest cover within 100 m of sites (Table 2.3). Species encountered only in forest (*C. stejnegerianus* and dendrobatids) were positively associated with local canopy cover. *Craugastor stejnegerianus* was also positively associated with forest cover at 250 m and 1000 m and negatively associated with forest cover at 500 m (Table 2.3). The occurrence of two glassfrogs, *C. pulverata* and *C. granulosa*, was positively associated with stream width and forest cover at 100 m buffer zones. Additionally, the occurrence of *C. granulosa* was negatively associated with local canopy cover and forest cover within 250 m. *Craugastor diastema* occurrence was positively associated with forest cover within 1000 m and *C. fitzingeri* occurrence was negatively associated with stream width and depth. Distance to edge was a significant predictor of *L. poecilochilus* occurrence. Likelihood ratio tests indicated that the models fit the data well ($P \leq 0.01$ for each species; Table 2.3). Homer-Lemeshow statistics also supported a good fit to the data ($P > 0.18$), except for *L. bolivianus* ($P = 0.002$). However, a step before the final model was fit, forest cover within 500 m was included in

Table 2.3. Logistic regression models for species occupancy in pasture and forest in relation to local and landscape characteristics with sign and significance for each variable included in final models. Likelihood ratio test chi-square value and p values are given to assess overall model significance.

	<i>Rmar</i>	<i>Edia</i>	<i>Cfit</i>	<i>Cste</i>	<i>Lbol</i>	<i>Lpoe</i>	<i>Epus</i>	<i>Hros</i>	<i>Cpul</i>	<i>Cgra</i>	<i>Dendrobates</i>
Local characteristics											
Canopy cover				+						- *	+
Stream width (m)			-	X	X	X	X		+	+	
Stream depth (m)			-	X	X	X	X				-
Landscape characteristics											
Distance to edge (m)						- *		-			
Forest cover 100 m	- *				- **	- **	- *		+	+	
Forest cover 250 m				+							
Forest cover 500 m				- *						- *	
Forest cover 1000 m		+	***	+							
Likelihood ratio test	11.123 ***	18.466 ***	11.171 **	35.084 ***	18.363 ***	38.805 ***	10.396 ***	8.556 **	9.262 **	17.541 **	10.492 **

* $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, x = variable not entered into model.

the model and was negatively associated with *L. bolivianus* occurrence. At this step, the Homer-Lemeshow statistic was not significant.

Ecological and life history traits

Multidimensional scaling (MDS) separated species into three ecological groups based on dissimilarities in life history and ecological traits (Fig. 2.4). The first MDS axis was highly correlated with all traits, reproductive habitat ($\rho = 0.920$, $P < 0.001$), activity period ($\rho = 0.727$, $P < 0.001$), clutch size ($\rho = 0.686$, $P < 0.001$), geographic distribution ($\rho = 0.670$, $P = 0.001$), and body size ($\rho = 0.573$, $P = 0.005$). The second MDS axis was correlated with activity period ($\rho = 0.590$, $P = 0.004$). The stress value was low (0.059) and the R^2 value was high (0.989), indicating that the axes represented a good fit to the dissimilarity matrix. Species within each of the three groups had several traits in common. Members of group 1 have small body sizes, clutch sizes, and geographic distributions, and a diurnal activity pattern. In contrast, members of group 3 have large body sizes, clutch sizes, and geographic distributions. Members of group 1 occurred only in forest, whereas members of group 3 occurred primarily in pasture. Members of group 2 have small to moderate body sizes and clutch sizes compared to groups 1 and 3. Many species in group 2 occurred in both forest and pasture, but four species were detected exclusively in pasture and two species were found only in forest (Figs. 2.3 and 2.4).

Ecological and life history traits explained variation in the pasture occurrence index among species ($P < 0.05$ for all traits except reproductive habitat; Table 2.4, Appendix). The best predictor of pasture occurrence was clutch size, explaining 66 % of variation in the occurrence index among species (Fig. 2.5). As clutch size increased, the

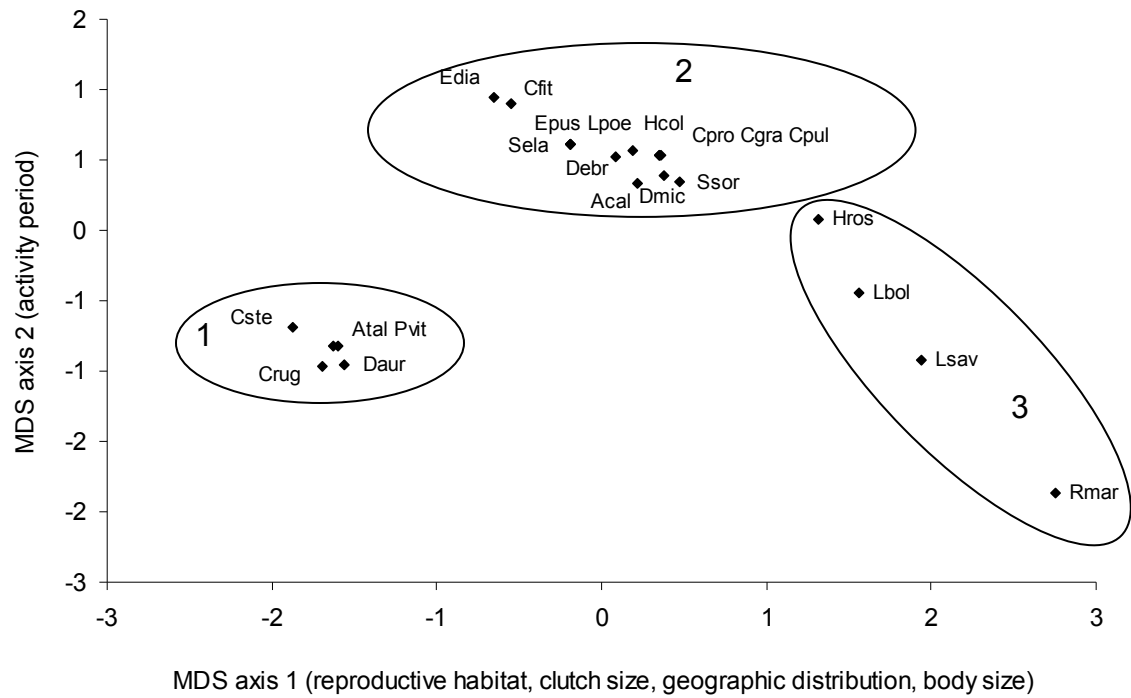
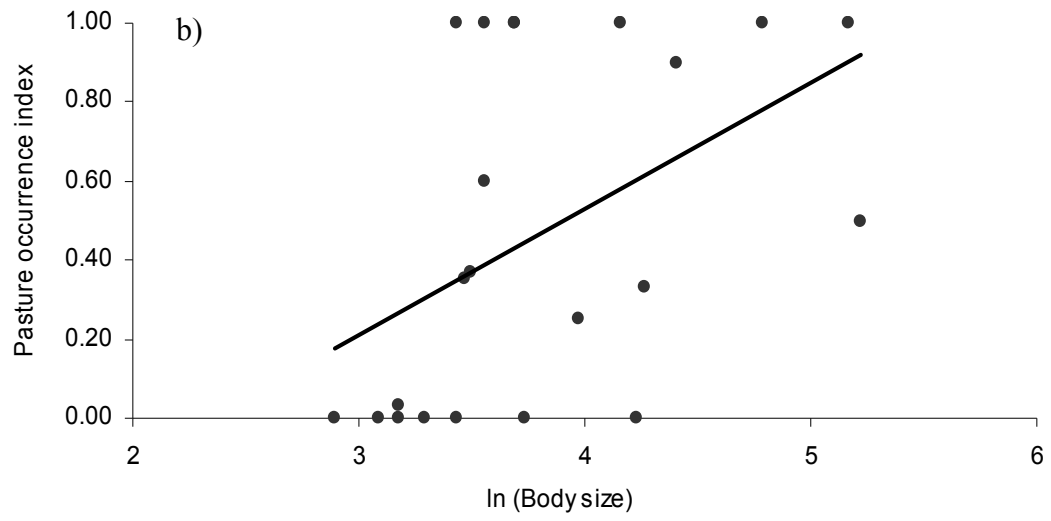
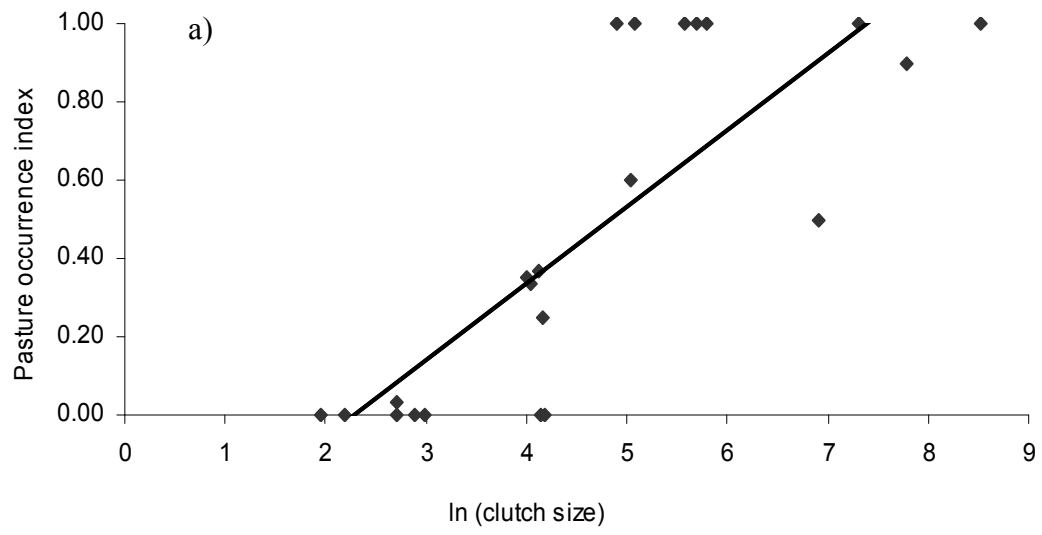


Figure 2.4. Plot showing non-metric multidimensional scaling of species by dissimilarities in ecological and life history traits (raw data in Appendix). Species are abbreviated by the first letter of the genus and first three letters of the species names. Ecological groups, numbered 1-3, are described in the text.

Table 2.4. Univariate results of linear regression analyses examining the effect of independent variables on the index of occurrence in pasture, $df = 1, 21$ for each variable.

Variables	Constant	Coefficient	r^2	F	P
Reproductive habitat	0.096	0.126	0.172	4.141	0.055
Reproductive mode	1.025	-0.194	0.362	11.371	0.003
ln (Geographic distribution)	-0.916	0.113	0.290	8.151	0.01
ln (Body size)	-0.748	0.319	0.223	5.746	0.026
ln (Clutch size)	-0.443	0.196	0.660	38.896	< 0.001



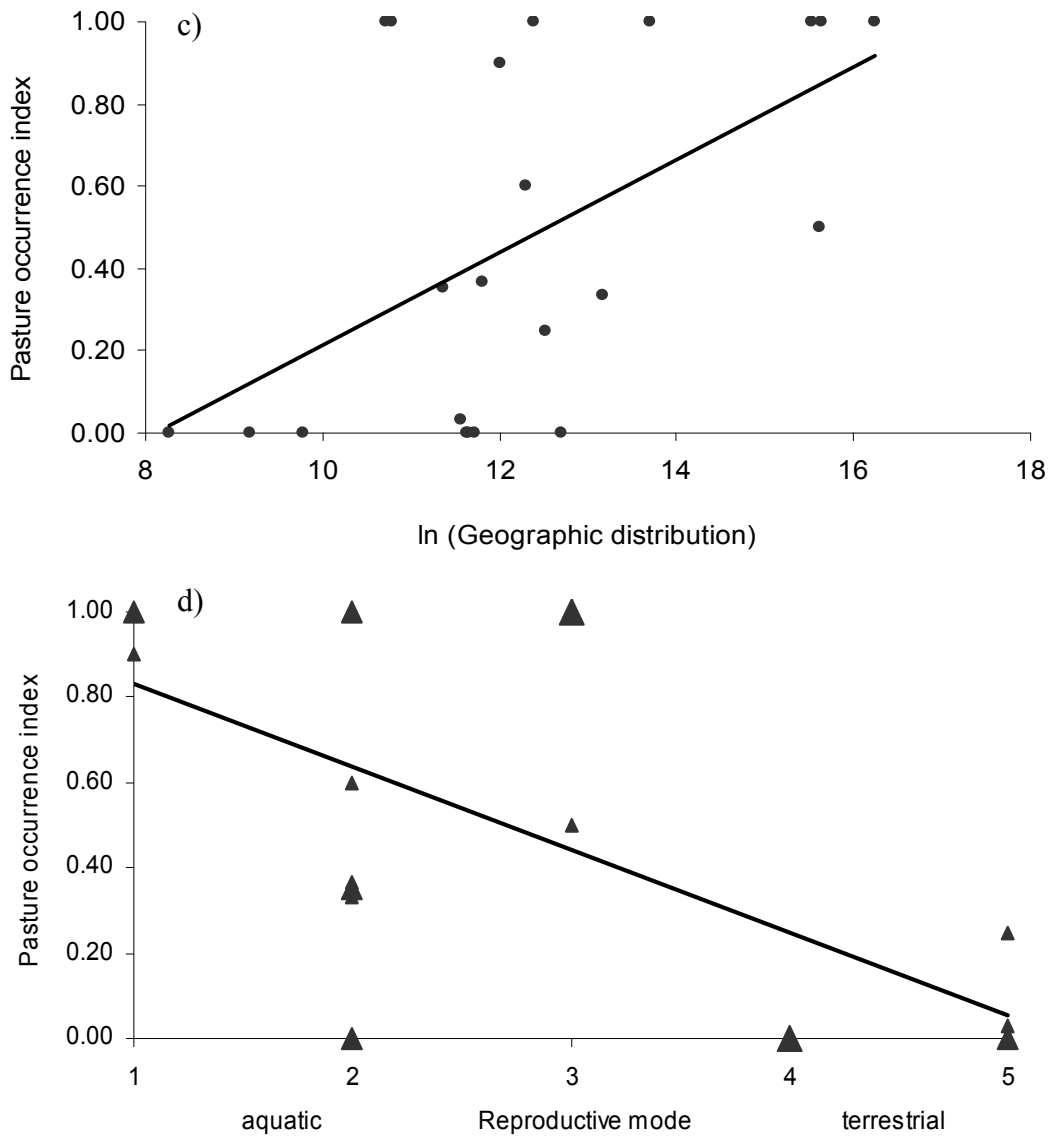


Figure 2.5. Relationships between the index of occurrence in pasture (calculation described in text) and ecological traits, a) clutch size, b) body size, c) geographic distribution, and d) reproductive mode. Symbol sizes for reproductive mode represent the number of species, small = 1, medium = 2, and large = 3.

pasture occurrence index increased, indicating that species with large clutch sizes were often detected in pasture, whereas species with clutch sizes under 50 eggs were not detected in pasture, with one exception, *E. diastema* (Fig. 2.5). Reproductive mode explained 36% of the variation in the pasture occurrence index, with increased occurrence in pasture among species with greater dependency on water for reproduction (Table 2.4, Fig. 2.5). Body size and geographic distribution explained less variation in the pasture occurrence index (< 30% each) compared to clutch size and reproductive mode, but the relationships were still significant. Large frogs and those with large geographic distributions had higher occurrence in pasture than small frogs and those with small geographic distributions (Fig. 2.5). Reproductive habitat explained little variation (17%) in the pasture occurrence index (Table 2.4). In the first multiple regression model, all variables were retained except body size (Table 2.5). The model explained 83% of the variation in pasture occurrence index. All variables in the model were highly significant ($P < 0.01$) except total geographic distribution ($P = 0.092$). In the second model, clutch size was the only variable retained, yielding the same result as a univariate model with clutch size (Tables 2.4 and 2.5).

DISCUSSION

Frog richness and composition

Species richness did not differ between forest and pasture habitats, but aquatic sites generally had higher richness than terrestrial sites. Opportunistic encounters, species detected outside of the 30-minute survey periods, and ICE estimates suggested that richness would increase in all habitats with additional survey effort. Precise

Table 2.5. Variables remaining in the final multiple linear regression models with t and P values. Model 1: $F = 20.987$, $P < 0.001$, $df = 4,21$, $R^2 = 0.832$, Model 2: same as univariate ln (Clutch size) model.

Variables	Coefficient	t	P
Constant	0.203	0.566	0.579
Reproductive habitat	-0.217	-3.828	0.001
Reproductive mode	-0.199	-3.688	0.002
ln (Geographic distribution)	0.050	1.788	0.092
ln (Clutch size)	0.186	5.315	< 0.001

measures of richness depend on sampling effort, the number of rare species, spatial distribution of species, and unbiased survey methods (Chazdon et al. 1998). If a large number of species are rare in my forest sites, a difference in richness between pasture and forest would become apparent only after a more extensive survey effort.

In contrast, species composition differed among all habitat types. Thirty-six percent of species were detected in both pasture and forest, 32% of species were detected only in forest, and the remaining 32% were detected only in pasture. My results demonstrate that pasture does not impede the dispersal or activity of all species. In fact, pasture was the only habitat in which some species were detected; whereas other species were restricted to forest. A study in Mexico found fewer species occupying pasture (57%) than forest-edge (67%) and forest interior (62%) sites (Urbina-Cardona et al. 2006). The quality of the matrix for particular species undoubtedly influences their occupancy patterns within the pasture-forest mosaic.

Site characterization and species occurrence models

Forest and pasture habitats differed in many characteristics that contribute to the overall quality of the abiotic environment for amphibians. Most notably, canopy cover on a local scale and forest cover at all buffer distances on the landscape scale differed between forest and pasture. In comparison to pasture sites, forest sites had more forest cover in surrounding buffers, lower diurnal air temperatures, and higher diurnal relative humidity, consistent with results of other studies (e.g., Williams-Linera et al. 1998). Although amphibians have adaptations to cope with unfavorable abiotic conditions, physiological limitations associated with their permeable skin and high evaporative water

loss (Shoemaker et al. 1992) may limit their activity patterns and distributions (Duellman and Trueb 1986).

Species occupancy within the forest-pasture mosaic was associated with environmental characteristics at different spatial scales. The occurrence of four species detected only in pasture (*R. marina*, *L. bolivianus*, *L. poecilochilus*, *E. pustulosus*) was negatively associated with forest cover near the study sites, whereas forest cover at larger spatial scales (250 – 1000 m buffers) was not important. Forest cover was correlated at all buffer distances, complicating my ability to conclude that any one scale is more important than other scales. However, the consistency of results for these four species suggests that they respond to the spatial configuration of the modified landscape at the local scale rather than larger spatial scales. Terrestrial pasture sites typically had isolated trees or living fences (i.e., lines of trees connected by barbed wire) within 100 m, possibly providing cover for these species. Additionally, all the pasture species retreat during the day into burrows under grass or leaf litter, or in mud (Savage 2002, T. Hawley *personal observations*). In contrast, the occurrence of species detected primarily or only in forest (*E. diastema*, *C. stejnegerianus*, and the dendrobatids) was positively associated with forest cover at larger spatial scales (250 and 1000 m) and/or canopy cover at the local scale. Glassfrog occurrence was positively associated with forest cover at 100 m, stream width, and *C. granulosa* was negatively associated with local forest cover. My results are consistent with studies conducted at temperate latitudes; therefore, patterns of species occurrence reflect species-specific preferences and tolerances. For instance, the occurrences of *Rana pipiens* and *Bufo americanus* are negatively associated with forest cover within 1 km of study sites, whereas two salamanders, *Ambystoma maculatum* and

the *A. laterale/A. jeffersonianum* complex are positively associated with forest cover (Guerry and Hunter 2002). Other studies (e.g., Otto et al. 2007, Piha et al. 2007) have also shown that forest cover influences occupancy patterns of species at different spatial scales.

Ecological and life history traits

Species exhibiting habitat preferences for pasture or forest shared similar ecological and life history traits. However, some pasture-tolerant species could not be distinguished from those intolerant of pasture by the traits examined in my analyses. For example, two glassfrogs were detected in both pasture and forest sites, whereas a different pair of glassfrogs were detected only in forest. My analysis was unable to identify a trait responsible for limiting their occupancy in pasture. However, some generalizations can be made and may be applicable to other frog assemblages in a mosaic of modified and intact habitats. For example, species in group 1 of the MDS analysis were small in size (all < 42 mm SVL except for *C. rugosus* (69 mm SVL)), with small clutch sizes (< 65 eggs/clutch), diurnal, and were only found in forest. In contrast, species in group 4 were large in size (> 82 mm SVL), with large clutch sizes (> 1000 eggs/clutch), nocturnal, and primarily found in pasture.

Variation in the pasture occurrence index was accounted for by various ecological traits, supporting results of the MDS analysis. Clutch size alone explained over 66% of the variation in pasture occurrence. These results suggest that species with large clutches will occur more often in pasture than species with small clutches. Large clutches may provide greater protection against whole clutch failure because it is more likely that some individuals will survive; however, small clutches usually have more energy invested per

egg and tadpoles hatched from large eggs typically have higher fitness (Kuramoto 1978). Energy investment per egg and clutch size is, in part, a function of environmental uncertainty (Brockelman 1975). Therefore, species with large clutches may be better suited for the fluctuating abiotic conditions in pastures than species with small clutches.

The addition of reproductive habitat, reproductive mode, and geographic distribution to the clutch size model explained an additional 17% of the variation in the pasture occurrence index. Species with reproductive modes that depend on water were more likely to occur in pasture than those with terrestrial reproductive modes. Ephemeral pools were abundant in pasture but rare in forest (T.Hawley, *personal observations*). Species that are able to tolerate the pasture abiotic environment during pre- and post-metamorph life stages can take advantage of the increased availability of breeding habitats in pasture. Species with large geographic distributions were more likely to occur in pasture than those with small geographic distributions.

Physiological tolerances will determine the ability of frogs to emigrate across and use modified habitats. Large body size may be advantageous in desiccating environments because of smaller surface area-volume ratios (Lee 1993). Broad thermal tolerance may allow species to succeed in disturbed environments (Pough et al. 1977, Hutchison and Dupré 1992). Other studies have identified traits that may predict amphibian population declines. Niche breadth is positively associated, whereas proximity to a species range boundary is negatively associated, with the occupancy of frogs in woodlot patches in an agricultural landscape in Indiana (Swihart et al. 2003). Amphibian body size, behavioral traits, and ontogeny were not important predictors of patch occupancy in least squares regression models (Swihart et al. 2003). Stream use and

habitat specialization are important in distinguishing amphibian species with declining populations from those with stable populations (Lips et al. 2003, Williams and Hero 1998). Additionally, low fecundity (Williams and Hero 1998) and large body size (Lips et al. 2003) are informative of species' sensitivity to decline. The former two studies involved frog assemblages that have undergone population declines due to chytrid (*Batrachochytrium dendrobatidis*) fungal infection; however, their findings, together with my results, suggest that ecological and life history traits can be useful predictors of a species vulnerability to disturbance.

Conservation implications

Results of my study indicate that pastures and forest may have equivalent frog species richness, but substantially different assemblage composition. Some species are capable whereas others are incapable of residing in a pasture matrix. While it is important to be aware of individual species responses, it is clear that a pasture matrix provides suitable habitat for some species. Additional research is needed to identify the mechanisms underlying occupancy patterns and to determine if species with large body sizes, clutch sizes, and geographic distributions are likely to exhibit high performance in other matrix habitats. If so, land managers will be able to predict species' responses to habitat modification. One of the greatest challenges for conservation is to improve the quality of the agroecological matrix such that individual organisms can move among forest fragments, thereby diminishing local extinctions (Vandermeer and Perfecto 2007). Meeting this challenge will ensure the long-term persistence of species that are intolerant of matrix environments in a landscape mosaic of modified and intact habitats.

CHAPTER 3

NONRANDOM BREEDING SITE SELECTION IS CONSISTENT WITH TADPOLE PERFORMANCE IN NEOTROPICAL PASTURES AND FORESTS

Some frog species thrive in modified habitats whereas others are restricted to undisturbed forest; however, the mechanisms underlying these occupancy patterns remain unclear (Marsh and Trenham 2001). A population may be unsustainable in a habitat because of reduced vital rates during any life cycle stage. The presence of adult frogs in a habitat does not indicate the suitability of the habitat for eggs, tadpoles, or juveniles (Cushman 2006). Yet most studies investigating frog abundance in modified habitats assume that adult occupancy patterns reveal the overall habitat quality for a particular species (i.e., Urbina-Cardona et al. 2006, Toral et al. 2002). This assumption is rarely tested experimentally, but is supported by the habitat selection theory, which predicts nonrandom patterns of patch occupancy based on differential fitness in each patch (Fretwell and Lucas 1970, Morris 2003). Here, I examine whether adult frogs select breeding sites in habitats where their tadpoles achieve the highest performance.

Amphibians typically require multiple habitats during different stages of their life cycle (Wilbur 1980). For example, pool-breeding species often undergo seasonal migration to their breeding sites and retreat to nearby terrestrial areas for cover and foraging (Semlitsch 2000). In modified landscapes, frog movements can be influenced by the configuration of forest fragments, matrix habitat composition, and habitat connectivity (Gascon and Lovejoy 1998). In addition, the availability of breeding sites can limit frog occupancy in different habitats (Zimmerman and Bierregaard 1986).

Landscape-level features, along with species-specific behavior and ecology, influence breeding site selection. Frogs may utilize breeding sites in matrix habitat near forest edges even if it is unsuitable for foraging and cover. For example, gray treefrogs likely undergo nightly migrations from forest into open sites to oviposit in pools (Hocking and Semlitsch 2007). In contrast, other species avoid matrix habitats because of physiological or behavioral constraints. For example, *Epipedobates femoralis* oviposits in pools in secondary forest, whereas *Osteocephalus taurinus* oviposits only in pools in primary forest (Gascon 1993). Breeding site selection can have fitness consequences for subsequent life cycle stages; metamorph and juvenile survival will be reduced if pools are isolated from suitable terrestrial habitat (Rothermel 2004).

Persistence in the matrix can be influenced by the extent to which each life cycle stage tolerates the abiotic environment. Compared to forests, pastures have higher air temperature and photon flux density, and lower humidity and soil moisture (Holl 1999). These abiotic characteristics result in edge effects, often penetrating more than 50 m into forest (Laurance et al. 2002). Similar abiotic characteristics influence the vital rates of eggs and tadpoles. Open tree canopy (low shade) can increase or decrease rates of tadpole development and growth rates depending on the species (Skelly et al. 2002, Halverson et al. 2003, Schiesari 2006). Ponds in open-canopy habitats have higher temperatures, dissolved oxygen concentrations, and productivity (Werner and Glennemeier 1999, Skelly et al. 2002, Schiesari 2006). Higher water temperatures in open-canopy pools can increase rates of tadpole growth (Werner and Glennemeier 1999, Skelly et al. 2002) and development (Ultsch et al. 1999). However, for many species a plateau may be reached where the benefits of increased growth and development will be

offset by reduced survival caused by extreme temperatures. This plateau, described as the critical thermal maximum, is between 38° and 42° C for a group of studied species (Ultsch et al. 1999).

I examined the mechanisms underlying interspecific variation in frog occupancy patterns by quantifying breeding site selection and tadpole performance in forest, edge, and pasture sites. I considered patterns of breeding site selection across the pasture-forest edge for several members of the frog assemblage in my study area. In addition, I studied two model species, *Dendrobates auratus* and *Engystomops pustulosus*, to gain insight about whether breeding site selection is nonrandom with respect to tadpole performance among habitats. Based on habitat selection theory (Fretwell and Lucas 1970), I expected tadpole performance to vary consistently with patterns of adult breeding site selection in forest, edges, and pastures.

METHODS

My study was conducted in the Osa Peninsula, Puntarenas Province, Costa Rica (8°26' N, 83°22' W). This region is characterized by distinct rainy (May to December) and dry seasons (January to April) with precipitation averaging 4000 to 6000 mm y⁻¹ (L. Gilbert, *unpublished data*). The study landscape is a mosaic of lowland wet forest (Hartshorn 1983) and pasture (40 years old), with a distinct edge separating the habitats.

Study species

The two model species, *Dendrobates auratus* and *Engystomops pustulosus*, were selected based on their abundance, contrasting life histories, and habitat preferences. *Dendrobates auratus* is a diurnal, territorial, forest specialist that lays eggs in small clutches (5-13) in terrestrial habitats. After eggs hatch, males carry the tadpoles to small

pools (e.g., palm fronds or tree cavities; Savage 2002). Metamorphosis occurs within five to 13 weeks. *Dendrobates auratus* is restricted to lowland forests (Savage 2002), however Toft (1985) observed individuals foraging in fields and fence rows adjacent to forest in Panama. *Dendrobates auratus* is a model for species in the region with similar life histories, including *Phyllobates vittatus* and *Allobates talamancae*. In contrast, *E. pustulosus* deposits large clutches of eggs (80-450) in foam nests across a wide array of aquatic habitats, including small temporary pools, hoofprints, and ditches (Savage 2002). Metamorphosis occurs within five to nine weeks (Savage 2002). *Engystomops pustulosus* is nocturnal and common in open and disturbed areas, as well as forest edges (Savage 2002). It is associated with forest in other parts of its range (Marsh et al. 1999). *Engystomops pustulosus* acts as a model for species with similar life histories such as *Leptodactylus bolivianus* and *L. poecilochilus*, which are common in the study area.

Abiotic environments

I quantified abiotic characteristics of each habitat to correlate with tadpole performance. Air temperature and relative humidity were measured hourly during part of the 2005 study period (41 days; 1 June – 11 July) using HOBO data loggers (Onset Computer Corporation, Pocasset, Massachusetts). A single HOBO logger was placed in forest (85 m from the edge), edge, and pasture (65 m from edge) habitats.

I quantified the light environment in each subblock of the tadpole performance experiment (Fig. 3.1) using hemispherical photographs (Nikon Coolpix 4500 digital camera with a Nikon FC-E8 fisheye converter lens; 180° field of view). The camera was mounted on a tripod 30 cm above the ground and oriented with magnetic north at the top of the image, allowing the incorporation of solar tracks in the photo analysis. I took four

photos in each subblock on overcast days (19-20 November 2006). I processed and analyzed the photos using Gap Light Analyzer, version 2.0 (Frazer et al. 1999), calculating global site factor (GSF). Global site factor can range from 0 (complete obstruction) to 1 (open sky) and is the percentage of incident radiation transmitted through a gap to a point below the canopy during a growing season (Canham 1988). I tested for heterogeneity in GSF by habitat (fixed) and block (random) treatment factors using analysis of variance (ANOVA).

In addition, I monitored water temperature hourly in one pool in each subblock using I-button temperature loggers (Maxim Integrated Products, Sunnyvale, CA) placed in plastic bags at a depth of 18 cm. I calculated mean daily maximum and minimum water temperature in each subblock for 30 days each year. I used only a subset of the total data because of logger malfunctions. I examined relationships between GSF and water temperatures by calculating Pearson product-moment correlation coefficients.

Breeding site selection experiment

I arranged seven plastic pools (10 L, 16 cm depth, 31 cm diameter) at fixed distances from the pasture-forest edge along three transects ($n = 21$ pools). Transects were oriented in different directions with respect to the edge and at least 200 m apart. Along each transect, I placed a pool at the edge (0 m), and at 10, 30, and 50 m into both pasture and forest. Pools were placed in the ground and surrounded by a barrier to prevent cows from entering. The lower edge of the barrier was elevated 15 cm above the pool, allowing frogs to enter. Frogs could also enter through two openings (30 cm wide) on either side of the pool. Pools contained two fiberglass screen ladders (10 cm wide) to

allow metamorphs to escape. Water and leaf litter accumulated within the pools naturally and overflow holes along the rim of pools prevented flooding.

I monitored pools for egg and tadpole presence every seven days for 20 weeks (26 June – 6 November 2006), by visually checking and dipnetting for tadpoles in pasture pools. In forest pools, I removed all leaf litter and water to thoroughly search for tadpoles because a large quantity of leaf litter had accumulated. I determined the presence of new cohorts differently in pasture and forest because of differences in the life history of species that colonized pools in each habitat. In pasture and edge habitats, I identified new cohorts by the presence of eggs or recent hatchlings. In the forest, I counted tadpoles each week and used increases in tadpole populations to determine whether a new cohort was present. This conservative approach may underestimate the number of new cohorts in forest pools, for instance, dendrobatids may deposit cohorts of tadpoles on more than one occasion during a week but all were counted collectively as a single new cohort.

Analysis

To determine if selection of breeding pools across the pasture-forest edge was equal, I used a chi-square goodness of fit randomization test to account for small sample sizes. I summarized the number of cohorts of each species in each pool at equal distances from the pasture-forest edge for analysis. Low sample sizes permitted chi-square tests only for *E. pustulosus* and members of the families Dendrobatidae and Aromobatidae. Data for species in these families were pooled because of their similar life histories. I calculated time to colonization for each pool and summarized data across transects at each distance. Statistical analyses examining differences in time to colonization at each

distance were not possible because of small sample sizes. Instead, I pooled time to colonization data within pasture and forest separately to examine if it differs by habitat type, using a two-tailed t-test. Edge pools (0 m) were excluded from this analysis.

Tadpole performance experiment

I used a randomized block design, with three blocks composed of three subblocks (each enclosed in a fence), one in forest, pasture, and at the forest-pasture edge (Fig. 3.1). A block design was used to reduce variation from genetic and environmental sources. Each block was positioned at a different cardinal angle with respect to the forest-pasture edge. Because of the landscape configuration, pasture subblocks were located 65 m from the edge, while forest subblocks were 80 m from the edge. Each experimental unit (10 L; 19 cm depth, 31 cm diameter artificial pool), was randomly assigned to a subblock and a location in each subblock. There were two pool replicates per subblock, for a total of 18 pools per species. *Dendrobates auratus* was studied in 2005 and *E. pustulosus* in 2006.

I filled each pool with stream water (6 L) and damp leaf litter (150 g). I inoculated each pool with zooplankton and phytoplankton (1 L water samples) collected from two ponds and filtered (1.0 mm²). Pools were covered with nylon mesh cloth to reduce introductions of insect or frog larvae and had overflow holes along the rim to prevent flooding. All pools within a block were prepared on the same day and remained in the edge subblock until the experiment began. On the day the experiment began, pools were transported to their appropriate position in each subblock and buried, with 4 cm of the pool top exposed above the soil surface.

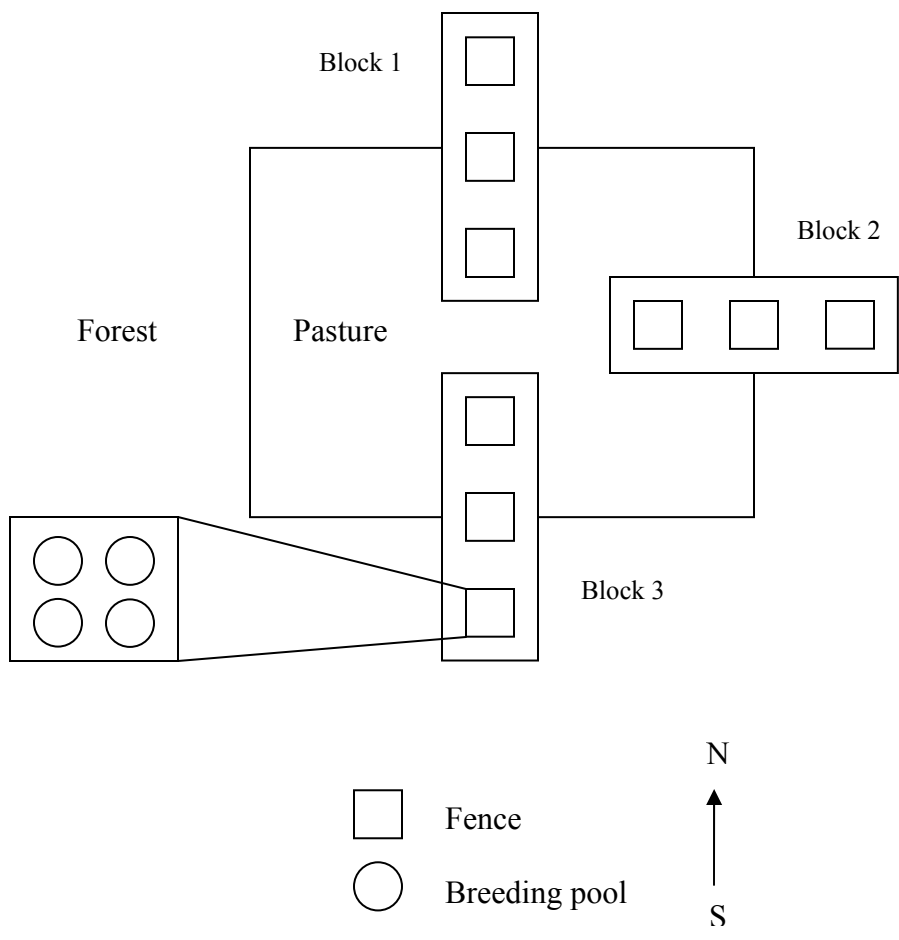


Figure 3.1. Randomized complete block design used for the tadpole performance experiment. Each block was composed of three subblocks, one in forest, pasture, and forest-pasture edge. Each treatment had two replicates within each subblock, for a total of six pools in each block and 18 pools/species in each experiment.

The experimental design remained largely the same between species, with a few exceptions. Because of the inherent difficulties in finding *D. auratus* eggs, I collected tadpoles from natural and artificial breeding sites (plastic cups placed in tree crevices). I sorted tadpoles into size classes (small, medium, and large) and standardized average tadpole size among pools in each block. I added 5 tadpoles/pool in block 1, and 4 tadpoles/pool in blocks 2 and 3. I digitally photographed tadpoles and determined tadpole size (mean total length) per pool digitally (Image J, version 1.34s; <http://rsb.info.nih.gov/ij/>: block 1, 1.85 ± 0.06 cm (mean \pm SE), block 2, 1.93 ± 0.06 , and block 3, 2.00 ± 0.03). All *D. auratus* tadpoles were in developmental stage 25 (Gosner 1960). I collected three *E. pustulosus* foam nests, laid the previous night, as a source of individuals for pools in each block and added 10 tadpoles (4 days old; Gosner stage 24 or 25) to their respective pools in the late afternoon. In 2005, I prepared the pools 3-4 days before introducing *D. auratus* tadpoles. In 2006, I prepared the pools 14 days before introducing *E. pustulosus* tadpoles. Blocks were started in a consecutive manner; in 2005, block 1 was started on 29 May, block 2 on 9 June, and block 3 on 18 July. In 2006, block 1 was started on 27 June, block 2 on 3 July, and block 3 on 10 July.

I measured tadpole survival on day 3 by removing tadpoles and thoroughly searching the leaf litter in each pool. Thereafter, I assessed survival every five days. When tadpoles reached stages 40-41 (Gosner 1960), I checked the basins twice daily for metamorphs (at least one emerged forelimb). Metamorphs were removed and held individually until their tail was absorbed, then weighed to the nearest mg. I ended the experiment on day 103 (in 2005) and day 60 (in 2006) because *D. auratus* require more time to develop than *E. pustulosus*. I considered all tadpoles that did not complete

metamorphosis to have perished. The response variables were proportion surviving to metamorphosis, metamorph size (mass), and larval period (days to metamorphosis).

Analysis

Differences in the experimental design for each species precluded interspecific statistical comparisons. All variables met parametric assumptions of normality and homogeneity of variance. I used ANOVA to examine heterogeneity among tadpole performance measures by habitat (fixed) and block (random) factors. When block did not have a significant effect on the dependent variable, I ran the analysis again without the block factor. Tukey's HSD post-hoc multiple comparisons tested for differences between treatments and blocks when ANOVA yielded a significant result. Because *D. auratus* had high mortality, I used a Kaplan-Meier survival analysis (Levesque 2007) to compare tadpole survival over time in pasture, edge, and forest treatments using the proportion of pools in each treatment with at least 50% of tadpoles surviving over time. Survivorship curves were compared among treatments using a log-rank test.

RESULTS

Abiotic environments

On average the maximum daily water temperature was 10° C higher in pasture than in forest and edge pools (35.2° C, 24.8° C, and 26.5° C, respectively), whereas minimum water temperature varied little among habitats (25.8° C, 24.0° C, 24.5, respectively). In forest and edge pools, water temperature fluctuated by an average of 1 – 2° C throughout the day; in contrast, it fluctuated by as much as 9° C in pasture pools. Maximum and minimum daily water temperatures in 2005 and 2006 varied little (0.439 ± 0.114 ; mean difference between years in each habitat \pm SE, $n = 3$). In both years, the

temperature range was larger in pasture (24 – 41° C) than in edge (23 – 29.5° C) and forest (23.5 – 26° C). Air temperature ranged from 23.9 - 27.3 °C (mean daily minimum - maximum) in forest, 23.5 - 27.8 °C in the edge, and 23.5 - 35.0 °C in pasture. Relative humidity ranged from 96 – 100 % in the forest (mean daily minimum – maximum), 94 – 100 % at the edge, and 61 – 100 % in the pasture.

Global site factor (GSF) was higher in pasture than in edge and forest ($F_{2,35} = 5764.851$, $P < 0.001$), but there was no difference between edge and forest ($P = 0.307$; Fig. 3.2). In addition, GSF differed among blocks ($F_{2,35} = 14.023$, $P < 0.001$); it was higher in block 3 than in block 1 or 2 ($P \leq 0.002$), but did not differ between blocks 1 and 2 ($P = 0.358$). Global site factor was positively correlated with both maximum ($r = 0.983$, $P < 0.001$) and minimum ($r = 0.914$, $P = 0.001$) water temperatures in each subblock ($n = 9$).

Breeding site selection experiment

Thirty-seven egg or tadpole deposition events occurred during the study period. Four species (*Engystomops pustulosus*, *Smilisca phaeota*, *Leptodactylus poecilochilus*, and *Hypsiboas rosenbergi*) deposited eggs in pasture and four species (*E. pustulosus*, *Phyllobates vittatus*, *Dendrobates auratus*, and *Allobates talamancae*) deposited eggs or tadpoles in forest (Table 3.1). No species deposited eggs or tadpoles in multiple pools across the pasture-forest edge. *E. pustulosus* and *P. vittatus* deposited multiple cohorts in pools on more than one transect. Single cohorts of *L. poecilochilus*, *H. rosenbergi*, and *D. auratus* were observed in different pools. Multiple cohorts of *A. talamancae* were found in the 50 m forest pool on transect one and single cohorts of *S. phaeota* were observed in three pasture pools along transect three (Table 3.1).

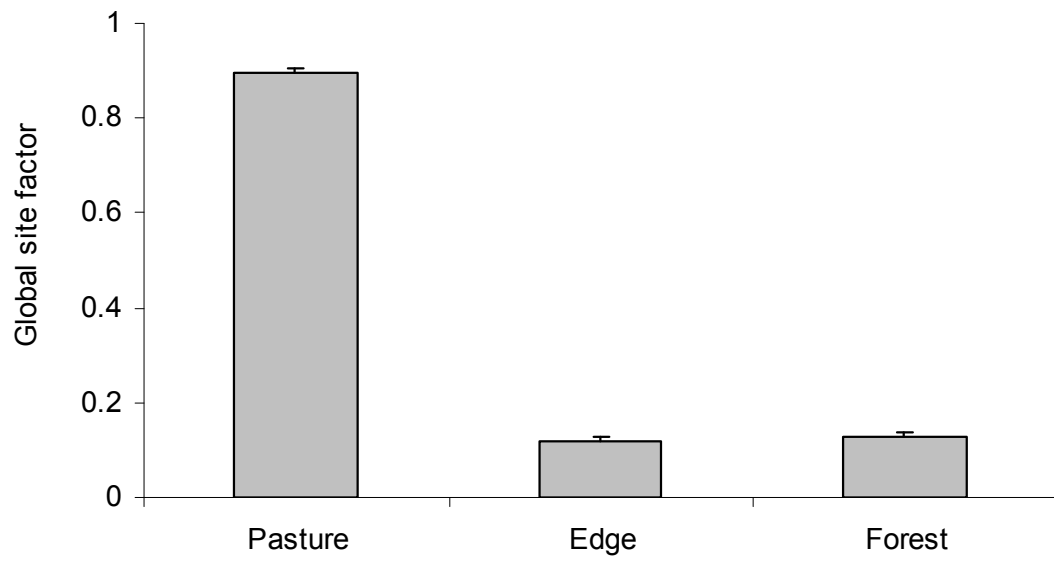


Figure 3.2. Global site factor calculated from hemispherical photographs in each habitat.

Table 3.1. Number of cohorts of each species^a colonizing pasture, edge, and forest pools at different distances from the pasture-forest edge, along three transects.

Transect	Pasture			Edge			Forest		
	50 m	30 m	10 m	0 m	10 m	30 m	50 m		
1	1 Lp	0	0	0	0	0	4 Pv, 4 At		
2	0	1 Hr	2 Ep	1 Ep	1 Ep	0	0		
3	1 Ep	4 Ep, 1 Sp	5 Ep, 1 Sp	1 Sp	1 Da	0	9 Pv		

^a Species abbreviations: At = *Allobates talamancae*, Da = *Dendrobates auratus*, Hr = *Hypsiboas rosenbergi*, Lp = *Leptodactylus poecilochilus*, Ep = *Engystomops pustulosus*, Pv = *Phylllobates vittatus*, and Sp = *Smilisca phaeota*.

The number of egg or tadpole depositions was greatest in the 50 m forest pool and 10 m pasture pool and lowest at the 30 m forest pool (Fig. 3.3). The number of oviposition events in pasture decreased with distance from forest. *Engystomops pustulosus* selected pools in pasture and near the edge ($X^2 = 20.00$, $P = 0.003$), while members of Dendrobatidae and Aromobatidae selected pools in forest, almost exclusively 50 m from the edge ($X^2 = 94.83$, $P < 0.001$).

Frogs deposited eggs or tadpoles in 12 of 21 pools. There was a trend of a shorter number of days to initial colonization at the 10 m pasture and 50 m forest pools and a longer number of days at the 50 m pasture and 0 m edge pools; however, because of low sample size, variation in the number of days to colonization was large (Fig. 3.3). Time to colonization in forest (59.5 ± 15.2 , $n = 6$) did not differ from pasture (49.0 ± 12.1 , $n = 4$; $t = -0.493$, $df = 8$, $P = 0.635$).

Tadpole performance experiment

Engystomops pustulosus survival was greater than 90% in all pools with the exception of one forest pool in block 3 (Fig. 3.4). Tadpole survival did not differ among habitats; however, metamorphs from each habitat differed in other performance measures (Table 3.2). Pasture metamorphs were 29% larger than those from the edge ($P < 0.001$) and 16% larger than those from the forest ($P = 0.043$) but edge and forest metamorphs did not differ ($P = 0.113$; Fig. 3.5, Table 3.2). Additionally, *E. pustulosus* tadpoles completed metamorphosis 3 – 4 d faster in pasture than those in edge or forest (Fig. 3.5, Table 3.2). Larval period was shorter in pasture than in edge ($P = 0.008$) and forest ($P = 0.001$) but did not differ between edge and forest ($P = 0.570$).

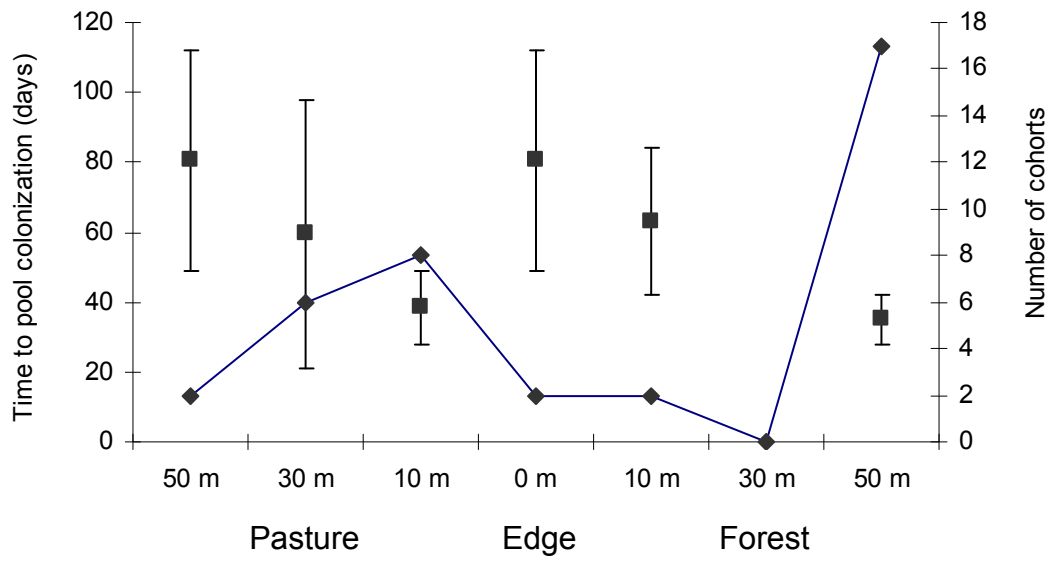


Figure 3.3. Time to colonization (mean \pm SE; squares) and number of cohorts (diamonds) in pasture and forest at different distances from the edge.

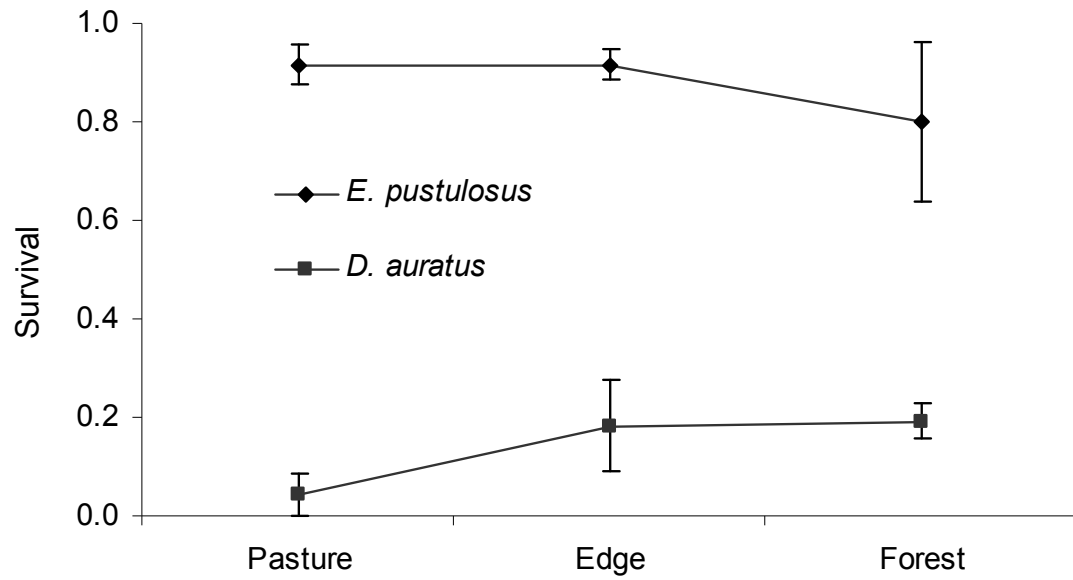


Figure 3.4. Proportion surviving (mean \pm SE) to metamorphosis for *Engystomops pustulosus* and *Dendrobates auratus* in pasture, edge, and forest.

Table 3.2. ANOVA results for survival, metamorph mass, and larval period for each species. Block factors are reported only if a significant result was found.

Variables	<i>F</i>	df	<i>P</i>
<i>Engystomops pustulosus</i>			
Survival			
Habitat	0.466	2,17	0.636
Metamorph mass			
Habitat	13.045	2,16	0.001
Larval period			
Habitat	11.380	2,16	0.001
<i>Dendrobates auratus</i>			
Survival			
Block	4.438	2,17	0.034
Habitat	4.192	2,17	0.039
Metamorph mass			
Habitat	5.454	2,9	0.037
Larval period			
Habitat	1.280	2,9	0.336

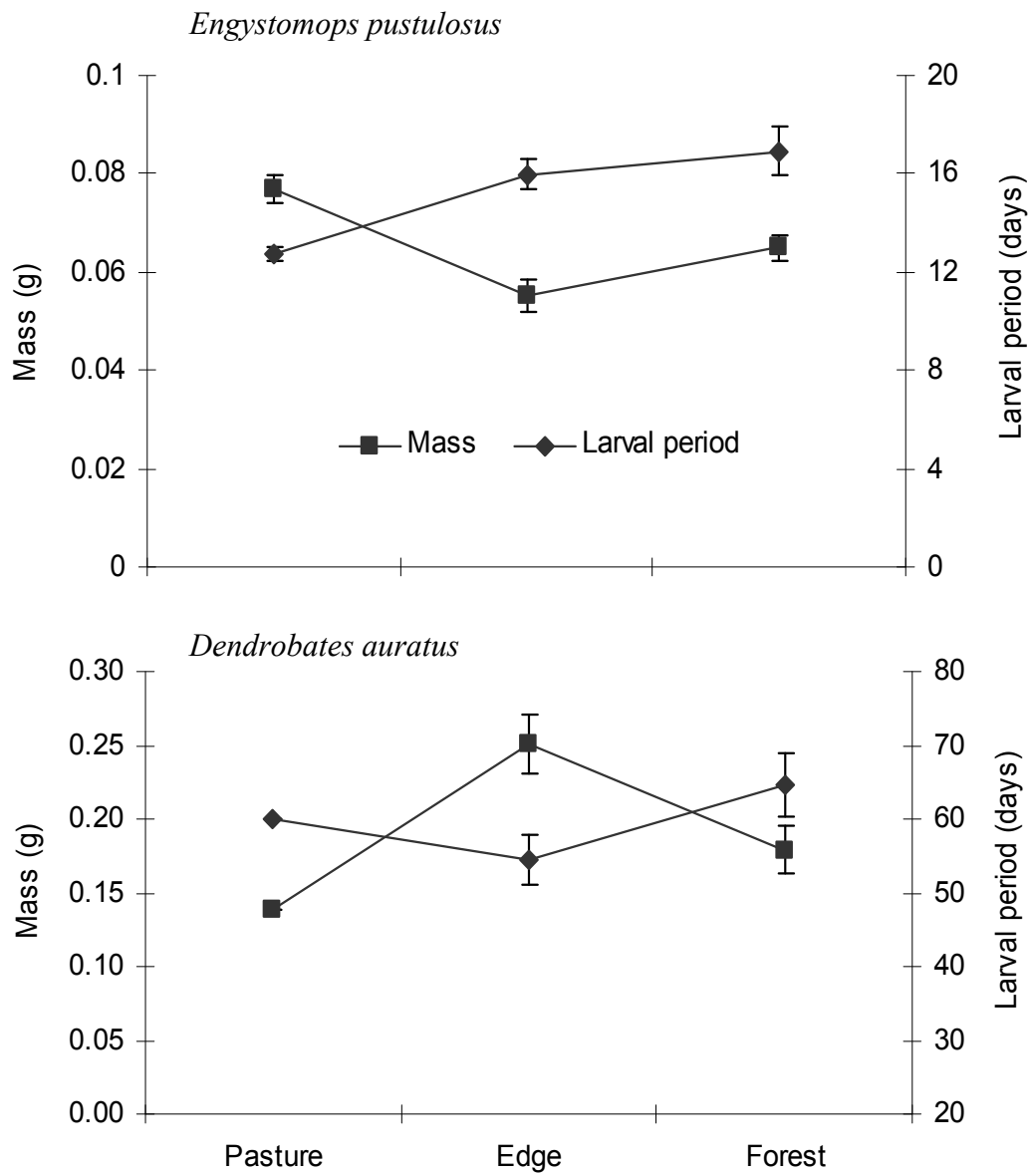


Figure 3.5. Metamorph mass (mean \pm SE) and larval period (mean \pm SE) for *Engystomops pustulosus* and *Dendrobates auratus* in pasture, edge, and forest.

Dendrobates auratus survival was less than 20% in all habitats (Fig. 3.4); only one tadpole survived to metamorphosis in pasture. Survival was marginally lower in pasture than in forest ($P = 0.056$) but did not differ between edge and forest ($P = 0.989$; Table 3.2). Survival in pasture was marginally lower than in edge (Fig. 3.4; $P = 0.072$). Additionally, survival in block 2 was higher than in block 3 ($P = 0.033$), but survival in block 1 did not differ from block 2 ($P = 0.759$) or block 3 ($P = 0.119$; Table 3.2). In edge and forest, 46 – 62% of tadpoles survived to the end of the experiment but did not complete metamorphosis. Within 28 days, $\geq 50\%$ of tadpoles were alive in only 33% of pasture pools (Fig. 3.6). After 60 days, survival in forest and edge began to decrease. Survival curves in pasture differed from those in edge and forest ($\chi^2 = 12.02$, $P < 0.001$, $df = 2$, $n = 18$) but did not differ between edge and forest (Fig. 3.6). Metamorphs from edge pools were 29% larger than those from forest and 45% larger than the single metamorph from a pasture pool (Fig. 3.5, Table 3.2). Larval period was long (46 – 78 d) and did not differ among habitats, although there was a trend towards shorter duration in edge pools (Fig. 3.5).

DISCUSSION

By linking adult breeding habitat selection with tadpole performance, my study goes a step beyond previous ones that assume habitat quality based on adult occupancy, by providing a quantitative fitness measure of habitat quality. Adult breeding site selection was nonrandom and consistent with tadpole performance across the pasture-forest continuum, in agreement with the habitat selection theory (Fretwell and Lucas 1970). *Engystomops pustulosus* selected pools in pasture and near the edge,

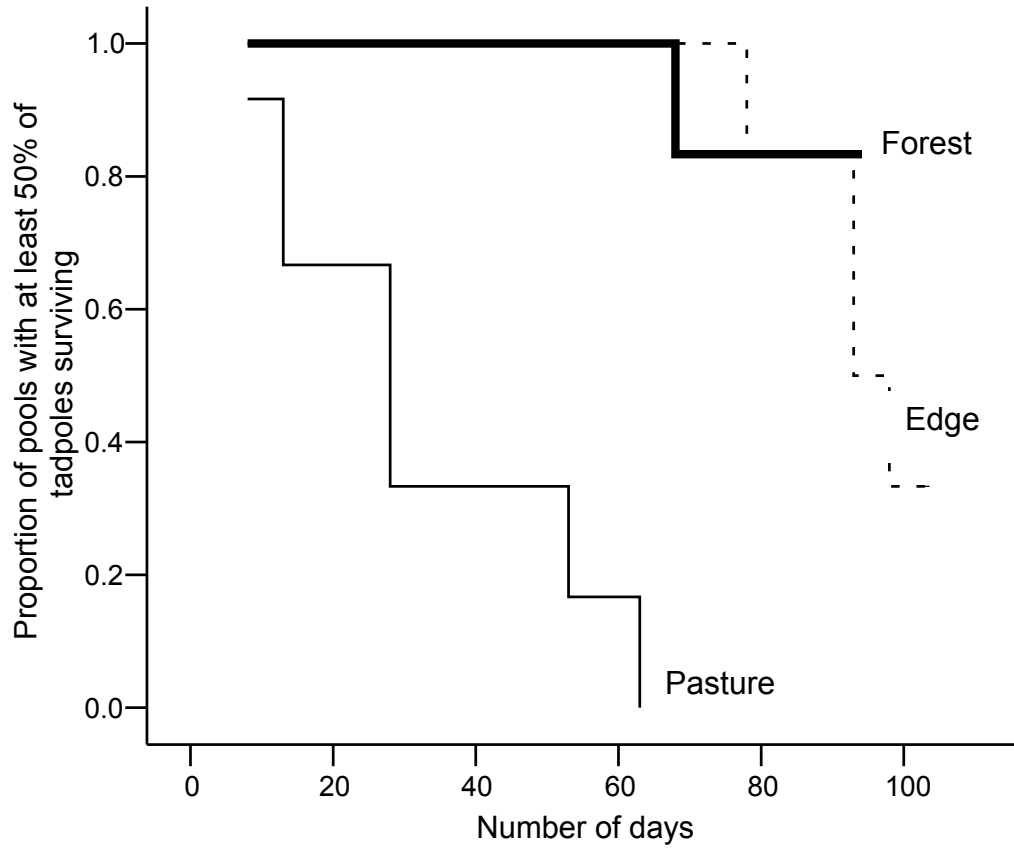


Figure 3.6. Kaplan-Meier survival estimates for the proportion of basins with at least 50% of *Dendrobates auratus* tadpoles surviving until the end of the experiment.

whereas *D. auratus* and species with similar life histories selected pools in forest. Although *E. pustulosus* tadpoles had equally high survival in each habitat, metamorphs developed faster and became larger in pasture than in edge and forest. In contrast, *D. auratus* tadpoles had higher survival and metamorphs were larger in edge and forest than in pasture. For both species, adults selected habitats where their tadpoles attained higher fitness.

High performance during the tadpole stage can lead to improved fitness during later life stages. For example, individuals that spend less time as tadpoles or become larger metamorphs may attain reproductive status faster and produce more offspring at a younger age (Smith 1987). Additionally, these individuals decrease mortality risks from predation and desiccation by spending less time in pools (Skelly 1996). Therefore, tadpole performance can result in higher adult fitness, with positive consequences for population growth in a particular habitat (Berven 1990).

Across all species, breeding site selection was consistent with patterns of adult occupancy in pasture and forest based on surveys in the region (Chapter 2) and habitat preferences of species (Savage 2002). *Engystomops pustulosus*, *S. phaeota*, and *H. rosenbergi* deposited eggs in pasture pools. Yet, in addition to open habitats, *E. pustulosus*, *S. phaeota* and *H. rosenbergi* also occupy forest (Savage 2002). *Smilisca phaeota* and *H. rosenbergi* may have higher tadpole performance in pasture than in forest, similar to *E. pustulosus*. My results suggest that species characterized as forest specialists will not use breeding pools in a pasture matrix, even near the forest edge. In contrast, many forest-specialists are encountered in Brazilian pastures (Tocher et al.

2001); however, it is not known whether pasture meets the resource needs of those species.

A species' presence in matrix habitat is sometimes influenced more by proximity to a suitable source habitat rather than the ability of the matrix to meet the resource needs of that species (Pulliam 1988). If the pasture did not meet the requirements of species in my study, I would expect my data to show an isolation effect, i.e., fewer clutches deposited in pasture pools distant from forest edge than near forest edge. There was an indication of an isolation effect on transect three, where *E. pustulosus* selected pasture pools near the forest edge and colonized these pools faster than those distant from the forest edge. However, the number of oviposition events was too small to test for an isolation effect and the distance was relatively short. Other studies have shown an isolation effect; for example, gray treefrogs prefer to oviposit in pools located close to forest edges rather than farther into clearcut habitats (Hocking and Semlitsch 2007).

My results suggest one of the specific mechanisms influencing patterns of species occupancy in pasture and forest by integrating abiotic heterogeneity among habitats with interspecific variation in breeding site selection and tadpole performance. The abiotic environment in pasture is characterized by greater light availability, higher air and water temperature, and lower relative humidity, compared to edge and forest environments, which were similar to one another. It is likely that pasture pools also had improved food quality for tadpoles, as has been documented in other open-canopy ponds (Schiesari 2006). Members of the Dendrobatidae and Aromobatidae may have avoided pasture because of physiological limitations. These species are likely prohibited from dispersing through pasture because of small body size and diurnal activity, which raise already high

rates of evaporative water loss (Shoemaker et al. 1992). Additionally, water temperature in pasture pools may have exceeded the critical thermal maxima of *D. auratus* tadpoles, resulting in high tadpole mortality at the beginning of the experiment. Most mortality events occurred during periods when water temperatures reached or exceeded 38.5° C. Surprisingly, one *D. auratus* tadpole survived to metamorphosis in pasture, indicating that some individuals are capable of tolerating high temperatures. My data are consistent with a laboratory study showing that *D. auratus* tadpoles reared at 30.4 °C have lower survival (10%) and longer larval periods (> 100 days) than those reared at 26.2 °C and 29.4 °C (Korbeck and McRobert 2005). My results raise concern for *D. auratus* persistence in a fragmented landscape. Local extinctions in forest fragments are inevitable (Vandermeer and Perfecto 2007) and the recolonization of areas where populations have been extirpated is dependent on dispersal events (Fahrig and Merriam 1994). If a *D. auratus* population in a forest fragment goes extinct, my results suggest that this species may not disperse to repopulate the fragment if it is surrounded by a pasture matrix.

In comparison, *E. pustulosus* adults selected breeding pools in pasture where their tadpoles had the highest performance. *Engystomops pustulosus* persist in pastures because water temperatures are tolerable and confer benefits of faster tadpole growth and development. My results suggest that *E. pustulosus* has flexibility in its behavior and physiology and that fitness advantages gained in pastures encourage adults to concentrate their reproductive efforts there.

Conclusions

Quantifying amphibian vital rates in modified and intact habitats can aid land managers in improving management strategies (Armstrong 2005) and in identifying specific life stages vulnerable to land use change. My results suggest that adults actively select breeding sites in habitat where their tadpoles have improved performance. These patterns influence frog occupancy patterns across a landscape mosaic of forest and pasture. Based on the two model species in my study, I suggest that species with life histories similar to *E. pustulosus* may maintain large populations in modified habitats because they thrive in open canopy abiotic environments. Those species may also be capable of surviving in forest, but with reduced performance compared to in pasture. The reverse pattern is not supported by my data. Species with life histories similar to *D. auratus*, that have high performance in forest, are likely incapable of sustaining viable populations in abiotic environments similar to that of a pasture matrix habitat.

CHAPTER 4

DO TREEFROG TADPOLES PERFORM BETTER IN AN AGRICULTURAL MATRIX, EDGE, OR FOREST?

The distribution of species within fragmented landscapes is influenced by whether species avoid, tolerate, or exploit matrix habitats (Gascon et al. 1999); however, we have a poor understanding of the mechanisms involved. Abiotic conditions in matrix and forest habitats may contribute to patterns of species' distributions by affecting different stages of their life cycles. For instance, adult frogs may be tolerant of abiotic conditions in matrix habitats, but the habitat may function as an ecological trap if survival of offspring is reduced (Gates and Gysel 1978, Schlaepfer et al. 2002). I studied the survival, growth, and development of the tadpoles of two hylid species in tropical pasture, edge, and forest habitats to add to our understanding of how anthropogenic changes in forest cover influence amphibian distributions.

The amount of forest cover influences pond conditions in several ways. Open canopy ponds have higher average temperatures (Werner and Glennemeier 1999, Skelly et al. 2002) and may undergo greater daily variation in maximum and minimum temperatures compared to closed canopy ponds. Dissolved oxygen concentrations are lower in closed canopy ponds than in open canopy ponds (Werner and Glennemeier 1999, Skelly et al. 2002). Additionally, food resources available to tadpoles are of a higher quality in open canopy ponds (Schiesari 2006); for example, periphyton are more diverse and abundant (Skelly et al. 2002). These abiotic and biotic variables are known to influence tadpole growth and development (Alford 1999).

Tadpole performance is influenced by a variety of abiotic and biotic factors. Growth and development rates are faster in high water temperatures (Skelly et al. 2002, Ultsch et al. 1999). However, extreme temperatures, usually more than 38° C, can decrease tadpole survival and cause catastrophic mortality of entire cohorts (Ultsch et al. 1999). Species may experience slightly reduced tadpole growth and survival in ponds with low dissolved oxygen content (Schiesari 2006). Tadpoles grow faster in ponds with higher food quality, measured as carbon-to-nitrogen ratios (Schiesari 2006). Rapid growth and development lead to larger metamorph size and shorter larval duration, which can benefit later fitness by allowing individuals to reproduce earlier than slow-growing individuals (Smith 1987, Pechenik et al. 1998).

Neotropical treefrogs of the family Hylidae are found in a variety of habitats, from primary closed canopy forest to disturbed open canopy areas (Duellman 2001). Some studies have revealed that hylids have higher species richness in disturbed habitats; for instance in Ecuador, hylid richness was greater in selectively cut forest than in primary forest (Pearman 1997). In Brazil, the majority of hylids found in continuous forest are also encountered in pasture and secondary forest (Tocher et al. 2001). Hylids typically breed in temporary or permanent ponds, but some species oviposit in small tree holes or along stream edges (Duellman and Trueb 1986). During non-breeding periods, pond-breeding amphibians typically occupy terrestrial habitat surrounding ponds. This necessitates migration to the aquatic habitat during the breeding season (Semlitsch 2000). Treefrogs may be capable of relatively long distance dispersal, as two small species (to 5.0 cm snout-vent length) are reported to disperse more than 2 km/year (*Hyla arborea*, 12.6 km/year, [Stumpel and Hanekamp 1986] and *Hyla regilla*, 2.5 km/year, [Reimchen

1991]). These observations suggest that the adults of some treefrogs are tolerant of disturbed habitats. This, together with previous work showing that increased temperatures result in faster tadpole growth and development, suggest that treefrogs may thrive in ponds in open canopy habitat.

To gain a better understanding of how forest cover influences tadpole performance and patterns of species' occupancy, I quantified the growth, development, and survival of *Agalychnis callidryas* and *Dendropsophus ebraccata* in forest, edge, and pasture pools. Specifically I asked 1) do tadpoles experience different abiotic environments in pasture, edge, and forest pools?, and 2) do tadpoles differ in survival, growth, and development in pasture, edge, and forest pools? Water temperatures should be highest in pasture pools because of more direct sunlight; therefore, I expected tadpoles of both species to grow larger and develop faster in pasture. I expected survival of both species to be lower in pasture pools because of the possibility of temperature extremes.

METHODS

I conducted this study in the Osa Peninsula, Puntarenas Province, Costa Rica (8°26' N, 83°22' W). This region has distinct rainy (May to December) and dry seasons (January to April) and precipitation averages 4000 to 6000 mm y⁻¹ (L. Gilbert, *unpublished data*). My study area is composed of lowland wet forest (Hartshorn 1983) and pasture (40 years old), with a distinct edge separating the habitats.

Tadpole performance experiment

Study species: I selected two hylids to study based on their abundance in the study area, ease of manipulation, and similar life histories. Both species are encountered in forest and disturbed habitats in the study region (Savage 2002, T. Hawley, Chapter 2).

In the rainy season, *A. callidryas* congregate in ponds, where females lay clutches (11-104 eggs) on leaf surfaces over water (Savage 2002). Larvae complete development within a minimum of 55 days (Vonesh and Warkentin 2006). In contrast, *D. ebraccata* frequents temporary ponds where it deposits variably sized clutches of eggs (15-296) on leaves close to the water surface. Metamorphosis occurs within four to six weeks (Savage 2002). Although the experimental venues (10 L artificial pools) in my study were small, I expected *A. callidryas* and *D. ebraccata* to be tolerant of the conditions because I have encountered their tadpoles in natural ponds of various sizes (T. Hawley, *personal observations*).

I used a randomized block design, with three blocks, each composed of three subblocks (fences), in forest, pasture, and at the forest-pasture edge (Fig. 4.1). A block design was used to reduce variation from genetic and environmental sources. Each block was placed at a different cardinal angle with respect to forest and pasture. Pasture subblocks were located 65 m from the pasture-forest edge and forest subblocks were 80 m from the edge. The experimental unit was an artificial pool (10 L; 19 cm depth, 31 cm diameter, with overflow holes along the rim) and I randomly assigned each pool to a subblock and position within each subblock. There were two pool replicates in each subblock, for a total of 18 pools/species.

I filled each pool with water from a nearby stream (6 L) and damp leaf litter (150 g). The remainder of the 10 L pools filled with rain water within a week. I inoculated each pool with zooplankton and phytoplankton (1 L) collected from two ponds and filtered using a fine mesh cloth (1.0 mm²). Pools were covered with nylon mesh to eliminate introductions of insect or frog larvae. All pools within a block were prepared at

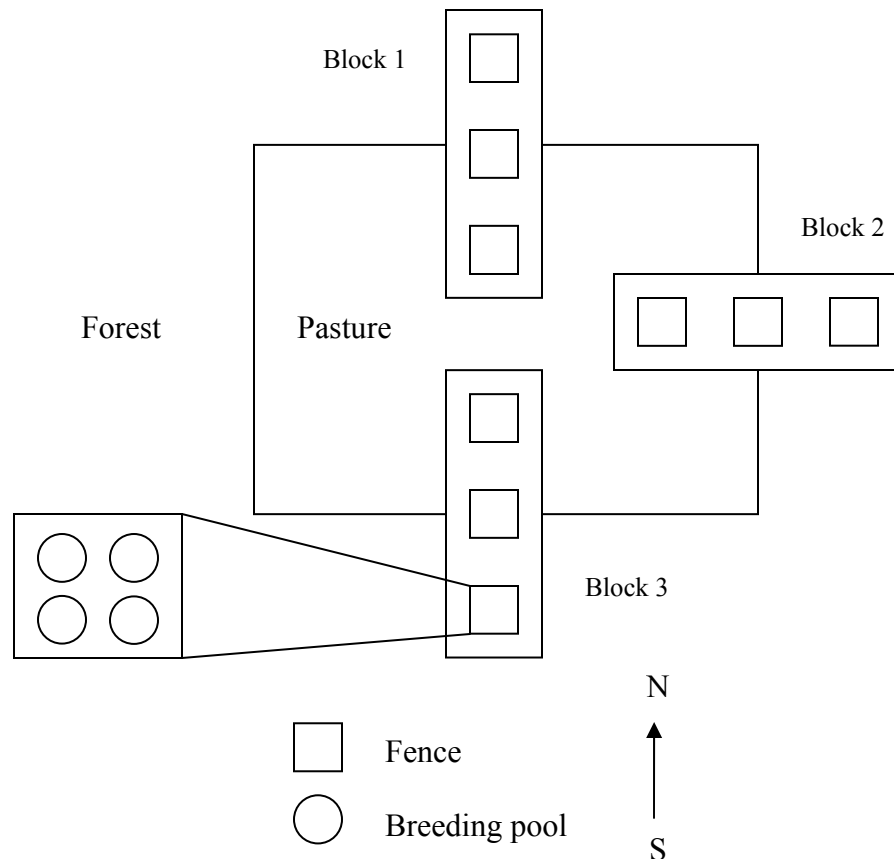


Figure 4.1. Randomized complete block design used for the tadpole performance experiment. Each block was composed of three subblocks, representing three habitats (forest, pasture, and forest-pasture edge). Each treatment had two replicates within each subblock for each species, for a total of six pools in each block and 18 total pools/species.

the same time and left for 14 days at the pasture-forest edge subblock until the experiment started. On the day the experiment began, pools were moved to the appropriate position within each subblock and buried in the ground, with approximately 4 cm of the top exposed above the soil surface.

The only difference in experimental design between species was that *A. callidryas* tadpoles were stocked at a density of 6/pool, while *D. ebraccata* were stocked at a density of 8/pool. This difference in stocking density was based on tadpole sizes and a preliminary experiment. Because of logistical issues, blocks were started in a consecutive manner; block 1 on 27 June, block 2 on 3 July, and block 3 on 10 July. For each block, I collected three to five clutches of eggs of each species. From this source of individuals, I haphazardly selected tadpoles (2-4 days old; stage 23 to 25 (Gosner 1960)) for each pool. Within each block, tadpoles were of the same age and similar developmental stage when introduced to pools. I digitally photographed tadpoles by groups added to each pool. Later, I determined initial tadpole size (mean total length) per pool using the software Image J, version 1.34s (<http://rsb.info.nih.gov/ij/>). I introduced tadpoles to their respective pools in the late afternoon to allow acclimation overnight.

I assessed tadpole survival on day 3 by removing tadpoles from each pool and thoroughly searching the leaf litter. Thereafter, I assessed survival every five days. On days 13, 23, 33, and 43, I digitally photographed tadpoles from each pool. I ended the experiment on day 43. The dependent variables were proportion surviving to metamorphosis, tadpole size (length), and developmental stage (Gosner 1960).

Differences in the experimental design for each species preclude interspecific statistical comparisons of results. I estimated mean tadpole length/pool on days 13, 23,

33, and 43 using the software Image J. I used a repeated measures analysis of variance (ANOVA) to test for variation in tadpole length by habitat (treatment) and block factors. The block factor was not significant so data were reanalyzed with only the habitat factor. I used Tukey's HSD post-hoc multiple comparisons tests to test for differences among treatments when ANOVA yielded a significant result. I transformed survival (sqrt arcsin) to meet parametric assumptions of normality and homogeneity of variance. Tadpole length did not require a transformation. The most developmentally advanced tadpole was in Gosner stage 36-40, with clear differentiation of hindlimb digits. Therefore, I estimated the developmental stage of each tadpole from photographs using two broad categories, 0 = no or barely visible hindlimb development (stages 25-27) or 1 = visible hindlimb development (stages 28+). I assessed variation in tadpole development among habitats qualitatively.

Abiotic environments

To demonstrate that qualitative differences in vegetation structure in pasture, edge, and forest treatments affect the abiotic environment, I measured water temperature and solar radiation in each habitat. I used a Nikon Coolpix 4500 digital camera with a Nikon FC-E8 fisheye converter lens (180° field of view) to take hemispherical photographs. I took four photos in each subblock on overcast days (19-20 November 2006). The camera was mounted on a tripod 30 cm above the ground and oriented with magnetic north at the top of the image, allowing the incorporation of solar tracks in the photo analysis. I processed and analyzed the photos in Gap Light Analyzer, version 2.0 (Frazer et al. 1999) calculating the variable global site factor (total solar radiation). Global site factor (GSF) can range from 0 (complete obstruction) to 1 (open sky) and is

the percentage of incident radiation transmitted through a gap to a point below the canopy (Canham 1988). I tested for heterogeneity in GSF by habitat (fixed) and block (random) factors using ANOVA. I examined relationships between the light environment and water temperatures by calculating Pearson product-moment correlation coefficients.

I monitored water temperature hourly in one pool in each subblock using I-button temperature loggers (Maxim Integrated Products, Sunnyvale, CA) in plastic bags at a depth of 18 cm. I calculated mean daily maximum and minimum water temperature in each subblock for 30 days (10 July – 9 August). Additionally, I measured dissolved oxygen content with a YSI DO200 portable instrument (YSI Environmental, Inc.) in three pools in each subblock on 19 -20 July between 14:00 and 17:00. I examined heterogeneity in dissolved oxygen content by habitat (fixed) and block (random) factors using ANOVA, after testing to ensure that the data met the assumptions of normality and homogeneity of variance.

RESULTS

Tadpole performance experiment

The length of *A. callidryas* tadpoles more than doubled in the first 13 days in pasture and edge and continued to increase but at slower rates until the end of the experiment (Fig. 4.2). In forest, tadpole length nearly doubled in the first 13 days, then growth slowed until tadpoles began to lose length in the final 10 days. *Agalychnis callidryas* tadpole growth was affected by time (Wilks lambda = 0.015, $F_{4,11} = 185.378$, $P < 0.001$) and its interaction with habitat (Wilks lambda = 0.106, $F_{8,22} = 5.716$, $P < 0.001$). Differences in *A. callidryas* tadpole growth among habitats were apparent by the first sampling date (Fig. 4.2, Table 4.1). Over the duration of the experiment, *A. callidryas*

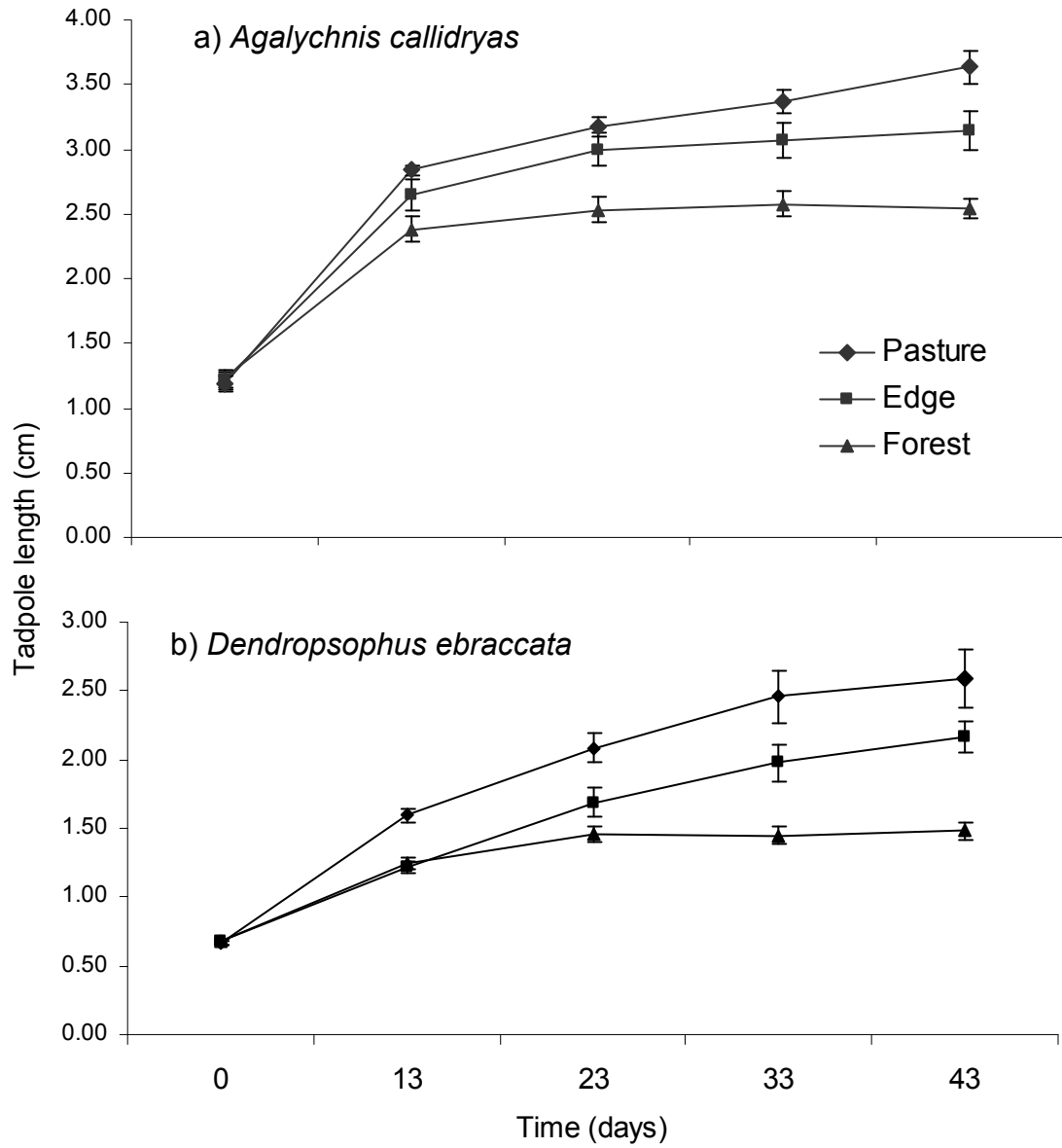


Figure 4.2. Change in a) *Agalychnis callidryas* and b) *Dendropsophus ebraccata* tadpole length (mean \pm SE) in pasture, edge, and forest, from the start (0 days) to the end (43 days) of the experiment.

Table 4.1. Results of repeated measures ANOVA for effects of time and habitat on *Agalychnis callidryas* and *Dendropsophus ebraccata* tadpole growth (length). Degrees of freedom for within-subjects effects are Greenhouse-Geisser corrected because the assumption of sphericity was violated.

	Sum of squares	df	<i>F</i>	<i>P</i>
<i>Agalychnis callidryas</i>				
Between-subjects effects				
Habitat	5.294	2	15.211	< 0.001
Error	2.436	14		
Within-subjects effects				
Time	41.635	1.673	339.285	< 0.001
Time x Habitat	2.080	3.347	8.477	< 0.001
Error	1.718	23.427		
<i>Dendropsophus ebraccata</i>				
Between-subjects effects				
Habitat	5.741	2	16.175	< 0.001
Error	2.662	15		
Within-subjects effects				
Time	23.346	1.314	185.853	< 0.001
Time x Habitat	2.809	2.628	11.181	< 0.001
Error	1.884	19.709		

tadpole length was larger in pasture than in forest ($P < 0.001$), larger in edge than in forest ($P = 0.012$), but did not differ between pasture and edge ($P = 0.100$). *Agalychnis callidryas* tadpoles had hindlimb development in four of five pools in pasture, one of six pools at the edge, and in zero of six pools in forest.

At least one *A. callidryas* tadpole survived until the end of the experiment in all pools, except one pool in pasture where mortality was 100 %. Survival in pasture (42 %) was less than half of survival in edge and forest (> 90 %, $F_{2,17} = 9.02$, $P = 0.003$; Fig. 4.3). Multiple comparisons tests revealed that survival in pasture was less than that in edge ($P = 0.007$) and forest ($P = 0.005$) but survival in edge and forest ($P = 0.988$) did not differ.

Dendropsophus ebraccata tadpoles nearly doubled in length in edge and forest and more than doubled in pasture in the first 13 days (Fig. 4.2). After 13 days, tadpole length continued to increase in pasture and edge but growth slowed in forest. Tadpole growth was affected by time (Wilks lambda = 0.017, $F_{4,12} = 169.163$, $P < 0.001$) and its interaction with habitat (Wilks lambda = 0.084, $F_{8,24} = 17.324$, $P < 0.001$). Differences in tadpole growth among habitats were evident by the first sampling date (Table 4.1, Fig. 4.2). During the first 23 days, tadpoles in edge and forest were similar in length, but after 23 days tadpoles in edge resembled tadpoles in pasture more than those in forest. Overall, tadpoles were larger in pasture than in edge ($P = 0.019$) and forest ($P < 0.001$) and tadpoles were larger in edge than in forest ($P = 0.050$). At the end of the experiment, *D. ebraccata* tadpoles had hindlimb development in three of six pasture pools, one of six edge pools, and zero of six forest pools. *Dendropsophus ebraccata* tadpole survival was high (> 87 %) in all habitats ($F_{2,17} = 1.011$, $P = 0.387$; Fig. 4.3).

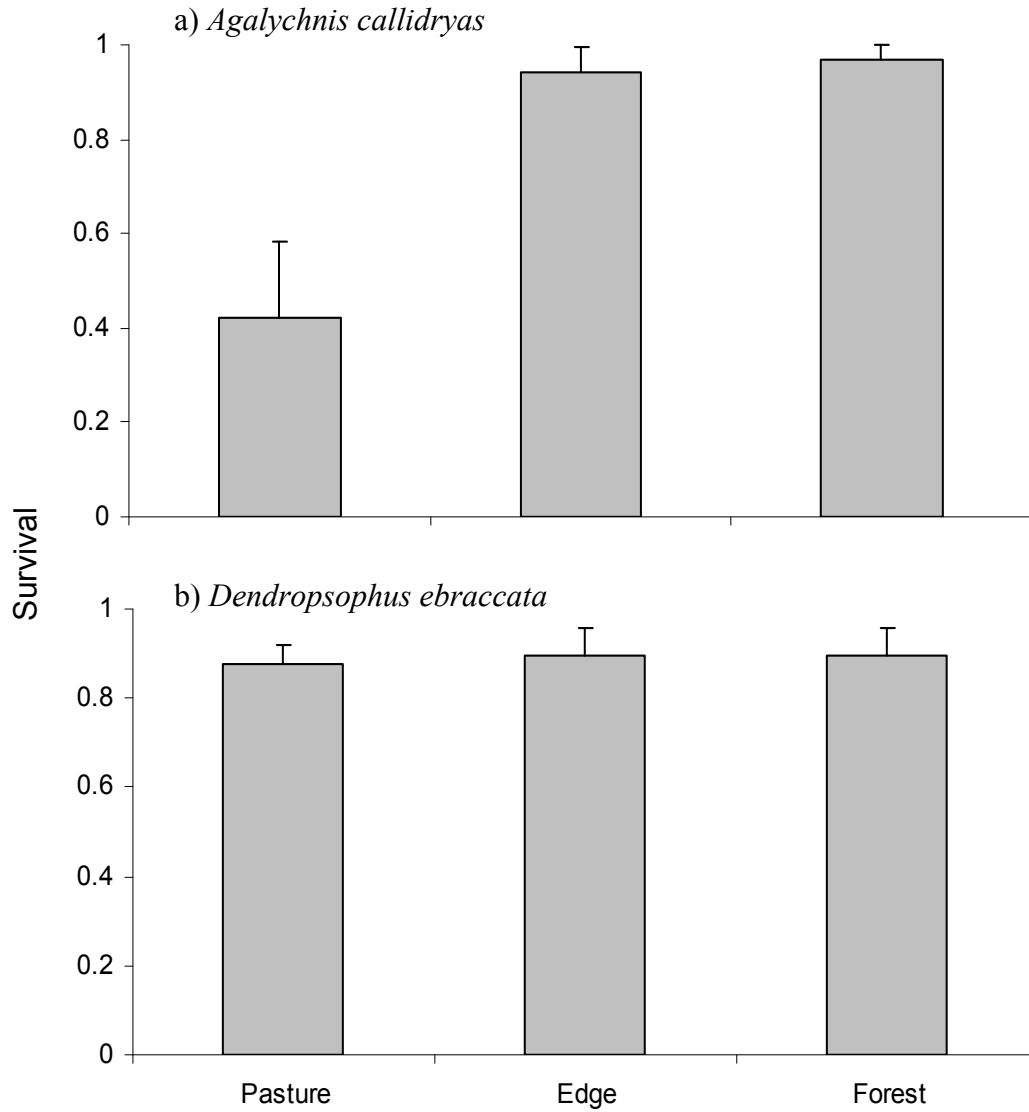


Figure 4.3. Proportion of tadpoles surviving (mean \pm SE) for a) *Agalychnis callidryas* and b) *Dendropsophus ebraccata* in pasture, edge, and forest at the end of the experiment.

Abiotic environments

Maximum daily water temperature was on average of 9.0 °C warmer in pasture pools than in forest pools, while minimum water temperature varied little among habitats. The daily temperature range (mean maximum – mean minimum) was greatest in pasture (33.7 – 25.2 °C) and minimal in edge (26.1 – 24.2 °C) and forest (24.6 – 23.8 °C) habitats. Dissolved oxygen content was higher in pasture pools (6.08 ± 0.26 mg/L) than in either edge (3.16 ± 0.24 mg/L) or forest pools (2.87 ± 0.33 mg/L; $F_{2,27} = 133.73$, $P < 0.001$). Dissolved oxygen content also differed among blocks ($F_{2,27} = 25.20$, $P = 0.005$). Global site factor (GSF) was higher in pasture than in edge and forest ($F_{2,35} = 5764.851$, $P < 0.001$), but there was no difference between edge and forest ($P = 0.307$; Fig. 4.4). In addition, GSF differed among blocks ($F_{2,35} = 14.023$, $P < 0.001$); it was higher in block 3 than in block 1 or 2 ($P \leq 0.002$), but did not differ between blocks 1 and 2 ($P = 0.358$). There was a strong positive correlation between GSF and both maximum ($r = 0.982$, $P < 0.001$) and minimum ($r = 0.911$, $P = 0.001$) water temperatures in each subblock ($n = 9$).

DISCUSSION

Tadpoles of *Agalychnis callidryas* and *Dendropsophus ebraccata* grew larger and faster in pasture than in edge or forest, in agreement with my expectations. However, *A. callidryas* survival was lower in pasture than in edge or forest, whereas *D. ebraccata* survival was equally high in all habitats. All measured abiotic variables showed marked differences in pasture compared to edge and forest habitats. Variation in abiotic variables among habitats led to differences in tadpole performance, likely contributing to patterns of frog occupancy across the landscape.

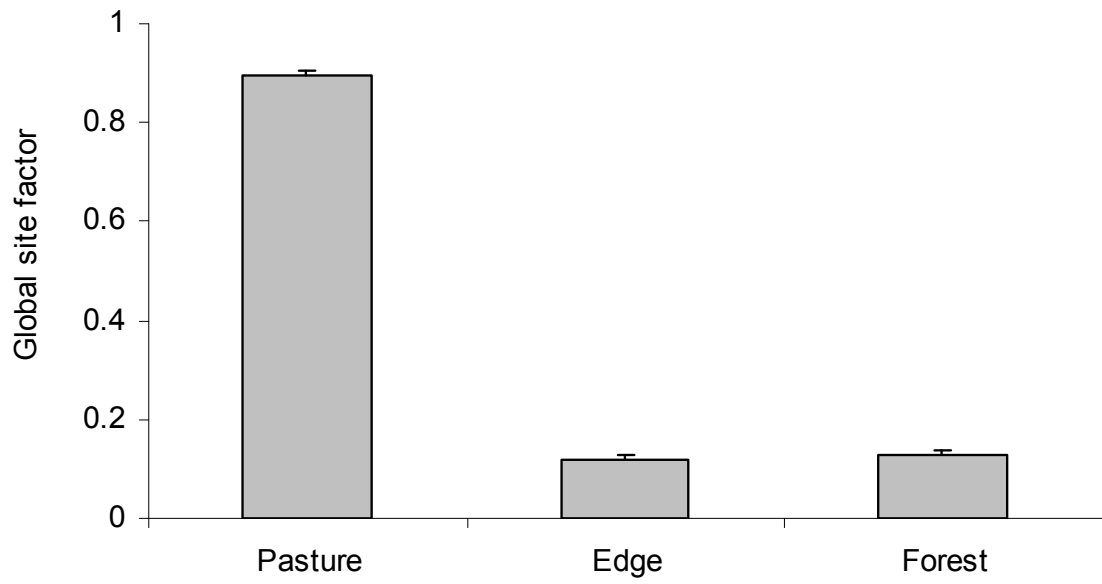


Figure 4.4. Global site factor (mean \pm SE) calculated from hemispherical photographs within each habitat.

The light environments in pasture, edge, and forest were quantitatively different, with solar radiation unobstructed by vegetation in pasture but highly obstructed by complex vegetation structure in edge and forest. Global site factor was highly correlated with average maximum and minimum water temperatures recorded in pools in each habitat. Tadpoles in pasture grew to a larger size over the 43-day experiment in comparison to those in edge and forest. This result is consistent with results of other studies; for instance, spring peepers, *Pseudacris crucifer*, are larger in size and more developed in wetlands with high GSF values (Halverson et al. 2003). A positive correlation between temperature and tadpole growth and development is well established from laboratory studies (e.g., Smith-Gill and Berven 1979, Álvarez and Nicieza 2002). Field studies conducted in open and closed canopy sites also reveal positive correlations between open canopies, temperature, and tadpole growth and development, with interspecific variation in the strength of the responses (Werner and Glennemeier 1999, Skelly et al. 2002, Schiesari 2006). Although dissolved oxygen concentrations were higher in pasture pools, a laboratory experiment suggests that this factor does not significantly influence tadpole growth (Schiesari 2006). Alternatively, variation in food quality may play a large role in determining tadpole performance in pasture, edge, and forest.

The quality of food resources available to tadpoles in open and closed canopy habitats may be the primary factor responsible for intraspecific and interspecific variation in performance (Schiesari 2006). There are positive relationships between light and temperature with the composition and abundance of periphyton (DeNicola 1996) and zooplankton (Covich and Thorp 1991). *Dendropsophus ebraccata* tadpoles are

macrophagous while *A. callidryas* tadpoles are suspension-feeders that feed in the water column and on detritus (Savage 2002). However, it has been suggested that we underestimate the variety of foods that tadpoles consume (Altig et al. 2007). Variation in food quality among habitats may be equally or more responsible for intraspecific variation in tadpole growth and development among habitats compared to water temperature. Enhanced food quality or quantity can offset the low growth and development rates that occur at low temperatures, but the strength of this response varies among species (Newman 1998, Werner and Glennemeier 1999, Skelly et al. 2002, Schiesari 2006). One laboratory study found that tadpole growth and development are depressed in low temperature pools compared to high temperature pools even when high quality food resources are available (Newman 1998). In my study, tadpole growth curves reached an asymptote after 23 days in forest, suggesting that the food remaining was sufficient to maintain the tadpoles but not sufficient to support continued growth. I did not directly quantify food quantity or quality in my study, but in future studies it would be helpful to do so in order to separate the effects of temperature and food quality on tadpole performance.

Dendropsophus ebraccata survived as well in open canopy as in closed canopy habitats, whereas *A. callidryas* survival was reduced in pasture compared to forest and edge. Extreme maximum daily water temperatures (≥ 37 °C) always preceded or occurred on the same day that *A. callidryas* deaths were recorded. *Agalychnis callidryas* survival in pasture may have been even lower if I had continued the experiment through metamorphosis, because tadpoles become more sensitive to temperature extremes in later developmental stages (Noland and Ultsch 1981). My results suggest that *A. callidryas*

tadpoles are less flexible than *D. ebraccata* in their ability to tolerate extreme temperatures. Therefore, reduced tadpole survival is likely a factor that limits the distribution of *A. callidryas* in pastures.

Tadpoles regulate their body temperatures behaviorally by moving to different areas of the aquatic environment (Brattstrom 1962, 1979), and as a result they may maximize developmental and growth rates (Smith-Gill and Berven 1979) and minimize exposure to extreme temperatures (Ultsch et al. 1999). In pasture pools in my study area, tadpoles may thermoregulate to take advantage of higher temperatures for growth and development benefits but escape to areas of the pool with more vegetation cover if temperatures begin to exceed their tolerance limits. However, small pasture pools, with little variation in vegetation cover, may represent an ecological trap for *A. callidryas*.

Another factor that likely influences the occurrence of leaf-breeding hylid frogs in pastures is increased mortality caused by egg desiccation compared to edge or forest pools. Both *D. ebraccata* and *A. callidryas* attach their egg clutches to vegetation above the surface of water. Desiccation is the main source of egg mortality for *D. ebraccata* at La Selva, Costa Rica (Roberts 1994) and it also causes mortality of *A. callidryas* egg clutches (Warkentin 2000). Pasture pools with emergent vegetation will vary in the amount of cover for egg clutches. Adult frogs must select oviposition sites to reduce the risk of mortality caused by clutch desiccation. For example, in Brazil, *Phyllomedusa tarsius*, a treefrog that breeds in pasture, secondary forest, and primary forest, has the lowest hatchling success in pastures, with mortality caused by desiccation and flooding (Neckel-Oliveira 2004).

My results suggest that adult breeding site selection represents a trade-off such that adults selecting closed canopy ponds may risk reduced fitness in the form of depressed tadpole growth and development rates, while adults selecting pasture ponds may risk reduced fitness measured as depressed tadpole survival. However, pastures can offer growth advantages to treefrog tadpoles that may improve the fitness of later life stages. These findings support the view that matrix habitats should be recognized as important to maintaining diversity in fragmented landscapes (Vandermeer and Perfecto 2007). A better understanding of the mechanisms underlying frog distributions will allow land managers to predict how species will respond before and address species-specific issues after land use change.

CHAPTER 5

TADPOLES ACT AS PREDATORS AND PREY IN TROPICAL EPHEMERAL PASTURE POOLS

The position tadpoles occupy in aquatic food webs is being redefined, moving from the long-standing but erroneous view that tadpoles are strictly herbivores and detritivores towards an appreciation of their potential as predators (Petranka and Kennedy 1999). Cannibalistic tadpoles have been known for some time (e.g., Bragg 1956, Crump 1983, Polis and Myers 1985). However, little attention has focused on understanding the incidence of predaceous tadpoles within multi-species assemblages and whether they influence the composition of assemblages in ephemeral pools. Unexpectedly, tadpoles may be the primary predators and regulators of heterospecific populations in some ephemeral pools (Petranka and Kennedy 1999).

Cannibalism is a behavior found in many animals and several factors influence its incidence. High animal density and low food availability increase cannibalism (Fox 1975, Polis 1981). Low food quantity and quality increase foraging activity, raising the probability of intraspecific encounters, and leaving animals weak and more vulnerable to predation (Polis 1981). These trends follow the foraging theory prediction that animals should expand their diets beyond the foods they typically consume when stressed during periods of low food availability (Pyke et al. 1977). Additionally, aggressive behavior can increase the incidence of cannibalism (Fox 1975). Cannibalism also has a genetic basis; within a species, some breeding strains have a higher tendency to cannibalize than others (Polis 1981). Finally, for tadpoles, species that are able to recognize kin are less prone to cannibalize siblings than non-siblings (Pfenning et al. 1993).

Carnivorous tadpoles are expected to benefit from the additional nutrients and minerals in their diets (Nagai et al. 1971, Crump 1983), leading to faster developmental or growth rates (Crump 1986, 1990, Heinen and Abdella 2005). Even larger benefits may accrue to cannibals than to tadpoles that consume heterospecifics because of increased conversion efficiency (Crump 1990). These benefits may transfer to the adult stage, as large metamorph size and faster developmental can improve later fitness (Smith 1987, Berven 1990, Pechenik et al. 1998). Costs of cannibalism include reduced inclusive fitness in related individuals (Crump 1983) and increased injury (Crump 1992). Yet these disadvantages may be offset by improved sibling fitness for those that complete metamorphosis. Additionally, whether these costs are compensatory or additive is questionable, as mortality is typically greatest during premetamorphic stages (Wilbur 1980).

Cannibalism and heterospecific carnivory can have demographic consequences for tadpole populations in ephemeral pools (Fox 1975, Polis 1981). Differential cohort survival can alter population size (Fox 1975, Polis 1981), size structure (Ziembra and Collins 1999), and age structure (Fox 1975). For instance, vulnerability to cannibalism is typically size-specific, with eggs and hatchlings preyed upon by more advanced developmental stages (Crump 1983, Petranka and Thomas 1995). Ultimately, heterospecific carnivory and cannibalism may influence species composition of tadpole assemblages in ephemeral pools.

There are few data on ecological interactions between and within members of tropical tadpole assemblages in ephemeral pools. In many neotropical regions, landscapes are dominated by a matrix of modified habitats that surround small forest

patches. These new environments create opportunity for the assembly of species that interact in potentially novel ways. Such species assemblages are composed of members that are able to succeed ecologically in the modified habitat at least in part because of phenotypic plasticity (Agrawal 2001). In tropical pastures, ephemeral pools are common, tadpole populations are dense and diverse, and food is limited. In these pools, species that have flexible feeding strategies are likely to have an advantage in attaining nutrients required for growth and metamorphosis.

My objectives were to examine the incidence of tadpole carnivory and the factors that influence its intensity among species that breed in ephemeral pools in tropical pastures. I addressed three questions. First, how prevalent are heterospecific carnivory and cannibalism among species that use ephemeral pools in pastures? Second, is the incidence of cannibalism influenced by the size and developmental stage of predators and prey? Third, do tadpole density, food quantity, and relatedness influence the incidence of cannibalism and tadpole performance?

METHODS

Study area and species

My study was conducted in the Rio Piro region of the Osa Peninsula, Puntarenas Province, Costa Rica (8°26'N, 83°22'W). This region is a mosaic of lowland wet forest (Hartshorn 1983), cattle pastures, and tree plantations. The Osa Peninsula is characterized by distinct rainy (May to December) and dry seasons (January to April) and precipitation averages 4000 to 6000 mm y⁻¹ (L. Gilbert, *unpublished data*).

I selected three species to study, *Smilisca phaeota*, *Engystomops pustulosus*, and *Leptodactylus poecilochilus*, because of their abundance, ease of manipulation, and

paucity of data on their tadpole ecologies. Additionally, all three species oviposit in ephemeral pools in pastures in the study area during the same time of year. *Smilisca phaeota* females deposit large clutches of eggs (1,600 to 2,000) in a surface film in shallow water (Savage 2002) and tadpoles require a minimum of 18 days to complete metamorphosis. During field observations I confirmed that *S. phaeota* tadpoles are facultative carnivores, consuming conspecific and heterospecific eggs and hatchlings, and scavengers, consuming remains of dead tadpoles and *Anolis* species. *Engystomops pustulosus* females lay 80-450 eggs in a foam nest (Savage 2002) and tadpoles complete metamorphosis in a minimum of 14 days (T.J. Hawley, *unpublished data*). Predators of *E. pustulosus* eggs include *Chaunus marinus* (Downie 1988) and *Leptodactylus savagei* (Kluge 1981). *Leptodactylus poecilochilus* females deposit eggs into a foam nest inside burrows at the edges of temporary pools (Savage 2002). Metamorphosis is expected within 15 days (Savage 2002), similar to a closely related congener, *L. fragilis*. There are no known predators of *L. poecilochilus* eggs. The natural tadpole densities of these species are not known; however, oviposition rates suggest that tadpole densities are high in pasture pools. For instance, over a period of 162 days (June 20 – November 28 2006), I counted a total of 141 *E. pustulosus* foam nests and 8 *S. phaeota* clutches in a pool (surface area 16.5 m²) that dried five times (T.J. Hawley, *unpublished data*). I did not search this pool for *L. poecilochilus* eggs because it would require destructive sampling; however, I noted that *L. poecilochilus* adults vocalized and tadpoles were collected there.

Experiment 1: Heterospecific carnivory and cannibalism in ephemeral pasture pools

I reviewed the literature to determine whether species that occupy pastures are known to exhibit carnivory during the tadpole life stage. The species list was compiled

from surveys in the study area from 2004 to 2006 (Chapter 2). Few data were available for *S. phaeota*, *E. pustulosus*, and *L. poecilochilus*, so I used a fully factorial experiment, with two fixed factors, predator and eggs, to examine the incidence of carnivory and preferences among these species. Predator had four levels, tadpoles of three species (*S. phaeota*, *E. pustulosus*, and *L. poecilochilus*) and a control with no tadpoles, and eggs had three levels, 20 *S. phaeota* eggs, 20 *E. pustulosus* eggs, or 10 eggs of each species. *L. poecilochilus* eggs were not studied because they are difficult to locate. Each treatment was replicated four times and controls were replicated three times, for a total of 45 experimental units, or cups. Controls estimated variation in egg mortality unrelated to treatment manipulations. Predator tadpoles were reared from eggs of at least four clutches on a leaf and plankton diet (*S. phaeota* and *E. pustulosus*) or collected from natural pools (*L. poecilochilus*). Egg prey originated from two clutches (*S. phaeota*) and at least three foam nests (*E. pustulosus*). Eggs were used only as prey, so I was not concerned about using a large number of source egg clutches for genetic reasons.

I filled plastic cups (480 ml) with 300 ml of water from a spring in the study area. I sorted tadpoles by size and added two individuals to each cup, attempting to standardize total tadpole biomass per cup. Tadpoles were starved for approximately 2.5 hours before eggs were added. My preliminary work suggested that additional time would cause tadpole fatigue. During the starvation period, I estimated tadpole size (length to the nearest 0.25 cm) using a rapid measuring technique that involved placing tadpoles in a petri dish over a grid (0.5 cm²). I also estimated developmental stage (Gosner 1960) using a hand lens. Tadpole size (mean length/cup) and developmental stage were the following: *S. phaeota*, 3.3 ± 0.2 cm (mean ± SD, n = 12 cups), stages 29 – 36 (range of

the mean stages of two individuals/cup, $n = 12$ cups), *E. pustulosus*, 1.9 ± 0.1 cm, stages 27 - 32, and *L. poecilochilus*, 2.4 ± 0.1 cm, stages 25 - 32. I assigned prey treatments to cups within each predator treatment at random. Because *E. pustulosus* males construct foam nests within which the eggs are suspended (Downie 1990), I mimicked how the eggs naturally occur by using a spoon to cut pieces of foam and counting the number of eggs by gently pressing the foam between plastic petri dishes. When eggs were added to cups, I added a decayed leaf for substrate and an alternative food source. After adding eggs to all cups, I observed tadpoles (one minute/cup) from a distance of 0.5 m and recorded their activity according to the following categories: inactive, swimming, interacting with eggs (chewing jelly or foam, gulping embryo), or foraging on the leaf. I counted the number of living eggs and predators after 24 and 48 hours.

Survival (proportion) in the control groups was 1.0 ± 0 (mean \pm SE); therefore, I did not analyze these data further because the assumption of homogeneity of variance would be violated. I used repeated measures ANOVA to examine variation in embryo survival among treatments at 24 and 48 hours. I summarized observational data by determining the number of cups with at least one tadpole feeding on eggs. I used SPSS version 12.0 (2003) for all statistical tests and tested for normality and homoscedascity of errors using Shapiro-Wilk's and Levene's tests. In the treatments where predators were exposed to both species at the same time, I measured preference of predator species by calculating Rodger's index of preference (Krebs 1999). Rodger's index can be applied in experiments that include more than one observation over time and if a food type is entirely consumed by the end of the experiment. Preference scores close to 1.0 indicate the most preferred items, with scores close to 0.0 indicating avoidance.

Experiment 2: Cannibalism as a function of predator size and prey stage

This experiment was a fully factorial 3 x 5 design with three *S. phaeota* tadpole size classes (small, medium, and large) and five *S. phaeota* embryo developmental stages (Gosner 1960; egg stages 10-12, egg stages 17, hatchling stage 22, hatchling stage 23, and hatchling stage 25, representing trials 1-5 respectively). I ran each trial, a set of all three tadpole size classes with a given level of prey, in succession as prey developed. Controls, consisting of embryos alone, and experimental treatments were each replicated four times. Tadpoles were reared from source populations as described in Experiment 1. I collected prey eggs from two clutches in natural pools on the morning of the first trial and maintained them in plastic containers in an open-air laboratory for use during later trials. I used the eggs only as prey; therefore, I was not concerned about low genetic replication.

I filled plastic cups (480 ml) with 300 ml of spring water. Tadpoles were sorted into three size classes by eye, randomly selected, and added to cups (2 individuals/cup). Tadpoles were held in cups without food for approximately two hours. During this starvation period, I estimated tadpole length to the nearest 0.25 cm using the same protocol as described above (range of mean size/cup across all trials: small, 1.4-1.7 cm, medium, 2.9-3.1 cm, and large, 3.4-3.8 cm). I estimated developmental stage (Gosner 1960) using a hand lens (range of mean developmental stage/cup (n = 20 trials for each size class): small, 25, medium, 28-32, large, 36-38). Tadpoles were naïve, or previously unexposed to conspecific eggs in trials 1-3, but recycled from earlier trials in trials 4 and 5.

I added embryos (20/cup) and one decayed leaf, to provide substrate and an alternative food source, to cups in each trial. Immediately after the embryos were added to all cups in a trial, I began an observation period (1 minute/cup) recording tadpole activity according to the following categories: inactive, swimming, interacting with prey (chewing jelly, gulping embryo), or foraging on leaf. After two hours, I observed tadpoles in each cup for another minute and counted the remaining embryos. After 24 hours, I counted the remaining tadpoles and embryos and estimated embryo developmental stage. I started Trial 1 on 1 September 2006 at 8:00 and Trial 2 at 16:00, Trial 3 on 2 September at 10:00, Trial 4 on 3 September at 7:00, and Trial 5 on 4 September at 8:00.

I summarized observational data across all treatments in each trial and calculated cup means for embryo survival within each treatment and control. Statistical analyses focused on Trials 1-3 as Trials 4 and 5 were not independent because predator tadpoles were reused from previous trials. All embryos survived in the controls, whereas mortality was 100% in each replicate of some treatments. Therefore, parametric assumptions of normality and homogeneity were violated and could not be corrected with a data transformation. I used non-parametric Kruskal-Wallis tests to examine the effects of prey stage and predator size on embryo survival.

Experiment 3: Effects of density, food availability, and relatedness on S. phaeota performance

This experiment was a fully factorial 2 x 2 x 2 x 2 design, with four fixed factors, tadpole density (low = 10 individuals, high = 20 individuals), plant food quantity (low = 50 g damp leaf litter, high = 150 g damp leaf litter), animal-based food quantity (presence

= addition of 30 *S. phaeota* eggs on day 14, day 19, and day 21, or absence = no eggs added), and relatedness (siblings or non-siblings). Tadpole densities ($265/\text{m}^2$ or $132/\text{m}^2$) were similar to the density of eggs in a natural pool ($218/\text{m}^2$) and much lower than the highest egg density ($> 10,000$ eggs/ m^2) measured in an artificial pool in the study area. A block design was used to reduce potential variation from genetic and environmental sources. Four blocks were arranged spatially, side by side, with each treatment represented once within each block, for a total of 64 experimental units.

The experiment was conducted in plastic basins (10 L in volume, 19 cm in depth, 31 cm in diameter) in a pasture. I filled each basin with water from a nearby stream (6 L), plankton (500 ml whole samples, collected from two natural pools, mixed, and filtered through 1.0 mm² mesh), and damp leaf litter on 30 August and covered them with a mesh top. The remainder of the 10 L basins filled with rainwater within one week. As basins were prepared, I randomly selected half to receive 50 g of leaf litter and half to receive 150 g of leaf litter. Accounting for the assignment of the plant-based food factor, I randomly assigned basins to treatments, a block, and a position within the block. On 3 September, I added another 500 ml of plankton (collected and prepared in the same manner as above) to each basin. Basins were covered throughout the experiment to eliminate introductions of insect or frog larvae and rainfall maintained water level throughout the experiment.

On 14 September, I collected four *S. phaeota* egg clutches, one from each of four different temporary pasture pools. I reared clutches in separate plastic basins until stage 23-24 (Gosner 1960) and introduced hatchlings into basins on 16 September (day 0). Sibling treatments within each block received hatchlings from the same clutch and each

block received siblings from a different clutch. Eggs added to basins in the animal food treatments were collected from pasture pools. After egg additions, early stage hatchlings were found in five of 32 basins during routine survival checks, indicating that some eggs had not been consumed. These hatchlings were left in the basins, but within two weeks most were no longer present and had likely been consumed by other tadpoles or died of natural causes. I monitored water temperature hourly using I-button temperature loggers (Maxim Integrated Products, Sunnyvale, CA) in plastic bags placed in one basin of each block.

I measured the initial survival of tadpoles on day 3, by removing tadpoles from each basin. Thereafter, I assessed survival every eight days. When tadpoles reached stages 40-41 (Gosner 1960), I checked the basins twice daily for metamorphs, defined as individuals with at least one forelimb emerged. Metamorphs were removed and held individually in plastic bags until the tail was absorbed, then weighed to the nearest mg. I ended the experiment on day 68, and considered all tadpoles that did not reach metamorphosis to have perished. I could not confirm whether individuals that disappeared from the basins during the experiment were directly killed by conspecifics or were scavenged after death from other causes; therefore, I treat these deaths as unknown sources of mortality.

I calculated five response variables for each basin, survival (proportion of tadpoles that became metamorphs), mortality (proportion of tadpoles that died during the experiment from an unknown cause), metamorph mass, total basin metamorph biomass, and duration of larval period (number of days to metamorphosis). I calculated basin means for metamorph mass and larval period for statistical analyses. I used a univariate

analysis of variance (ANOVA) to examine variation in survival (arc-sin transformed) and univariate analysis of covariance (ANCOVA) to examine variation in total biomass (log-transformed) among fixed treatment factors and the random block factor. For both response variables, the block factor was not significant, so the analysis was rerun excluding block. Survival on day 35 was a covariate in the biomass analysis because metamorph mass and larval period are influenced by tadpole density (Wilbur and Collins 1973). The survival covariate included living tadpoles and metamorphosed individuals as survivors; it represented the average tadpole density experienced in each basin throughout the experiment because it was near the midpoint of 68 days. The mortality response variable could not be normalized, so a nonparametric Kruskal-Wallis test was used to assess variation among treatments.

Analyses evaluating variation in metamorph mass and larval period were intended to be conducted using a fully factorial four-way ANOVA. However, no tadpoles became metamorphs in one treatment (high density, low plant food quantity, no animal food, siblings) and metamorphs emerged from only one of four replicate basins in two other treatments. Therefore, the final dataset was a subset of what was expected. The reduced size of the dataset precluded a complete four-way analysis and lowered the power of three-way interactions. To address the main hypotheses, I selected two subsets of the dataset that permitted full analyses but excluded levels of some treatments (after Milliken and Johnson 1984). The first subset examined treatments at high plant food quantity and all density, animal food, and relatedness levels and their interactions. This analysis addresses whether tadpole density influences metamorph mass and larval period when animal-based food is added and tadpoles are siblings or non-siblings. The second subset

examined treatments at low density but all animal food, plant food, and relatedness levels and their interactions. This analysis addresses whether foods of different sources and quantities influence the growth and development of siblings and non-sibling tadpoles. I used an ANCOVA for both analyses, with survival on day 35 as the covariate. To achieve a normal distribution and remove heteroscedasticity, I log-transformed larval period and metamorph mass.

RESULTS

Experiment 1: Heterospecific carnivory and cannibalism in ephemeral pasture pools

Eleven species were found in pastures within my study area from 2004 to 2006; based on previous literature, six of these species are facultatively carnivorous and/or cannibalistic during the tadpole stage (Table 5.1). My results indicated that *S. phaeota*, *E. pustulosus*, and *L. poecilochilus* can be facultative cannibals and/or heterospecific carnivores. In total, of eleven species that use ephemeral pools for tadpole development, eight are potential predators of eggs and/or tadpoles. Several of these species are known to consume eggs or tadpoles of more than one member of the assemblage.

Egg survival differed among predator species (Fig. 5.1; $F_{2,27} = 186.969$, $P < 0.001$) but did not differ between prey species ($F_{2,27} = 0.931$, $P = 0.406$). *Smilisca phaeota* tadpoles consumed all *E. pustulosus* and *S. phaeota* eggs within 48 hours, whereas *E. pustulosus* tadpoles consumed few conspecific or *S. phaeota* eggs in 48 hours (Fig. 5.1). *Leptodactylus poecilochilus* tadpoles consumed *E. pustulosus* eggs at a slower rate than *S. phaeota* eggs, but after 48 hours the proportion consumed of each species did not differ. Egg survival decreased with the amount of time the embryos were available to predators (24 or 48 hours; $F_{1,27} = 98.910$, $P < 0.001$) and all interactions among time,

Table 5.1. Known cases of facultative carnivory and cannibalism during the tadpole life stage of species detected in pastures in the study area during survey work in 2004 and 2006 (Chapter 2). Data on prey species are from the literature and current study (** indicates that there are no data pertaining to studies of carnivory in this species). Breeding habitat association data are from Savage (2002) and survey work (Chapter 2), EP = ephemeral pools and L = lagoons. Abbreviations for conditions, L = lab, N = natural. Abbreviations for prey species are: *Chaunus marinus* (Cm), *Agalychnis callidryas* (Ac), *Hypsiboas rosenbergi* (Hr), *Dendropsophus ebraccata* (De), *Scinax elaeochroa* (Se), *Smilisca phaeota* (Sp), *Leptodactylus savagei* (Ls), and *Engystomops pustulosus* (Ep).

Tadpole predator	Habitat	Prey	Prey stage	Conditions	Source
Bufo					
<i>Chaunus marinus</i>	EP, L	Cm Ep	egg egg	L, N L	Hearnden 1992 Downie 1988
Hylidae					
<i>Agalychnis callidryas</i>	EP, L	Ac, De, Se	egg	L	Roberts 1994
<i>Hypsiboas rosenbergi</i>	EP, L	Hr	egg, tadpole	N	Kluge 1981
<i>Dendropsophus ebraccata</i>	EP, L	Ac, De, Se	egg	L	Roberts 1994
<i>D. microcephala</i>	EP, L	**			
<i>Scinax elaeochroa</i>	EP, L	**			
<i>Smilisca phaeota</i>	EP	Sp Ep	egg, hatchlings egg	L, N L, N	Hawley present study Hawley present study
Leptodactylidae					
<i>Leptodactylus bolivianus</i>	EP, L	**			
<i>Leptodactylus savagei</i>	EP, L	Cm, Hr, Ep, Sp, Ls	hatchlings egg	L N	Heyer et al. 1975 Muedeking and Heyer 1976
		Hr	egg, tadpole	L, N	Kluge 1981
		Ac, De, Se	egg	L	Roberts 1994
<i>L. poecilochilus</i>	EP	Ep, Sp	egg	L	Hawley present study
<i>Engystomops pustulosus</i>	EP	Hr Ep, Sp	egg, tadpole egg	N L	Kluge 1981 Hawley present study

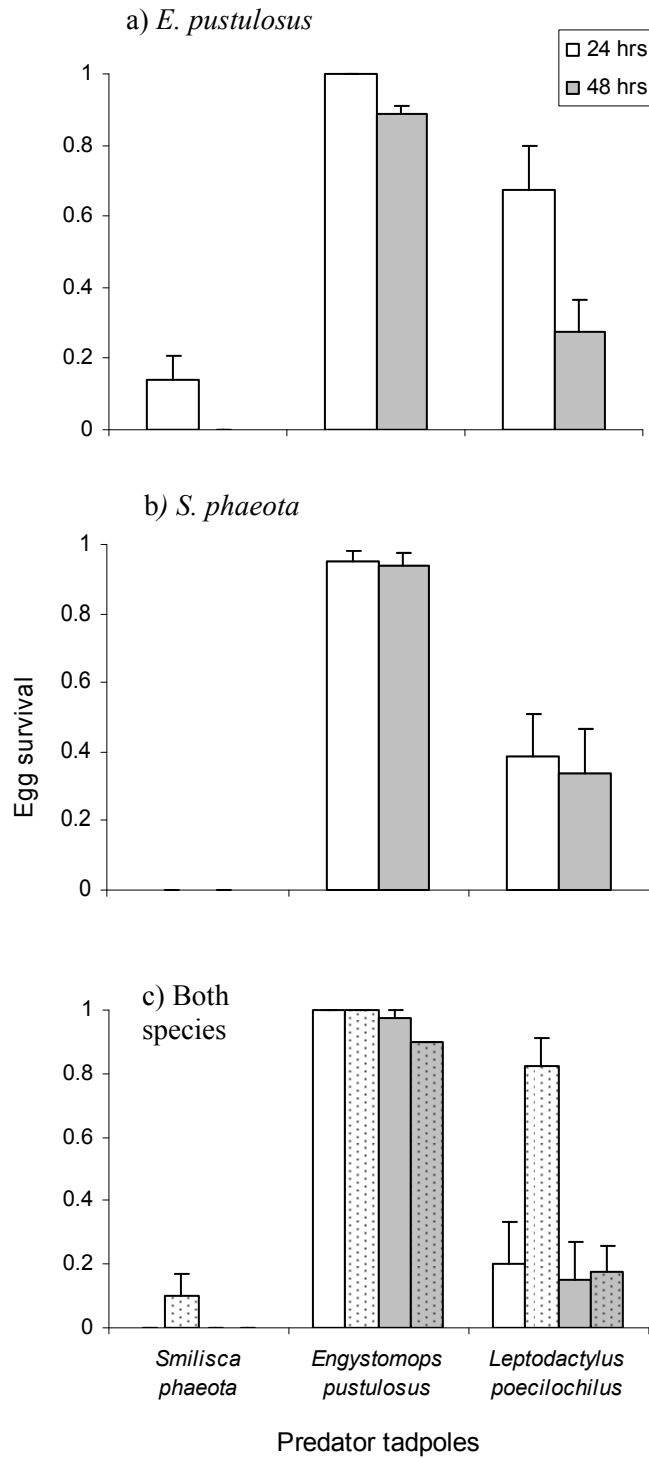


Figure 5.1. Egg survival (mean \pm SE) after exposure to predator tadpoles for 24 (white bars) and 48 (shaded bars) hours a) *E. pustulosus*, b) *S. phaeota*, and c) both species combined; open bars are *S. phaeota* and spotted bars are *E. pustulosus*.

predator, and prey species were significant. After 48 hours, the remaining embryos were in Gosner stage 23; *S. phaeota* hatchlings were actively swimming and *E. pustulosus* hatchlings were swimming or suspended within the remaining foam.

When tadpoles were presented with equal numbers of *E. pustulosus* and *S. phaeota* eggs, each species showed a different pattern of consumption (Fig. 5.1c). *Smilisca phaeota* tadpoles consumed all conspecific eggs and nearly all *E. pustulosus* eggs within 24 hours. Within 48 hours, *S. phaeota* preference scores were similar, indicating no preference (conspecific preference score = 1.0, *E. pustulosus* preference score = 0.93). In contrast, *E. pustulosus* tadpoles did not consume any eggs within 24 hours and few eggs of either species within 48 hours. The preference index indicated that *E. pustulosus* preferred conspecific tadpoles (score = 1.0, *S. phaeota* score = 0.25). *Leptodactylus poecilochilus* tadpoles showed a preference for *S. phaeota* eggs in 24 hours, but the strength of preference lessened over the next 24 hours (Fig. 5.1c; *S. phaeota* score = 1.0, *E. pustulosus* score = 0.49).

Paralleling the results of prey survival, tadpoles demonstrated different activity patterns after being presented with eggs. During the observation period, at least one *S. phaeota* tadpole in each cup interacted with *S. phaeota* prey and prey of both species, while only half of cups had at least one *S. phaeota* tadpole interacting with *E. pustulosus* prey alone. *Smilisca phaeota* tadpoles chewed on the jelly coat of conspecific eggs until it was broken, and then they began gulping the entire egg into their buccal cavity. Sometimes, after ingesting an egg into the buccal cavity, the egg would be expelled out of the mouth and then the tadpole would continue the pumping action, ingest the egg, and eventually pass the egg into the esophagus. A similar pattern was observed with *E.*

pustulosus eggs, but would begin with *S. phaeota* tadpoles orienting themselves vertically with their heads upright and chewing through the foam underneath eggs. *L. poecilochilus* tadpoles ingested eggs similar to the pattern observed with *S. phaeota*; more cups had at least one tadpole interacting with *S. phaeota* prey alone (70%) or both species (100%) than with *P. pustulosus* alone (50%). *E. pustulosus* tadpoles did not interact with any prey during the observation periods, but they were observed chewing on the jelly coats of eggs of both species on other occasions.

Experiment 2: Cannibalism as a function of predator size and prey stage

Smilisca phaeota embryo survival was dependent on tadpole size ($X^2 = 23.853$, $df = 2$, $P < 0.001$); survival was lowest in the presence of medium- and large-sized tadpoles (Fig. 5.2). Small-sized tadpoles caused less embryo mortality of all developmental stages. Tadpoles caused 54 – 100% embryo mortality (Trials 1 and 2), but mortality was more variable (2 – 95%) at the early hatchling stage ($X^2 = 5.890$, $df = 2$, $P = 0.053$). Mobile hatchlings (0.7 cm in length; stage 25) in Trial 5 had nearly 100% survival with all tadpole sizes (1.25 – 4.00 cm in length; Fig. 5.2).

In the majority of cups in Trials 1 and 2, at least one tadpole interacted with prey during the first observation period (75% and 92% respectively). However, no tadpoles interacted with prey in the first observation period of the other trials. Interactions between tadpoles and egg prey were similar to those described in Experiment 1. On average across all trials, the majority of mortality occurred within the first two hours of exposure of embryos to tadpoles.

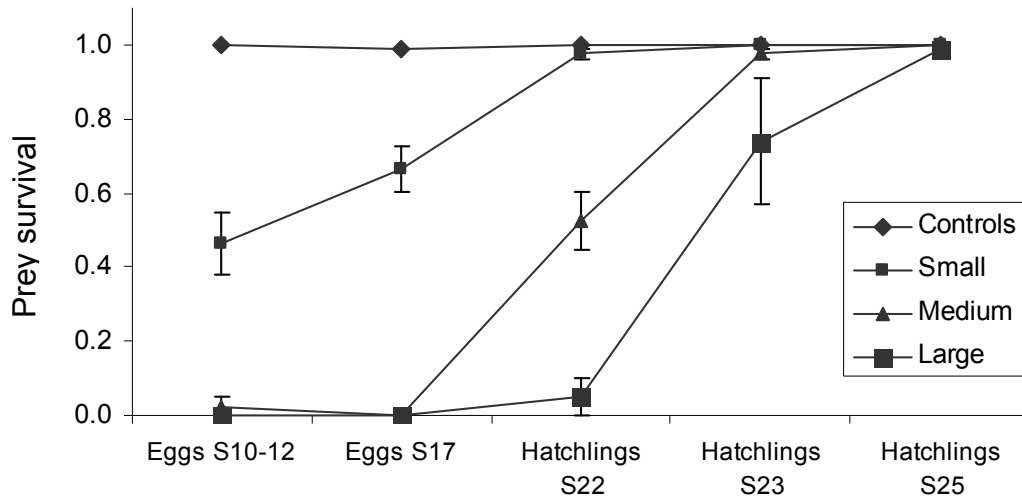


Figure 5.2. Survival (mean \pm SE) of *S. phaeota* embryos and hatchlings, as indicated by Gosner developmental stages (S; Gosner 1960), exposed to tadpoles of different sizes. Controls contained only embryos.

Experiment 3: Effects of density, food availability, and relatedness on S. phaeota performance

Water temperature did not vary among blocks; maximum daily water temperature was $34.39 \pm 0.46^{\circ}\text{C}$ (mean \pm SE; range of mean values in each block = $33.64 - 34.87^{\circ}\text{C}$, $n = 67$ days per block) and minimum was $24.19 \pm 0.09^{\circ}\text{C}$ (range = $23.82 - 24.36^{\circ}\text{C}$, $n = 67$ days per block).

Survival to metamorphosis was higher in high plant food than in low plant food quantity treatments and in low tadpole density than in high density treatments (Table 5.2, Fig. 5.3). One or more metamorphs were collected from 72% of basins (46 of 64). There was an interaction between plant food and density factors, with highest survival in high plant food, low density treatments and lowest survival in low plant food, high density treatments (Fig. 5.3). Survival to metamorphosis did not differ between animal food or relatedness treatments (Table 5.2). A higher proportion of tadpoles died of unknown causes in low plant food quantity than in high plant food quantity treatments (Fig. 5.4; $X^2 = 39.187$, $df = 1$, $P < 0.001$). Density was a marginally important factor ($X^2 = 3.589$, $df = 1$, $P = 0.058$), but animal food and relatedness did not influence ($X^2 = 0.100$, $df = 1$, $P = 0.752$; $X^2 = 0.231$, $df = 1$, $P = 0.631$, respectively) the proportion of dead tadpoles.

Total metamorph biomass was about five times larger in the high plant food, low density treatment compared to the three other combinations of density and plant food (Table 5.2, Fig. 5.3). Biomass was smallest in the low plant food, high density treatment, creating an interaction between plant food and tadpole density factors (Table 5.2). There was a trend towards larger total biomass in treatments with animal food compared to

Table 5.2. ANOVA results for survival to metamorphosis and ANCOVA results for total biomass with all main effects and the covariate half survival; interactions are shown if $P < 0.10$.

Response variable	<i>F</i>	df	<i>P</i>
Survival to metamorphosis			
Density	142.748	1,63	<0.001
Plant food	157.410	1,63	<0.001
Animal food	1.435	1,63	0.237
Relatedness	0.876	1,63	0.354
Plant food x Density	63.271	1,63	<0.001
Plant food x Relatedness x Density	2.960	1,63	0.092
Biomass			
Day 35 survival (covariate)	1.595	1,63	0.213
Density	91.306	1,63	<0.001
Plant food	107.622	1,63	<0.001
Animal food	1.724	1,63	0.196
Relatedness	1.420	1,63	0.239
Plant food x Density	48.907	1,63	<0.001

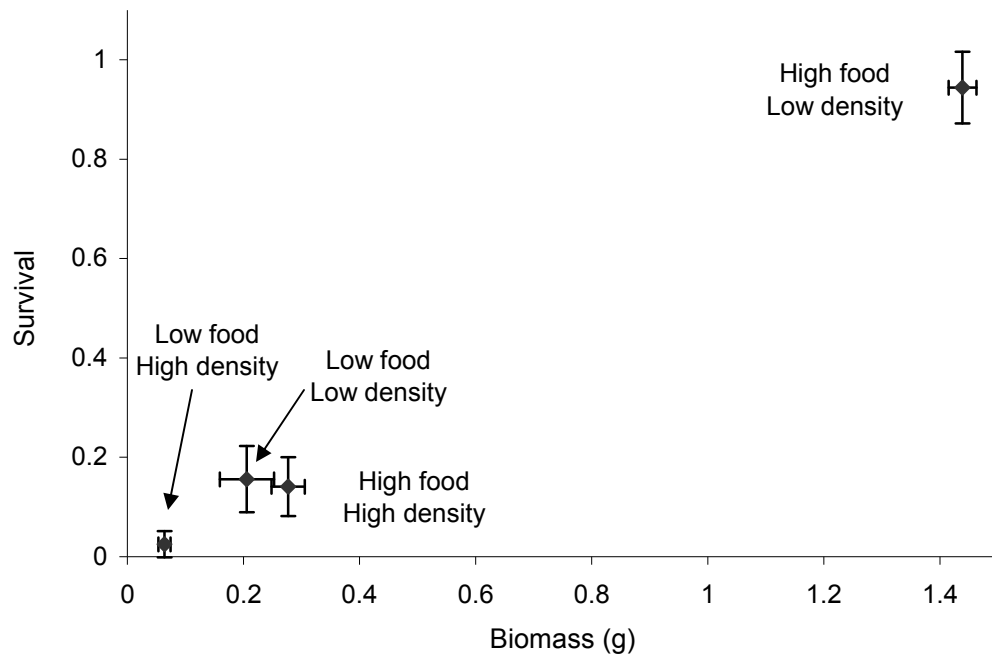


Figure 5.3. *Smilisca phaeota* total biomass (g) and survival (mean \pm SE) in different plant food and density treatments.

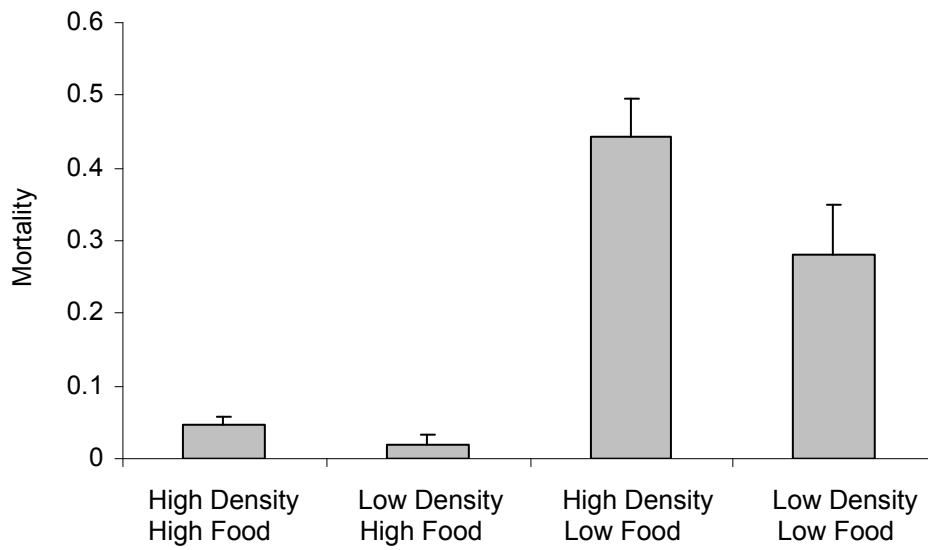


Figure 5.4. The proportion (mean \pm SE) of *S. phaeota* tadpoles that perished during the experiment in different plant food and density treatments.

those without animal food (at equivalent density and plant food amounts), but this trend was not statistically significant.

Metamorph mass was largest in treatments with low tadpole density and high plant food treatments (Fig. 5.5a,b, Table 5.3). Metamorphs in high plant food, low density treatments were 50% larger than those in high plant food, high density treatments. Additions of animal-based food and relatedness did not affect metamorph mass (Table 5.3). The survival covariate had a significant effect on metamorph mass in treatments with low tadpole density but not in treatments with high plant food (Table 5.3). Metamorphs in high plant food, low density treatments completed metamorphosis about 16 days earlier than those in low plant food, low density treatments (Fig. 5.5b, Table 5.3). Larval period did not differ between density treatments when plant food quantity was high. Additionally, larval period was not affected by the survival covariate, animal-based food, or relatedness factors (Table 5.3).

DISCUSSION

I have shown that many tadpoles are potential predators of hetero- and conspecifics in ephemeral pools in pastures in the Osa Peninsula, Costa Rica. Further, I identified prey and predator size and developmental stage as factors important to cannibalism intensity for *S. phaeota*. Additionally, tadpole density and plant food quantity influence *S. phaeota* tadpole performance and likely influence the incidence of cannibalism. In the following discussion, I consider how egg and tadpole carnivory may be common in ephemeral tropical pasture pools, how species differ in their ability to be predators and in their vulnerability as prey, and the factors that influence cannibalism

Table 5.3. Subset analyses of covariance at high plant food quantity (Subset 1) and low tadpole density (Subset 2). No interactions of factors yielded significant results. Metamorph mass and larval period were log-transformed prior to analyses.

	Metamorph mass			Larval period		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Subset 1						
Day 35 survival (covariate)	0.522	1,29	0.478	0.001	1,29	0.975
Density	68.995	1,29	<0.001	2.064	1,29	0.166
Animal food	0.310	1,29	0.584	0.064	1,29	0.802
Relatedness	0.004	1,29	0.948	0.005	1,29	0.944
Subset 2						
Day 35 survival (covariate)	10.862	1,25	0.004	0.959	1,25	0.341
Plant food	22.959	1,25	<0.001	20.355	1,25	<0.001
Animal food	0.067	1,25	0.799	0.763	1,25	0.395
Relatedness	0.029	1,25	0.867	0.039	1,25	0.846

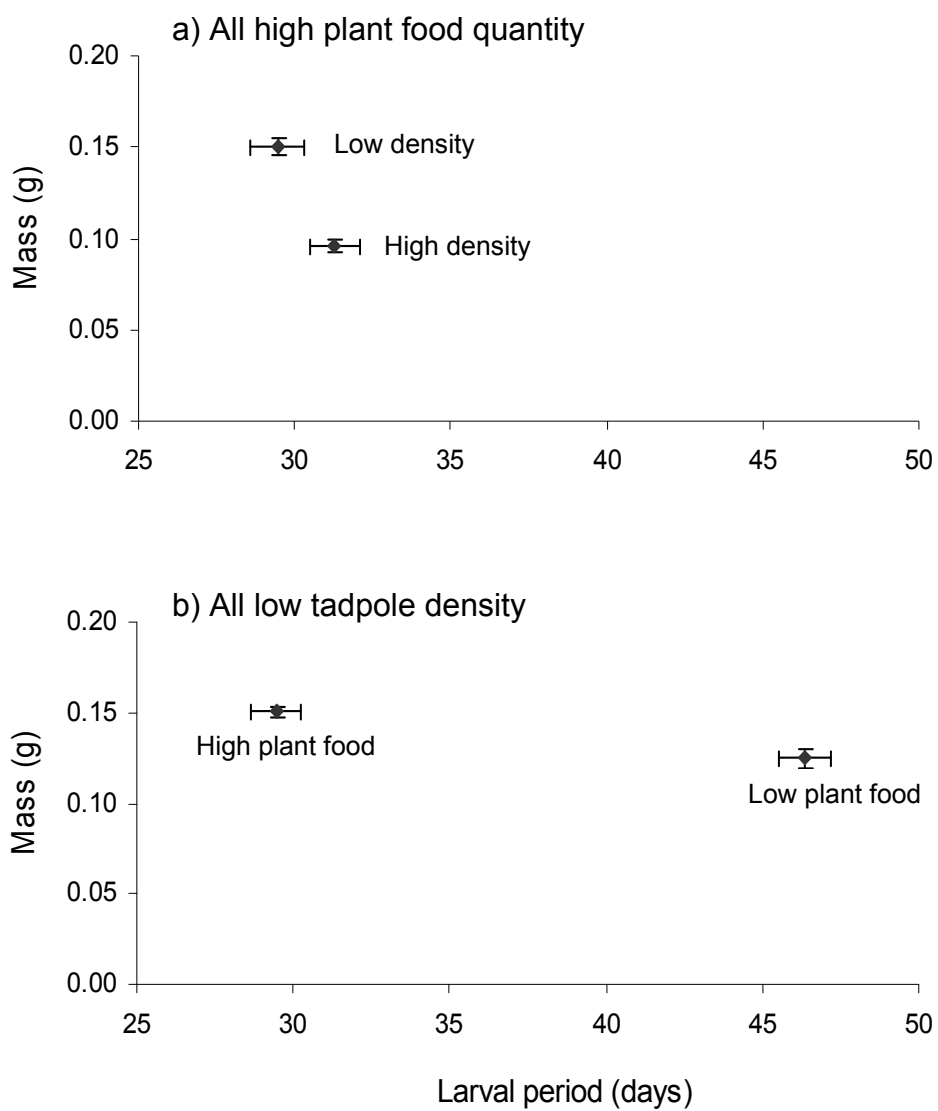


Figure 5.6. *Smilisca phaeota* metamorph mass and larval period (mean \pm SE) in different density and plant food treatments.

intensity. My results indicate that tadpole predators are capable of altering conspecific and heterospecific population sizes and in this manner may influence the composition of tadpole assemblages in pools in my study area.

Of eleven species that commonly occur in pasture pools within my study area, eight are known consumers of frog eggs and four of the eight also consume the hatchling or tadpole stages (Table 5.1). Seven of the eight species are known predators of more than one species of the eleven member assemblage. The three unstudied species are most common in water bodies with longer hydroperiods than the studied species (Table 5.1). To my knowledge, only one other study has examined tadpole predator-egg prey interactions among multiple species from pond assemblages (i.e., Magnusson and Hero 1991). The majority of those species, from undisturbed forest ponds with eight to nine month hydroperiods, consumed eggs and demonstrated variation in egg palatability and predator voracity (Magnusson and Hero 1991). Their study along with my current study suggest that facultative carnivory and cannibalism may be common foraging modes for tropical tadpoles and may involve multiple species represented within tadpole assemblages.

In my study, tadpole species differed in their preference and voracity for *S. phaeota* and *E. pustulosus* eggs. Egg and tadpole predation intensity in ephemeral pools may reflect variation in the ease of consumption as a result of differences in tadpole predator morphology (Crump 1992). Some oophagous species have reduced or no tooth rows, wide gapes, powerful jaws, and short digestive tracts (Noble 1929). *Smilisca phaeota* tadpoles have well-developed jaw sheaths (Savage 2002), but comparative morphological data are needed to understand how morphology influences the incidence

of carnivory among the studied species. *Smilisca phaeota* and *L. poecilochilus* showed more aggressive behavior and faster rates of egg consumption in comparison to *E. pustulosus*, suggesting that these species may have morphologies that facilitate carnivory. Differences in preference and voracity for eggs among predators will influence the relative abundance and composition of species in ephemeral pools through differential survival of new egg cohorts. Additionally, the first species to occupy a pool will have a survival advantage over others if it is predaceous.

Other factors that may influence the intensity that eggs and tadpoles are predated include the fecundity and reproductive mode of the prey species. The evolution of reproductive modes across several anuran families shows a trend towards independence from water (Goin 1960). Factors favoring this evolution could involve avoidance of competition and predation, and unpredictability in aquatic environments (Crump 1974, McDiarmid 1978). Additional driving factors could be the avoidance of cannibalism (Crump 1983) and heterospecific predation in ephemeral pools. *Engystomops pustulosus* and *L. poecilochilus* both construct foam nests which can provide at least some protection from predation (Downie 1990). *Leptodactylus poecilochilus* adults go a step further by constructing burrows at pool edges, where they deposit their eggs in foam nests (Savage 2002). The burrow likely isolates eggs from predators and offers protection from desiccation until the eggs or tadpoles are washed out (as with *E. pustulosus* (Downie 1990)). The initial preference *L. poecilochilus* tadpoles showed for *S. phaeota* eggs may be a result of the difficulty in penetrating the foam surrounding *E. pustulosus* eggs. Additionally, high fecundity, for example, that of *S. phaeota*, may act to reduce clutch mortality risk by satiating predator appetites. Ecological implications of species-specific

prey vulnerability to predation by tadpoles include variable relative abundance and composition of species in pools caused by differential mortality.

Cannibalism intensity was also dependent on predator and prey size and developmental stage, consistent with other studies (e.g., Crump 1983). Implications for tadpole populations in natural pools include the influence of oviposition timing on cohort survival. If a clutch is deposited in a pool that has a cohort already established, the new clutch may suffer complete mortality. Therefore, there may be selective pressures to be the first to colonize newly established breeding sites (priority effects) or to avoid ovipositing in pools that have established populations (Resetarits and Wilbur 1989).

Plant food quantity had a larger effect on the proportion of tadpoles that died during the experiment than tadpole density. Both low food availability and high tadpole density results in reduced larval health and increased aggression, leading to increased cannibalism in *Ambystoma macrodactylum* (Wildy et al. 2001). High tadpole density increases food competition resulting in increased stress that may favor cannibalism as an alternative food source. Although competition and non-lethal aggression were not measured in my study, large size variation among tadpoles in basins with high mortality was observed, suggesting intense competition. Stress caused by both low food quantity and high density could have led to a weakening of inferior competitors, a disparity in tadpole sizes, and eventual death followed by scavenging or predation of smaller tadpoles by larger individuals. The results of experiment 2 argue against the possibility that large-sized tadpoles consumed small-sized tadpoles. However experiment 2 did not test how the frequency of cannibalism is affected by density of individuals of the same age cohort.

Additional research is needed to determine whether individuals die of competition or of direct predation by larger tadpoles.

Smilisca phaeota metamorphs from basins where a large proportion of tadpoles died (suggesting a high incidence of cannibalism or scavenging) did not attain fitness benefits, such as larger mass or faster development, compared to other metamorphs. Low food quantity likely depressed tadpole growth and any increased cannibalism or scavenging was not sufficient to compensate for food deficiency. In addition, metamorphs from basins supplemented with conspecific eggs did not have a larger mass or shorter larval period. The lack of a treatment effect may be caused by an insufficient number of eggs added or a non-optimal time of egg additions. Cannibalistic *A. tigrinum* larvae did not consume siblings after the first 30 days of growth, suggesting that there may be a limited time period during which growth can be accelerated by conspecific consumption (Lannoo et al. 1989). Other studies have shown mixed results for how cannibalism influences developmental rate. For example, *Ambystoma tigrinum* cannibals developed slower (Rose and Armentrout 1976) or faster (Lannoo et al. 1989) than non-cannibals. Crump (1990) demonstrated that a cannibalistic diet does increase metamorph mass but does not change larval period duration in an experiment that controlled for the energy content and dry mass of food types. A similar experiment is needed to determine if cannibalism results in fitness benefits for *S. phaeota*.

My results suggest that *S. phaeota* tadpoles do not recognize siblings or that a reduction in inclusive fitness is offset by the benefits of cannibalism. *Spea bombifrons* and *S. multiplicata* are able to recognize kin and avoid cannibalism (Pfennig et al. 1993, Pfennig 1999). However, both of these species have cannibalistic morphs, specialized to

a carnivorous diet. Kin recognition may be less important in species with omnivorous diets.

I observed complex relationships between treatment factors and metamorph mass. Metamorphs from high density treatments were smaller than those in low density treatments and at low density, metamorphs from high plant food quantity treatments were larger than those in low plant food quantity treatments. Plant food quantity was the only treatment factor that had an effect on larval period, with tadpoles in treatments with low food quantity developing the slowest. This pattern is well known (Travis 1984, Leips and Travis 1994) but can be complicated by changes in food availability during the larval period (Wilbur and Collins 1973, Alford and Harris 1988).

Conclusions

My study supports the view that we need to reconsider the functional roles of tadpoles in aquatic systems (Petranka and Kennedy 1999). My results suggest that predation risk in ephemeral pools can be high, potentially comparable to that in permanent ponds, but caused by tadpoles rather than fish and insect larvae. In unpredictable environments, with conditions of high tadpole density, low food quantity and quality, and the possibility of desiccation, species with versatile foraging modes may be favored. My findings suggest that the morphology of predators, reproductive mode of prey, and size and developmental stage of both predators and prey influence predation intensity.

Currently, the study of tadpole functional roles is particularly urgent because of our poor understanding of the ecological consequences of amphibian population declines and extinctions (Altig et al. 2007). I add that such study is warranted because of the

creation of novel tadpole assemblages as a result of habitat loss and the spread of exotic species. Habitat fragmentation causes genetic and demographic changes at the population level, as well as alterations in trophic interactions, resulting in community level consequences (Hoffmeister et al. 2005). Heyer et al. (1975) postulated that anthropogenic activities in the Osa Peninsula would increase the amount of habitat available for *L. savagei*, a species with facultatively carnivorous tadpoles, and therefore, its population size should increase. My results indicate that tadpoles influence age and size structures of conspecific and heterospecific populations through differential predation and therefore, may influence the relative abundance and composition of tadpole species in pools.

Future studies should examine how tadpole foraging modes vary across habitat types and whether such behavior is correlated across situations as behavioral syndromes (Sih et al. 2004). This will allow the examination of whether land use change results in different risks of egg and tadpole predation and how species cope with such change. Future research should quantify the amount of nutrients available in pools and the ability of tadpoles to assimilate nutrients from different sources. Finally, studies should examine how these patterns translate into variation in the composition of species represented in tadpole assemblages in ephemeral pools in intact and human-modified habitats.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Amphibians are more threatened than other terrestrial vertebrates, with 37% of neotropical species at risk because of habitat loss, and more species at risk because of other factors (IUCN et al. 2006). Recent studies have highlighted the importance of understanding the spatial context in which amphibians respond to habitat modification and the mechanisms underlying amphibian occupancy patterns within modified landscapes (Cushman 2006, Gardner et al. 2007). I addressed these issues in my study conducted in the southeast Osa Peninsula, Costa Rica, where the loss and modification of forest have accelerated over the past 30 years (Sanchez-Azofeifa et al. 2002). Here, I highlight the conclusions of my study, discuss conservation implications, and suggest future work. The organization of this section follows that of the flowchart (Fig. 1.1) summarizing consequences of habitat modification for amphibians.

Patterns of habitat modification

I. Change in abiotic conditions

- Pastures have greater incident radiation (measured as global site factor) and less overstory canopy cover than edges or forest (Ch. 2, 3 & 4)
- Pastures have higher mean air temperature, lower mean relative humidity and greater daily variation in both variables than edges or forest (Ch. 2, 3 & 4)
- Artificial pools in pastures have higher mean water temperatures, greater daily variation in water temperatures, and higher dissolved oxygen content than pools in edges or forest (Ch. 3 & 4)

- Abiotic conditions (incident radiation, overstory canopy cover, air and water temperatures, and relative humidity) in edges and forest are similar (Ch. 2, 3, & 4)
- Streams in pastures are on average wider and deeper than streams in forest (Ch. 2)
- Small ephemeral pools are common in pastures but not in forest (Ch. 2)
- In a landscape mosaic of pasture and forest, forest cover surrounding sites in forest decreases as buffer zones increase in diameter, whereas forest cover surrounding sites in pasture generally increases as buffer zones increase in diameter (Ch. 2)
- In a landscape mosaic of pasture and forest, distance to forest-pasture edge may be longer for forest than for pasture sites (Ch. 2)

II. Change in biotic conditions

- Amphibian richness may not differ between forest and pasture habitats (Ch. 2)
- Amphibian composition differs between forest and pasture habitats (Ch. 2)
- Some frog species are restricted to either forest or pasture, whereas other species are found in both habitats (Ch. 2 & 3)
- Species may be more likely to breed in pasture pools located close to the forest edge than those located more distant from the forest edge (Ch. 3)
- In pasture pools, tadpoles that prey on eggs and tadpoles may be capable of altering conspecific and heterospecific population sizes, thereby influencing the composition of tadpole assemblages (Ch. 5)

III. Change in vital rates

- *Engystomops pustulosus* tadpoles survive equally well in pasture, edge, and forest but tadpoles develop faster and attain a larger metamorph size in pasture than in edge or forest (Ch. 3)
- *Dendrobates auratus* tadpoles survive poorly in pasture compared to edge and forest, rate of tadpole development does not differ among habitats, and metamorphs are largest in edges (Ch. 3)
- *Agalychnis callidryas* tadpole survival is lower in pasture but tadpole development is faster and metamorphs attain a larger size in pasture compared to edge or forest (Ch. 4)
- *Dendropsophus ebraccata* tadpole survival is high in all habitats, but tadpole development is faster and metamorphs are larger in pasture than in edge or forest (Ch. 4)
- Breeding habitat that adult frogs select along forest-pasture transects varies by species and site selection is consistent with high tadpole performance for *Engystomops pustulosus* and *Dendrobates auratus* (Ch. 3)

Species-specific traits

I. Behavior

- Breeding site selection by adult frogs may be influenced by whether abiotic conditions prohibit or facilitate their movement through forest and pasture (Ch. 3)
- Species of tadpoles in ephemeral pools in pasture differ in their voracity as predators of hetero- and conspecific eggs and tadpoles (Ch. 5)

- Species of eggs and tadpoles in ephemeral pools in pasture differ in their vulnerability as prey to other tadpoles (Ch. 5)
- If the first species to colonize ephemeral pools in pastures is predaceous during the tadpole stage, it may gain survival advantages over later-colonizing species because it will have more food options (Ch. 5)

II. Life history and ecology

- Compared to species occupying forest, those occupying pasture have larger body sizes, larger mean clutch sizes, larger geographic distributions, and reproductive modes with greater dependency on water (Ch. 2)
- Species capable of high tadpole performance (survival, growth, and development) in pastures may also perform well in forest, but species that have high performance in edge and forest may not perform well in pasture (Ch. 3)

III. Physiology

- Abiotic conditions in pasture may be tolerable or intolerable to adults (Ch. 3) and tadpoles depending on the species (Ch. 4 & 5)
- High water temperatures in pasture pools may exceed the critical thermal maxima of some species resulting in low tadpole survival (e.g., *Dendrobates auratus*; Ch. 3 and *Agalychnis callidryas*; Ch. 4)
- High water temperatures in pasture pools may be tolerable for some species, improving their performance and resulting in fitness benefits during later life stages (Ch. 3 & 4)

Mechanisms for model species

Here I will state the mechanisms supported by my data for the species featured in my experiments. The mechanism underlying occupancy patterns of *Engypstomops pustulosus*, a pasture-specialist, and species with similar life histories (*Leptodactylus bolivianus* and *L. poecilochilus*) involves increased air and water temperatures in pasture compared to edge and forest (Fig. 6.1). Species-specific traits that contribute to the mechanism include adult and tadpole behavior, adult life history and ecological traits, and tadpole physiology. Adults selected breeding pools in pasture and tadpoles consumed conspecific and heterospecific eggs. These species have large clutch sizes and deposit their eggs in foam nests. I measured high tadpole performance in pasture, edge, and forest habitats, suggesting that the physiology of these species is flexible. However, development was fastest and metamorphs were largest in pasture, likely leading to population growth in pastures.

The mechanism underlying occupancy patterns of *Dendrobates auratus*, a forest-specialist, and species with similar life histories (*Phyllobates vittatus* and *Allobates talamancae*) also involves increased air and water temperatures in pasture compared to edge and forest (Fig. 6.2). These species selected breeding pools exclusively in forest and have small clutch sizes, a reproductive mode that requires terrestrial egg development, and small geographic distributions. I measured decreased tadpole survival and growth in pasture compared to edge and forest, suggesting that the physiology of these species is inflexible. Overall, my data suggest that these species are not capable of survival in a matrix habitat such as pasture, leading to the restriction of their populations to forest patches.

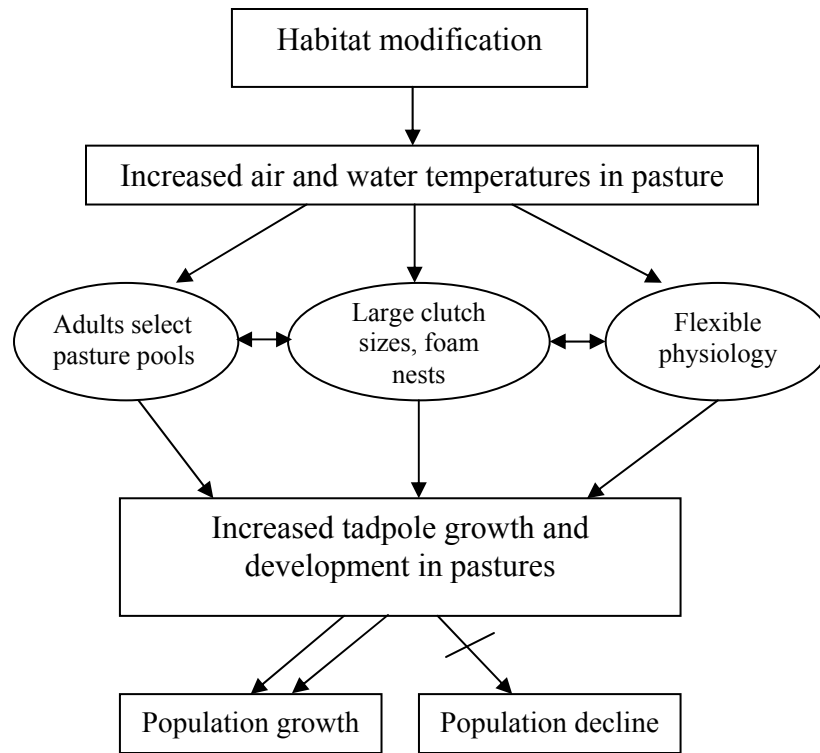


Figure 6.1. The proposed mechanism leading to population growth in pastures for *Engystomops pustulosus* and species with similar life histories, such as *Leptodactylus bolivianus* and *L. poecilochilus*.

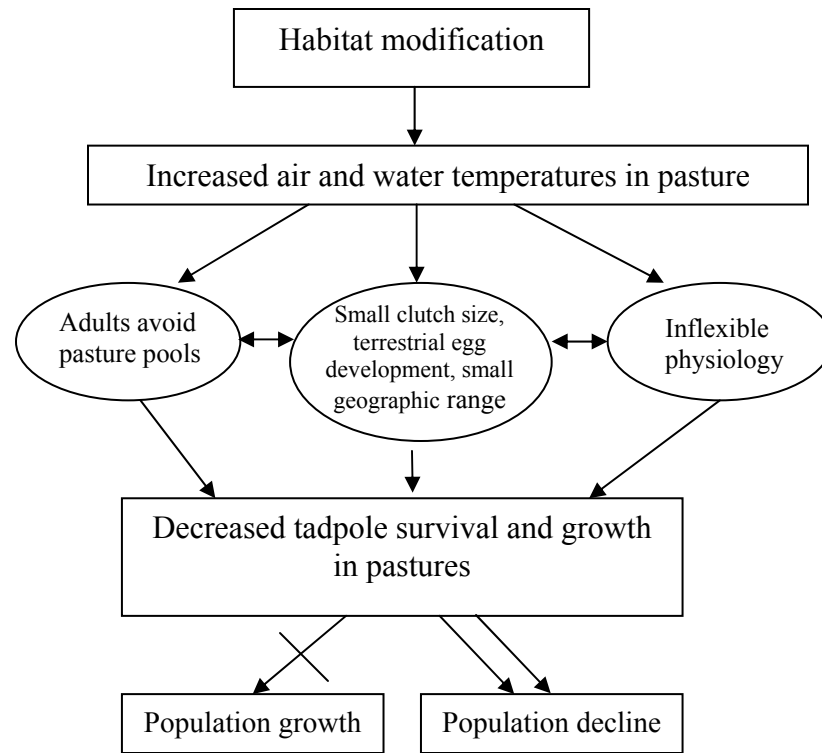


Figure 6.2. The proposed mechanism leading to population decline in pastures and restriction to forests for *Dendrobates auratus* and species with similar life histories, such as *Phyllobates vittatus* and *Allobates talamancae*.

Conservation implications

Results of my study have several implications for amphibian conservation in the tropics. The quality of matrix habitats influences the performance and persistence of species. Pastures can support some amphibian species during the egg, tadpole, and adult stages. Therefore, pasture matrix habitats contribute to regional frog diversity, perhaps most effectively when isolated trees and forested corridors along streams are present. Conservation managers should work with policy makers to improve existing land-use regulations and with land owners to educate about the importance of these regulations for maintaining amphibian diversity.

Selection of breeding sites by adult frogs was associated with high tadpole performance. Therefore, presence of adults in pasture and use of breeding pools there indicate that pastures are suitable for sub-adult life stages. This supports conclusions of studies that equate adult frog presence in habitats to the ability of the habitat to meet the resource needs of the species. If my conclusions can be generalized to include other systems, conservation managers may be able to use adult frog presence as a proxy for habitat suitability for species with confidence.

Habitat configuration was important to both species occupancy and movement patterns. The occurrence of species detected only in pastures was negatively associated with forest cover on small spatial scales, indicating that pasture-restricted species may be dependent on the presence of forest at larger spatial scales. In contrast, the occurrence of species detected mainly or exclusively in forest was associated with forest cover on small and large spatial scales, suggesting that these species require large tracts of forested land. Therefore, forest may be vital to species restricted to pasture or forest but at different

spatial scales. For forest-restricted species, the matrix must be managed to encourage frog movement among forest fragments, by improving the quality of forested corridors, especially along streams and rivers.

Finally, my study highlights the need for conservation managers to have access to data on life history and ecological traits of each species when preparing land-use plans. The acquisition of such knowledge is crucial for the conservation of amphibians and it deserves additional effort, especially in tropical regions. Life history traits, such as clutch size and reproductive mode, are useful predictors of the tolerance of a species to matrix habitats. If my findings can be generalized to other assemblages of frogs in other habitats, conservation managers will be able to predict how species composition will change as a result of habitat modification, and they will be able to better focus their efforts to protect particular species after habitat modification has occurred.

Future work

I intend to extend my dissertation research in several directions. First, I will examine the usefulness of ecological traits in predicting response to habitat modification by conducting a meta-analysis of published studies that compare species diversity in modified and intact habitats. Second, I will continue experiments that examine the diets of tadpoles to determine whether carnivory occurs with increased incidence in human-modified habitats. I will use stable isotopes to examine the incidence of carnivory among tadpoles in natural pools in forest and pasture. Finally, I will collaborate with others to model the dynamics of populations of the model species in my study in different habitats. In order to determine the long-term consequences of habitat modification for particular species, it is necessary to understand the relative contributions of each life stage to

overall population growth in different habitats (Biek et al. 2002). This will allow conservation managers to prepare effective strategies to protect species with declining populations by targeting the most vulnerable life stages.

Appendix. Species detected in 82 forest and pasture sites in the Osa Peninsula, Costa Rica. For each species, the index of occurrence in pasture (n = 39 sites in pasture and n = 39 sites in forest after standardization), and ecological and life history traits are given. Literature sources are provided for clutch size; sources for other traits are described in the text.

Species	Family	Occurrence in pasture	Reproductive habitat ^a	Reproductive mode ^b	Body size (mm)	Geographic		
						distribution (km ²)	Clutch size	Source
<i>Rhinella marina</i>	Bufonidae	1.00	5	1	175	11320528	5000	Zug 1983
<i>Eleutherodactylus diastema</i>	Brachycephalidae	0.03	1	5	24	104339	15	Savage 2002
<i>Craugastor fitzingeri</i>	Brachycephalidae	0.25	1	5	53	274642	65	Savage 2002, Quijoano et al. 2002
<i>C. rugosus</i>	Brachycephalidae	0.00	1	5	69	9735	65	estimated based on <i>C. fitzingeri</i>
<i>C. stejegerianus</i>	Brachycephalidae	0.00	1	5	22	17887	15	estimated based on <i>E. diastema</i>
<i>Leptodactylus bolivianus</i>	Leptodactylidae	1.00	5	3	120	6234976	1500	Savage 2002
<i>Leptodactylus poecilochilus</i>	Leptodactylidae	1.00	2	3	40	240804	161	Hawley unpubl. data
<i>Leptodactylus savagei</i>	Leptodactylidae	0.50	5	3	185	6104991	1000	Muedeking and Heyer 1976
<i>Engystomops pustulosus</i>	Leiuperidae	1.00	2	3	35	897849	265	Savage 2002
<i>Agalychnis callidryas</i>	Hylidae	0.33	3	2	71	519236	58	Savage 2002
<i>Dendropsophus ebraccata</i>	Hylidae	0.60	3	2	35	220739	156	Savage 2002
<i>Dendropsophus microcephala</i>	Hylidae	1.00	3	2	31	5594718	133	Roberts 1994
<i>Hypsiboas rosenbergi</i>	Hylidae	0.90	5	1	82	165072	2400	Kluge 1981
<i>Scinax elaeochrous</i>	Hylidae	1.00	2	2	40	45265	326	Roberts 1994
<i>Smilisca sordida</i>	Hylidae	1.00	4	1	64	48779	295	Hawley unpub. data
<i>Centrolene prosoblepon</i>	Centrolenidae	0.00	4	2	31	324437	20	Savage 2002
<i>Cochranella granulosa</i>	Centrolenidae	0.35	4	2	32	87034	55	Savage 2002
<i>Hyalinobatrachium colymbiphyllum</i>	Centrolenidae	0.00	4	2	27	123696	63	Savage 2002
<i>Cochranella pulverata</i>	Centrolenidae	0.37	4	2	33	135350	62	Savage 2002, Hawley 2006
<i>Allobates talamancae</i>	Aromobatidae	0.00	2	4	18	116005	9	Junca et al. 1994
<i>Phyllobates vittatus</i>	Dendrobatidae	0.00	2	4	24	3885	18	Savage 2002
<i>Dendrobates auratus</i>	Dendrobatidae	0.00	2	4	42	111944	7	Savage 2002

^a Reproductive habitat: 1 = terrestrial, 2 = ephemeral pools, 3 = permanent pools, 4 = streams, 5 = > 1 habitat type.

^b Reproductive mode: 1 = eggs deposited in water, 2 = eggs on vegetation over water, 3 = eggs in a foam nest, 4 = eggs laid on ground, tadpoles transported to a water body, 5 = eggs laid on the ground and undergo direct development

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