Project Anuran Phase II: Preliminary Report

An ecological research project concerned with the assessment and monitoring of anuran populations in the region around Las Cuevas, Chiquibul Forest Reserve, Belize

Phase II : Preliminary Report

BY

Toby Gardner, Matthew Bol, Fiona Bucknill, Norberto Coc, Emily Fitzherbert and Neil Hart

September 2001

Project Anuran
Institute of Ecology and Resource Management
University of Edinburgh
Darwin Building
Kings Buildings
Mayfield Road
EH9 3JU
Edinburgh, Scotland
Email: projectanuran@hotmail.com
Website: www.projectanuran.org

Summary

In the last 20 years the scientific community has become increasingly concerned following a growing number of reports documenting global amphibian declines. Observations of frequently catastrophic declines in areas of the world previously thought to be undisturbed by man, has led to the suggestion that human-induced global agents are responsible. The fact that amphibians play an integral role in the natural stability and functioning of many ecosystems, points to the potentially drastic consequences of large scale population declines. Furthermore, due to their bi-phasic lifestyle, existing in both aquatic and terrestrial habitats, coupled with their highly permeable skin, they can act as excellent indicators of environmental health and resilience - a fact that bears worrying implications for humans in light of their increasing demise. In order to resolve the phenomenon of declining amphibian populations, and consequently take appropriate conservation action, their is a desperate need for long-term and intensive monitoring programs. Two of the most important areas in which we lack an adequate understanding, is in the separation of natural and stochastic population fluctuations from real population declines, and secondly, the relative importance of local environmental factors over regional or global influences in determining amphibian population dynamics and reproductive activity. The aim of Project Anuran is to undertake a comprehensive monitoring program of an entire community of amphibians across a suite of study sites, in the region of the neotropical research station at Las Cuevas, Belize. This report presents a preliminary overview of field work conducted during the second phase of the project. Intensive surveillance of monitoring sites established in 2000 has provided further information on diversity and relative abundance, and patterns of vocalisation activity of local populations. Comparison of data between years will allow for an improved understanding of the both the temporal and spatial variability in species assemblage composition, population size, and reproductive activity. Alongside an insight into the results collected during Phase II, an outline is given as to the future project output and directions of data interpretation. It is re-emphasised that undergraduate research projects stand in a strong position to provide the necessary effort, resources, expertise, and enthusiasm required to provide the thorough ecological assessments that are needed to understand, predict and ultimately prevent the loss of amphibian populations.

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Acknowledgments

This project could never have moved beyond conception if it was not for a huge amount of help and support from a great number of people, trusts and institutions. The members of the project are extremely grateful to the very generous support of the following benevolent organisations, trust funds, companies and individuals. If it was not for the existence of such bodies, this project (nor any other similar) could never have become a reality: **Barnson Bequest (UoE), British Ecological Society, Davis Expedition Trust (UoE), Edinburgh Trust No.2, Gichrist Educational Trust, James Rennie Bequest (UoE), Life Support Training Services, Royal Geographical Society, Royal Scottish Geographical Society, Weir Fund for Field Studies (UoE).**

A great number of individuals have been instrumental in the development and success of this project. Our achievements are greatly attributable to the immense support and encouragement that we found wherever advice was sought. Thanks go to all the staff of the RGS Explore 99 for giving us the initial inspiration. A great number of people have gone beyond their call of duty to offer advice and support, particular thanks goes to Mr John Anthony and the staff of the photography lab in the Darwin building, Dr Sutton, Dr Knapp and Dr Penn of the Museum, Dr Aulay Mackenzie of the University Essex, Professor John Grace, Dr Graham Russell, and Mrs Margaret Jackson of the University of Edinburgh. We are especially indebted to Mr Peter Stafford of the Natural History Museum (London), Professor Julian Lee of the University Miami, Dr Jack Meyer of the MAYAMON (DAPTF), and Mr John Wilkinson (DAPTF) for their considerable advice on herpetological matters and their kind patience in the face of ceaseless emails and pleas for help. Professor Peter Furley of the University of Edinburgh gave unstinting support throughout the development and execution of the project, to him we are especially grateful. For most welcome assitance in the field as well as amusing companionship we are grateful to Hedley Twiddle, Marion Laurenceau and Anja Fiedler. Our time in Belize was made immensely enjoyable and rewarding thanks to the considerable efforts of the staff of the research station; Mr Chris Minty, Mr Nicodemus (Chapal) Bol, Mrs Celia Bol, and Mr Enrique Saquil. To all the above and countless more unnamed people, thank you.

Introduction

Human alteration of the global environment has triggered what is widely regarded as the sixth major extinction event in the history of life (Chapin *et al.* 2000). In the majority of cases proximal causes of species extinction can easily be identified, and reports are concentrated in areas most severely affected by man's activities. It is therefore with particular alarm that scientists have been increasingly reporting global declines in populations of one particular taxonomic group - the amphibians, in areas previously thought to be intact from human disturbance. The first such declines, and perhaps some of the most widely cited examples are those of the Golden Toad - Costa Rica (*Bufo periglenesi*), Harlequin Frog - Costa Rica (*Atelopus varius*) (Crump *et al.* 1992; Pounds & Crump 1994), the Cascade's Frog - California (*Rana cascade*) (Fellers & Drost 1993), and the Yellow and Red-legged frogs - California (*Rana mucosa* and *Rana aurora*) (Blaustein & Wake 1990). Highlighting the potential susceptibility towards population declines of the entire taxonomic group are reports of both reductions in population size as well as local extinction events occuring at the regional level across whole communities of amphibians; Atlantic forests of Brazil (Heyer *et al.* 1988), Central Valley California (Drost & Fellers 1996; Fisher & Shaffer 1996), Eastern Australia (Laurance *et al.* 1996), and the Monteverde forests of Costa Rica (Lips 1998; 1999). The candidate agents proposed to explain such declines are varied, but include; habitat modification (Alford & Richards 1999); UV-B radiation (Blaustein *et al.* 1994; Anazalone *et al.* 1998; Crump *et al.* 1999), environmental acidification (Freda & Dunson 1986; Beebee 1990), predation following exotic introduction (Fellers & Drost 1993; Hecnar & M'Closkey 1996), disease (Laurance *et al.* 1996; Lips 1998, 1999), and global climate change (Pounds *et al.* 1999; Pounds 2001). However, recent advances in research suggest that the majority of observed declines are unlikely to be the result of a small number of independent global agents, but rather a complex interaction of local processes in the context of human-induced environmental change (Pounds 2001; Kiesecker *et al.* 2001). Cause for concern following such reports of amphibian declines comes from two main justifications. Firstly, amphibians are integral components of many ecosystems, playing key roles in the maintenance of trophic dynamics, nutrient cycling and other fundamental ecosystem functions (Woolbright 1991; Beebee 1996; Pearman 1997). Secondly, and perhaps of more immediate concern for humans, is that due to their unique ecology and physiology amphibians are recognized to be excellent indicator species of environmental health and resilience (Blaustein & Wake 1990; Diamond 1996). The fact that amphibians as a group have remained largely unchanged since the era of the dinosaurs implicitly highlights man's overwhelming impact on the global environment, and emphasizes what may be potentially disastrous consequences if their demise continues unabated.

The first serious recognition afforded by the scientific community to the potential for a global decline in amphibian populations was in the establishment of a Declining Amphibian Populations Task Force under the Species Survival Commission of the IUCN in 1990 (Wake 1991). The following decade has not witnessed any abatement in the concern expressed in either the scientific (Wake 1998) nor the public community (Morell 2001). Despite this concern there are three main questions surrounding amphibian declines in which a large degree of uncertainty remains; (1) How to determine real declines from natural and stochastic population fluctuations? (2) Whether human-induced agents can be isolated as the potential cause of declines? (3) Whether global agents are responsible for the majority of observed declines? Through assessing the natural levels of variability inherent in amphibian populations across not only the temporal but also the spatial and taxonomic scales Project Anuran aims to contribute the answering of Question 1 - to improve our ability to predict and resolve real population declines as separate from natural fluctuations. Furthermore consideration of both a suite of monitoring sites and focal species, allows an insight into the third question - the relative strength of local environmental and biotic factors over more regional or global influences in determining amphibian population dynamics and reproductive activity. In light of increasing demands on available resources it is essential that we understand such phenomenon so as not to invoke unnecessary conservation and management action - a result that could also severely compromise support for conservation action in other, real situations (Pechmann *et al*. 1991).

Project Anuran is an undergraduate long-term research project, representing the collaborative efforts of biological science students in both the University of Edinburgh, Scotland and in Belize. Through the intensive monitoring of an entire anuran (Amphibia: Anura) fauna at the tropical site of Las Cuevas, Belize, we aim to contribute to the general goals outlined above by providing information on the population dynamics, reproductive behaviour, and environmental requirements of all local species throughout each wet season. Our study serves to complement existing work by concentrating on both an area and species assemblage that remain largely unstudied. Due largely to the spatial distribution of amphibian biologists, their remains a desperate need for studies in the diverse tropics (Wake 1991; Pearman *et al*. 1995; Wake 1998; Houlahan *et al*. 2000), and in particular the neotropics where amphibian diversity reaches its peak (Lee 1996). Although notable work has been done in high altitude neotropical sites (e.g. Guyer 1990; Lips 1998), very little has been done in lowland forest regions - of which Belize contains some of the most pristine and extensive stretches in Central America (Furley 1998). However, despite this the ecology of many amphibian species in Belize remains largely unknown (P.J.Stafford[1] pers comm, J.R. Meyer[2] pers. comm.). The DAPTF are presently co-ordinating a monitoring program entitled the Maya Forest Anuran Monitoring Program (MAYAMON), as part of a large, internationally funded biological monitoring project of the entire Selva Maya region (Carr & de Stoll 1999). The information collected by Project Anuran is fed into a regional picture collated by MAYAMON using data from studies throughout Belize, the southern states of Mexico, and the Péten of Guatemala. Our study site is able to provide a good comparison against many more disturbed areas in other parts of the region. However, we feel that perhaps our most significant contribution to an increased understanding of the declining amphibian phenomenon, is due to the fact that undergraduate projects such as ours are able to offer a considerable amount of time, personnel, and resources to allow for an effective comprehensive monitoring program - an achievement that is beyond the reach of many professional ecologists for very practical reasons of time and logistics. Although ideally, monitoring projects need to cover at least a few adult generations, both funding constraints and the urgency of the situation often preclude this approach. It is therefore optimal to have a program which focuses on a whole community of amphibian species, over a number of breeding sites, and incorporates consideration of detailed abiotic and biotic environmental variables on a frequent basis.

During Project Anuran Phase I (2000) standard protocols of vocalisation surveys, and nocturnal visual searches were used to collected detailed data on the diversity, relative abundance, and habitat associations of anuran populations local to Las Cuevas. Detailed surveillance of the temporal patterns of reproductive activity provided an insight into the temporal partitioning of peak vocalisation activity. This data provides an excellent baseline against which future population assessments can be compared. During Project Anuran Phase II (2001) we have collected data on the same populations across the established monitoring sites. The quality of data output has been improved due to a significant increase in the number of site survey repeats. Furthermore the addition of two extra vocalising species sites has allowed for an increased understanding of the ecology of the focal amphibian community. Due to the fact that the leaf litter anurans are extremely difficult to assess quantitatively (Pearman *et al*. 1995), a number of permanent transect lines were established to allow comparison between alternative methods of monitoring for this group (plots, traps and transects). The purpose of this preliminary report is to

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[1] Mr Peter Stafford, Research Biologist, Natural History Museum (London)
[2] Dr Jack Meyer, Co-ordinator DAPTF Maya Forest Anuran Monitoring Program (MAYAMON)

provide an insight into the nature of the data collected during Phase II, a comparative overview between results from the two years, and an outline of the future output of Project Anuran.

Aims and Objectives

Research Aim

"To contribute to the understanding of the ecology and conservation of amphibian populations via an intensive monitoring program of the anuran (Amphibia: Anura) fauna at the neo-tropical site of Las Cuevas, Belize (N16º44' W88º59')."

Research Objectives

1. To monitor changes in assemblage composition, relative abundance, and nocturnal patterns of vocalisation activity of vocalising anurans, throughout the wet season, across nine breeding sites in the vicinity of Las Cuevas.

2. Further to Objective 1, to monitor differences in abiotic and biotic environmental variables (weather, flora, physical pond attributes), between breeding sites, species assemblages, and across time.

3. Using a number of complementary methods, to monitor the diversity, relative abundance, and habitat associations of leaf litter and stream dwelling species. Further to consider the relative effectiveness of standard methods used to assess these groups

Further Aim

"To consider efforts to establish firm links with a collaborative student group from Belize, with an aim to consolidating closer ties between the student communities from both countries who are concerned about ecology and conservation management in Belize."

Methodology and Approach

Site description

Our work is conducted in the vicinity of Las Cuevas Forest Research Station (N16º44' W88º59'), Belize. The station is maintained as a joint initiative between the Natural History Museum (London) and the Forestry Department of Belize, and is situated in the Chiquibul Forest Reserve, north of the main Maya Mountains divide. It stands at roughly 500 m a.s.l. with an annual rainfall of between 1500 and 2000 mm, with vegetation consisting of mostly lowland subtropical moist forest (Hartshorn *et al.* 1984). Since the collapse of the Mayan civilisation c. AD 750-1000 there have been no permanent inhabitants of the region around Las Cuevas, although recent years have seen temporary disturbance due to logging, chicle harvesting, and the natural impact of hurricanes.

Our methodological approach falls under two directions; that for vocalising species (Field Study 1), and that for non-vocalising species (Field Study 2).

Field Study 1: Vocalising species

Most anuran species, especially in the tropics congregate *en masse* around breeding sites (Duellman & Trueb 1994; Beebee 1996), making the monitoring of vocalising choruses often the most effective technique by which to assess a population (Alford & Richards 1999). Nine breeding sites were used, representative of a number of different microhabitats around Las Cuevas. Seven of these sites represent the original sites established during Phase I[3], whilst two more were added during the present stage. Pond site selection was made following advice from local guides and night exploration of the forest. Each pond was surveyed at least 5 times (with the exception of Puzzle Pond which was dry for most of the season), at roughly even intervals throughout the study period of 13th July to 3rd September 2001. Each survey ran from 1900 hours until at least 0200 hours, depending on the duration of frog reproductive activity. Measurements taken at the start of each survey night included; depth of pond, diameter of longest dimension, pH water sample, and a summary weather report. Further environmental measurements were taken every half hour from 1900, including air temperature, water temperature, and duration of rainfall during the previous half hour. These environmental measurements follow standard recommendations for monitoring amphibian species (Heyer *et al.* 1994), the MAYAMON protocol (Meyer 1999), and a number of recent monitoring projects analogous to ours (e.g. Guyer 1990; Gascon 1991). Alongside the environmental measurements half hourly recordings were made of the diversity, relative abundance and calling activity of all frog species present. Measures of abundance followed the MAYAMON protocol (Meyer 1999). This method uses audible abundance Vocalisation Categories (VC) to assess the relative abundance of each species during the first fifteen minutes of each hour. It is important to note here that although these categories are quite broad, they allow for a rough but *accurate* and *repeatable* relative measure of the number of frogs of each species that are present. It is important here not to sacrifice accuracy for numerical precision - i.e. it is better to be roughly accurate than incorrectly precise. Measures of vocalisation activity of each species were also recorded to allow for an assessment of the temporal dynamics of reproductive activity. This was done using a measure (analogous to the above) of Vocalisation Intensity (VI) - the frequency of calls of an average individual of each species from across the first fifteen minutes of each half hour. One call was taken as each noticeably distinct vocalisation per individual, a definition which serves well for the majority of species, but with some notable exceptions. Identification of vocalisations followed a CD produced during Project Anuran Phase I, and an audio cassette produced by J.R.Meyer and J.C.Lee[4]. Observer teams per survey night were selected at random to minimise the contribution of observer bias to the results. Note that in comparison to Phase I, the intensity of the monitoring regime is increased in both the number of survey nights and the frequency of recordings per survey night.

Vocalisation Category (VC)	**VC definition**	**Vocalisation Intensity (VI)**	**VI definition**
1	1-5 individuals	**A**	1 or <1 calls per 15 minutes
2	6-20 individuals	**B**	2-14 calls per 15 minutes
3	21-50 individuals	**C**	5-10 calls per 5 minutes
4	>50 individuals	**D**	2-5 calls per minute
		E	6-10 calls per minute
		F	11-20 calls per minute
		G	21-40 calls per

[3] One Phase I site, Cockspur Pond, was discarded from future surveillance due to the small number of repeat survey nights obtained during 2000, and its small physical size.
[4] Professor J.C. Lee, University of Miami, Florida.

			minute
		H	>40 calls per minute

Values observed for the entire night were; maximum and minimum temperature, duration and number of rainfall events, and phase of moon. For each pond an assessment of the floral diversity was recorded. This was done by mapping the area surrounding the pond and noting all tree species with a DBH > 10 cm within 10 m of the pond edge. Floral mapping of this nature allows an insight into the contribution that the local plant species composition has towards structuring the local amphibian species composition. Pond site individual and location maps are to be included in the Phase II main report.

In addition to the standard survey protocol outlined above, all anecdotal recordings of these species were noted separately, detailing location, time of day, species type and number, and any relevant natural history notes.

Field Study 2: Non-vocalising and leaf litter species

The monitoring of leaf litter and stream dwelling frogs is subject to a variety of methods the effectiveness of which can vary greatly between geographic locations, amphibian species assemblages and local habitat conditions (Pearman *et al.* 1995). To allow for a comparison in the effectiveness of different methods in assessing inter site variation in species composition and relative abundance, 6 permanent transect lines were established to compare to the random plot data collected during 2000. Transect lines were stratified around areas presumed to be of both high diversity (creeks and the river), and areas noted to be ecologically distinct in terms of forest habitat (see forthcoming main report). Each line was cut to be 500 m in length and 2 m in width. Transect lines were surveyed by two people (selected at random), who walked slowly down the line each disturbing the leaf litter / branches on one half of the transect. Search effort was standardised using a time restraint of one hour per transect, producing an average searching speed of 0.5 km per hour. All individuals sited were noted with respect to species, number, location (nearest 50 m), time and any relevant behavioral observations. Transects were surveyed both during the day and night, at intervals which were roughly evenly spaced throughout the study period. A minimum of 3 repeats was conducted per transect.

To complement the transect lines, four drift net fences were erected following the arrangement described in Heyer *et al* . (1994). Each array utilised thick polythene sheeting and a minimum of 6 19 litre buckets. The polythene sheeting was laid to incorporate a 2 inch trench margin, and at a height of 1.5 m. As for the transect lines the position of the four trap arrays was stratified with respect to areas of high species richness and the ecological distinctness of the local habitat. Identification of specimens for both transect lines and traps followed the relevant guides; Lee (1996), Meyer & Foster (1996), Campbell (1998), and Lee (2000).

In addition to the standard survey protocol outlined above, all anecdotal recordings of these species were noted separately, detailing location, time of day, species type and number, and any relevant natural history notes

Weather

Daily recordings were made at 0900 of rainfall volume and duration for the previous 24 hours, maximum and minimum temperature, relative humidity and cloud cover. These were taken using standard equipment from the weather station at the Las Cuevas site. To allow for consideration of the spatial variability in rainfall patterns, two more rain guages were established for the latter half of the project - at the Aguada (4 km East LCRS), and Millionario (5 km West LCRS).

Results: Initial findings

Overall species richness

Family	Species
Leptodactylidae	*Eleutherodactylus chac*
	Eleutherodactylus laticeps
	Eleutherodactylus sabrinus
	Eleutherodactylus rhodopis
Bufonidae	*Bufo campbelli*
	Bufo marinus
	Bufo valliceps
Ranidae	*Rana berlandieri*
	Rana juliani
	Rana vaillanti
Hylidae	*Agalychnis callidryas*
	Agalychnis moreletii
	Hyla ebraccata
	Hyla loquax
	Hyla microcephala
	Hyla picta
	Smilisca baudinii
	Smilisca cyanostica
Microhylidae	*Gastrophryne elegans*
Centrolenidae	*Hyalinobatrachium fleischmanni*
Rhinophrynidae	*Rhinoprhynus dorsalis*

Table 1: Total anuran diversity found at Las Cuevas during Phase II

Table 1 presents the overall species richness found present at Las Cuevas during Phase II. A total of 21 species represents one more than found during Phase I (2000), and once more confirms the site to be one of the most diverse throughout Belize with respect to amphibian fauna (Miller & Miller 1995). Two species were found during this phase that were not discovered during Phase I - *Hyalinobatrachium fleischmanni* and *Eleutherodactylus rhodopis*. The former is an unsurprising addition in light of the discovery of a creek during the present phase - as the species requires flowing water to breed (Lee 2000). However, *E.rhodopis* the Polymorphic Robber Frog is a somewhat more exciting addition, having been described by Meyer & Foster (1996) as having only been recorded once in Belize 50 years ago. Lee (2000) describes it as a poorly known uncommon species found in humid lowland forest characteristic of the Peten of neighboring Guatemala - suggesting that its absence from Belize records is perhaps more due to a lack of detailed studies in the Maya Mountains as opposed to any real ecological obscurity. One species *E.sandersoni* was found during Phase I but not again during the this phase. However, owing to a recent revision of the taxonomy of the *E.rugulosus* group (Campbell & Savage 2000), it is likely that the record from 2000 represents a mis-identification.

Field Study 1: Vocalising species

A total of 53 survey nights were undertaken during this field study, covering all nine monitoring sites, and amounting to 371 recorded hours. This represents an increase of 30 nights over Phase I due to an increase in man-power and concentration of effort on vocalising species over nocturnal plot searches. With respect to the aims and objectives of this project, the benefits of an increase in the number of survey repeats are considerable - in that it allows for an increased understanding and resolution of the natural levels of inter-night variation in the patterns of species assemblage, relative abundance, and both intra and inter-specific reproductive activity patterns.

Data has been collected in a number of different directions; with respect to patterns of diversity, relative abundance, vocalisation activity, and environmental associations. Furthermore, following the completion of this present phase all these factors can be compared between 2000 and 2001. This section serves to outline examples of interesting and important directions of data interpretation - directions which will be expanded and discussed in the forthcoming main report.

It is of interest to consider data on vocalising species both with respect to between site differences and with respect to between species differences (see Main Report Phase I[5]). Table 2 presents species presence and absence data from across all Phase II survey nights in a two-way species by site matrix. Analogous data from Phase I is included to allow for comparison. This table provides the potential for a number of different analytical considerations. With respect to breeding sites it identifies the variation in local species composition between the two years, and also the variation in the *extent* of a species presence at any one site (i.e. proportion of site survey nights found). It is interesting to note just from a preliminary insight that the diversity of each pond has increased at all sites during Phase II (equivalent at Warree Pond), and further that the number of different sites each species was recorded in increased for all species except *R.berlandieri*. In light of an increase in the number of site repeats this could be partly attributable to an improved 'coverage' of all potential active inhabitants at each site. However, it is also possible that significant interannual variation exists in breeding site utilisation by different species - support for which comes from the high number of repeats of particular sites, and accompanying anecdotal observations of species assemblages on non-survey nights. Consideration of variability in breeding site use is of considerable interest and importance in attempting to understand amphibian population dynamics, and as far as we are aware has seen little if any investigation in the tropics. Secondly, Table 2 provides an insight into spatial and temporal variability in species presence in the Las Cuevas area, across all study sites. A measure of the extent of the *temporal* presence of each

[5] The main report from Project Anuran Phase I is available on request for readers who do not already hold a copy.

species is given by the proportion of nights recorded, and at the local scale the proportion of nights recorded per pond site. Additionally, a measure of the extent of the *spatial* presence of each species is given by the proportion of total pond sites at which each species was recorded. It is important to note that a number of ponds (namely Millionario, Warree Pond and the Aguada) were surveyed for a particularly high number of repeats. This allows for a detailed consideration of these particular sites - useful for example in comparing environmental variables to vocalisation activity patterns without the confounding effects of different habitat types between different activity nights for any one species.

Advancing on from simple presence and absence comparisons, Table 3 provides a preliminary comparison of the relative abundance of each species between 2000 and 2001, in addition to comparing the temporal extent or persistence of nocturnal vocalisation activity. The sum of the maximum Vocalisation Categories recorded for each study site gives the closest indication of the relative abundance of reproductively active individuals of each species across all sites. It is clear from

	Tapir Pond	Millionario	Guave Pond	Brooke Pond	Warree Pond	Marshy Pond	Puzzle Pond	Aguada	Coral Pond	Total number ponds observed in	Total number of survey nights observed in
Agalychnis callidryas	4 (0)	4 (0)	6 (3)	4 (n/a)	6 (3)	5 (3)	4 (3)	7 (3)	5 (n/a)	9 (5)	43 (15)
Agalychnis moreletii	0 (0)	0 (0)	1 (0)	3 (n/a)	5 (3)	3 (0)	4 (3)	6 (3)	4 (n/a)	7 (3)	25 (9)
Hyla loquax	5 (3)	7 (2)	4 (2)	2 (n/a)	0 (0)	3 (3)	0 (2)	2 (3)	4 (n/a)	6 (6)	22 (16)
Hyla microcephala	5 (0)	5 (1)	3 (2)	1 (n/a)	0 (0)	3 (3)	0 (0)	0 (0)	0 (n/a)	5 (3)	16 (7)
Hyla picta	3 (1)	1 (0)	3 (2)	0 (n/a)	0 (0)	3 (2)	1 (0)	0 (0)	0 (n/a)	5 (3)	11 (5)
Hyla ebraccata	0 (0)	0 (0)	0 (0)	0 (n/a)	0 (0)	5 (3)	0 (0)	2 (0)	0 (n/a)	2 (1)	7 (3)
Smilisca baudinii	4 (1)	4 (1)	3 (1)	1 (n/a)	0 (0)	0 (1)	1 (0)	1 (0)	0 (n/a)	6 (4)	13 (3)
Smilisca cyanostica	0 (0)	0 (0)	0 (0)	0 (n/a)	0 (0)	0 (0)	1 (0)	0 (0)	0 (n/a)	1 (0)	1 (0)
Gastrophryne elegans	0 (0)	0 (0)	0 (0)	1 (n/a)	0 (0)	1 (1)	1 (0)	0 (0)	1 (n/a)	4 (1)	4 (1)
Rana berlandieri	3 (1)	5 (2)	0 (2)	0 (n/a)	0 (0)	0 (3)	0 (1)	2 (0)	0 (n/a)	3 (5)	10 (8)
Bufo valliceps	2 (1)	2 (1)	1 (0)	0 (n/a)	0 (0)	0 (1)	0 (0)	0 (0)	0 (n/a)	3 (3)	5 (3)
Rhinophrynus dorsalis	1 (0)	0 (0)	2 (0)	1 (n/a)	0 (0)	0 (0)	1 (0)	6 (0)	0 (n/a)	3 (3)	11 (0)
Total number of repeats per pond	5 (2)	9 (3)	6 (3)	5 (n/a)	7 (3)	5 (3)	4 (3)	7 (3)	5 (n/a)		**53 (21)**
Total species diversity of each pond	8 (5)	7 (5)	8 (5)	7 (n/a)	2 (2)	8 (8)	7 (4)	6 (4)	3 (n/a)		

Table 2: Comparison of species presence and absence data between 2000 and 2001 across all species and study sites. The species-site matrix gives the number of survey nights each species appeared at each site. Summary site rows give the total number of survey nights conducted at each pond, alongside the total

Table 3 that a number of species are shown to have increased notably in their relative abundance with *A.callidryas*, *A.moreletii*, *H.picta*, *S.baudinii*, *G.elegans* and *R.dorsalis* all more than doubling in their index score. This result is likely to be due to both an increase in the number of survey nights - thus increasing the potential of recording rare peak breeding events, but also an increase in the ecological and geographical range of each species with nearly all species being found in more breeding sites during 2001 than during 2000 (see Table 2).

	Summed maximum Vocalisation Categories (2001)	**Summed maximum Vocalisation Categories (2000)**	**Number of hours heard calling as a % of the total number of recorded hours across all study nights (2000)**	**Number of hours heard calling as a % of the total number of recorded hours across all study nights (2000)**	**Number of anecdotal observations**
Agalychnis callidryas	20	10	67	62	50-100
Agalychnis moreletii	15	8	37	39	10-20
Hyla loquax	13	8	34	51	20-50
Hyla microcephala	12	10	28	43	10-20
Hyla picta	11	5	16	24	20-50
Hyla ebraccata	4	3	15	7	10-20
Smilisca baudinii	12	4	17	19	50-100
Smilisca cyanostica	1	0	0.5	0	26
Gastrophryne elegans	6	1	3	0.02	350-400
Rana berlandieri	3	3	14	33	10-20
Bufo valliceps	4	4	5	24	20-50
Rhinophrynus dorsalis	8	0	6	0	10-20

Table 3: Comparison of relative abundance and vocalisation activity data between 2000 and 2001 across all species and study nights. To allow for a comparison of data from 2001, Brooke and Coral pond are excluded. The first two columns give the sum (across pond sites) of the maximum Vocalisation Category that was recorded for each species at each site. This index provides a summary statistic for the overall *relative* abundance of each species in the vicinity of Las Cuevas. Also given is the total amount of time each species was recorded for as a percentage of the total number of recorded hours (287 in 2001, and 175 in 2000). Finally, an estimate of the number of anecdotal observations for each species on non-survey nights is provided.

The second index presented in Table 3 is the percentage of total survey hours in which each species was recorded. This gives a measure of both the spatial and temporal 'generality' of a species - i.e. the breadth of breeding sites, climatic conditions, and time of season in which a species is noted to be reproductively active. Their are a few notable changes between 2000 and 2001 which are worth highlighting in this preliminary assessment. *H.loquax, H.microcephala,* and *R.berlandieri* have all exhibited a significant decrease in the percentage of reproductively active time. Consultation with Table 2 can help to apportion these differences to a change in either number of sites a species was recorded in (e.g. *R.berlandieri*), or a change in the number of site specific survey repeats a species was recorded in (e.g. *H.loquax*). However, other species exhibit a remarkable similarity in the percentage of reproductively active time - notably *A.callidryas* and *A.moreletii*. As noted above both of these species have been found in significantly higher numbers during 2001, although it appears that the temporal pattern and concentration of their vocalisation activity has changed little. It is of relevance to note here that as for

Phase I a number of species are inadequately represented through the standard survey protocol as highlighted by the discrepancy between their relative audible abundance (VC) and frequency of anecdotal sightings. The two most distinct examples are *G.elegans* and *S.baudinii* - both classic examples of explosively breeding species (Wells 1977).

Figure 1 provides a summary of the proportion of survey nights in which a species was heard at each of the four potential maximum Vocalisation Categories (VC). Figure 2 allows comparison of the same data for 2000. This provides a measure of the between night variation in relative abundance for each species; for example whether the reproductive activity of a species is characterised by a prolonged level of activity at low numbers, or at the other extreme, by short bursts of activity at high numbers. Furthermore, when considered in conjunction with Table 2 the number of repeats each species was recorded at each VC can be calculated.

Combined Vocalisation Category Scores across all study sites (2001)

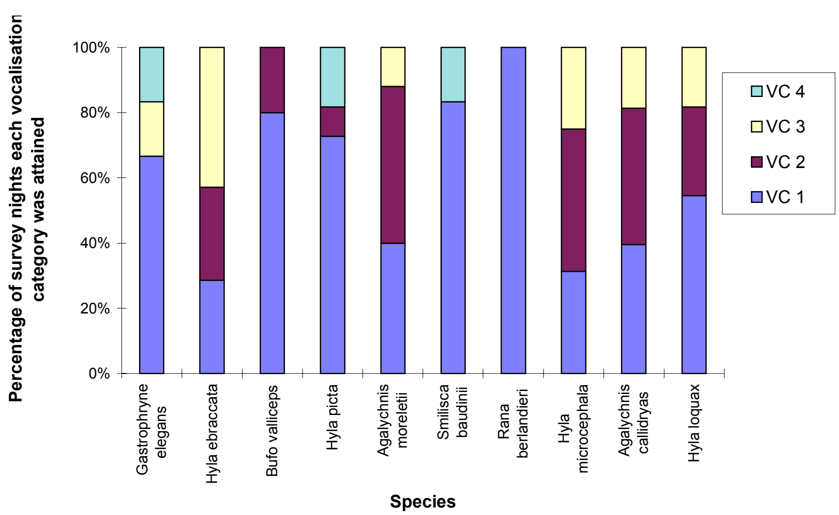

Figure 1: The proportion of survey nights recorded when each VC was attained as a maximum recording for each species (2001). The percentage value is taken from the total number of nights each species was recorded in.

Combined Vocalisation Category Scores across all study sites (2000)

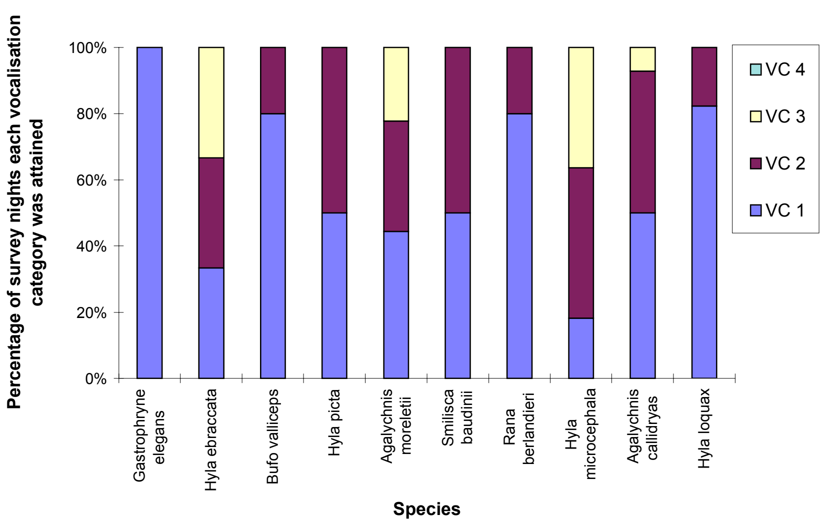

Figure 1: The proportion of survey nights recorded when each VC was attained as a maximum recording for each species (2000). The percentage value is taken from the total number of nights each species was recorded in.

As noted above a number of our study species are notably explosive in their breeding activity patterns. This is clearly shown from Figure 2 with *S.baudinii*, *G.elegans* and to a great extent *H.picta* are characterised in their patterns of vocalisation activity by having the majority of nights recorded being at a very low abundance (VC = 1) with a few crucial nights when each species was present in super abundance (VC = 4). It is of relevance to note that these peak breeding events were concordant with intense rainfall events (see below). A number of species show a remarkable similarity in the proportional ratio of maximum Vocalisation Categories recorded across survey nights when compared between 2000 and 2001 - notably *A.callidryas*, *A.moreletii*, *B.valliceps* and *H.microcephala*. This suggests a greater degree of reliability or predictability in the calling patterns of these species in comparison to others. *H.loquax* however, differs notably between years, and although it is found at fewer sites and over fewer hours during 2001 than 2000 (Table 2 and Table 3), it was found more frequently at higher levels of relative abundance during 2001.

The above paragraph introduces another direction for data analysis not addressed in this preliminary report (to allow for conciseness), and that is in assessing temporal patterns and partitioning of reproductive activity patterns. The half-hourly data on the relative abundance and vocalisation intensity of each species can be combined to give an index of vocalisation activity. Patterns of vocalisation activity can be compared between species to isolate any interspecific temporal partitioning, the strength of which can then be compared between years. Furthermore vocalisation activity patterns can be compared with respect to different species assemblages, habitat types and climatic conditions. The use of half-hourly recordings during this phase allows for an improved resolution of any underlying patterns over results obtained during Phase I. Due to the high level of variability in reproductive activity in amphibian populations, few species lend themselves to rapid, yet thorough sampling (Heyer *et al.* 1994) - a fact which largely explains the deficit of comprehensive amphibian studies in much of the tropics. Consideration as to the importance of the optimal allocation time and resources to budget constrained monitoring projects, highlights the importance of understanding temporal peaks of amphibian activity in allowing rapid and reliable site assessments. Furthermore, aside from logistical efficiency considerations, an understanding of the diversity of reproductive patterns and modes is vital to understand the pattern and organisation of amphibian communities (Donnelly & Guyer 1994). An understanding of community patterns is central in reaching an understanding of the underlying determining mechanisms (Levin 1992) - and in the context of declining amphibian populations, the underlying stability.

Field Study 2: Non-vocalising and leaf litter species

A total of 40 transect line surveys were conducted, or an equivalent to 20 km of transect searched across all six sites. Table 4 gives the summary data on the species richness and relative abundance found at each transect site. This overview provides data on inter-site variations in species composition and relative abundance as well as a comparison between day and night searches. A number of noteworthy results worthy of highlighting here include observations of sympatry between *B.valliceps* and *B.campbelli*, further recordings of *E.rhodopis* (see above), and relatively high numbers of *E.laticeps* in comparison to a complete absence of its apparently more common relative *E.chac* (Lee 2000). Aside from its use in the monitoring work of Project Anuran, this transect data forms part of a collaborative study with a research group from the University of Davis Caifornia, under Professor Tim Caro. This group are interested in comparing vertebrate diversity between different sites characterised by the presence of different umbrella and flagship species in determining their efficacy in capturing overall animal diversity in problems of reserve design.

Data from the four pitfall trap lines is given in Table 5. This data serves to highlight the serious inadequacy of pitfall traps in their use for censusing leaf litter anurans. Collaborating this years and last years data on the monitoring of leaf litter species we now stand in a position to compare the effectiveness of different standard methods for the assemblage at Las Cuevas.

	Total number trap nights	Rana juliani	Rana vaillanti	Eleutherodactylus laticeps	Gastrophryne elegans	Smilisca cyanostica	Trap success (individuals per trap night)
Creek	161	4	1	0	1	1	0.04
Mirage Pond	114	0	0	0	1	0	0.009
Raleigh Camp	90	0	0	0	0	0	0
Fifty Hectare Plot	250	0	0	2	3	0	0.02
	615	4	1	2	5	1	**0.02**

Table 5: Trap data from across five sites each with a pitfall trap array with between 6-10 buckets.

Weather recordings

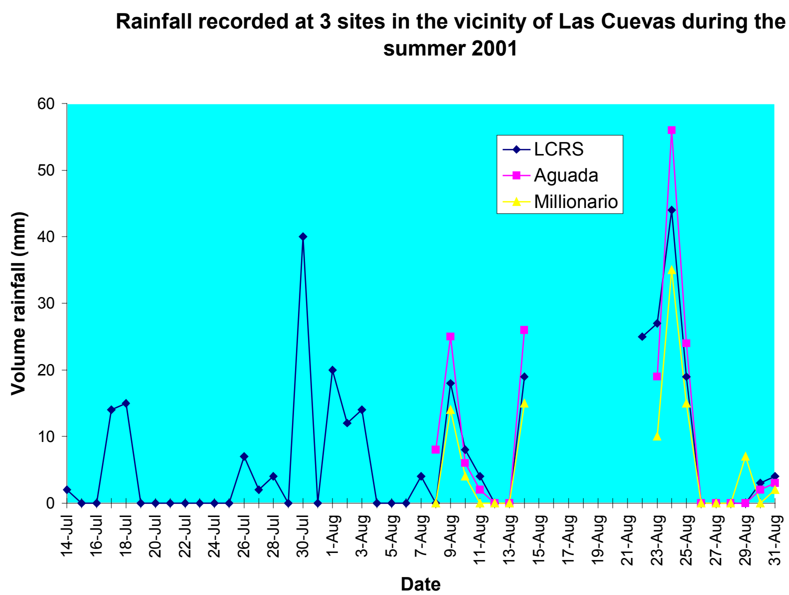

Figure 3: Rainfall at Las Cuevas during 2001. Measurements are taken from three locations (see Methods). The blank section represents an enforced period of departure from the station due to Hurricane Chantal.

Figure 3 presents the rainfall collected at Las Cuevas during the Phase II study period. The notably dry nature of this summer is highlighted by a total of only 347 mm fallen during all July and August - the two months which are supposed to exhibit some of the highest levels of rainfall in the area.(N.Bol pers. comm.[6]). Following anecdotal evidence that recent years have seen changes in the *spatial* as well as temporal patterns of rainfall events prompted us to monitor the rain volume from a 3 different sites along a 8 km line. Since water regimes are of critical importance to amphibian physiology and population dynamics (Gascon 1991; Ingler & Voris 1993; Pounds & Crump 1994) and have been implicated in a number of recent population declines (e.g. Laurance *et al.* 1996; Lips 1999), it seems vital that they are afforded careful consideration in any monitoring program such as ours.

[6] Mr N.Bol. Research Station Operations Manager

	Creek I (Night)	Creek I (Day)	Creek II (Night)	Creek (II) Day	River (Night)	Winstons (Night)	Winstons (Day)	San Pastor (Night)	San Pastor (Day)	Millionario (Night)	Millionario (Day)	Total number of each species	Total number anecdotal observations
Rana juliani	10	1	8	0	1	0	1	0	0	0	0	21	13
Rana vaillanti	9	4	1	0	36	0	0	0	0	0	0	50	2
Rana sp.	3	2	0	1	0	0	0	0	0	0	0	6	2
Bufo valliceps	0	0	0	0	0	1	0	0	0	2	2	5	28
Bufo campbelli	0	0	0	0	0	0	0	0	0	0	4	4	27
Bufo marinus	0	0	0	0	2	0	0	0	0	0	0	2	3
E. sabrinus	6	5	0	0	3	0	0	0	0	0	0	14	4
E.laticeps	1	0	0	0	0	1	1	0	0	1	0	4	8
E.rhodopis	0	1	0	0	0	0	0	1	0	0	0	2	2
G.elegans	0	0	0	0	0	0	0	1	0	1	0	2	350-400
H.fleischmanni	0	2	0	0	0	0	0	0	0	0	0	2	0
Total number species per transect	5	6	2	1	4	2	2	2	0	4	2		
Total number of repeats per transect	5	4	3	3	2	4	3	5	3	5	3	**40**	
Total distance walked per transect (km)	2.5	2	1.5	1.5	1	2	1.5	2.5	1.5	2.5	1.5	**20**	

Table 4: Summary transect data from across all six transect lines.

Future output of Project Anuran Phase II

- A main project report due September 2002. This report will form part of a comprehensive series of assessments of the amphibian populations local to Las Cuevas, following each year of Project Anuran field work. The data outlined here will be analysed with respect to between site and between species differences in species richness, relative abundance, and reproductive activity patterns. Particular attention will be paid to comparing the variability in such patterns both between years (2000 and 2001) and between spatial scales (i.e. comparing a pattern observed at one site to the analogous pattern observed across all site combined). Furthermore, consideration will be given to the role of environmental factors (climate, pond attributes, amphibian species assemblage composition) in determining patterns of species presence, relative abundance, and reproductive activity. Appropriate analysis will also be conducted on the leaf litter data, and comparisons made as to the efficiency of different survey methods. The report will be presented in the context of an up-to-date review of other associated research, and advances in the declining amphibian problem.
- A number of articles to appear in appropriate scientific journals. We feel that a number of results from this project are worthy of separate consideration for publication. Areas in which we have acquired particularly interesting data of sufficient quantity include; (1) the temporal partitioning of vocalisation activity of different species, (2) inter-specifc and environmental factors which can contribute to spatial and temporal variability in species presence, relative abundance, and vocalisation activity pattterns (more data for certain species), and (3) a comparison of different approaches for monitoring of leaf litter and stream dwelling amphibians.
- Final completion of the project website (www.projectanuran.org). Due to technical difficulties this has taken longer than expected, however it should see completion by early November of this year. Alongside a copy of the Phase I final report it will include taxonomic descriptions of each species accompanied by a digital audio recording taken during 2000.
- Lecture circuit. We are planning for all project members to give a variety of presentations on the nature, importance, and results of our work. Audiences will include; University Student Societies, University Departments and Seminar Groups, supporting Trust funds and organisations, and appropriate national conferences - e.g. the BES Winter meeting.

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TREASURY REPORT FOR PROJECT ANURAN 2001			
	Type	*Information*	*Amount (£)*
PRE-DEPARTURE	Flights	Mexico City Flight	1775.20
	Insurance		250.00
	Photographic Equipment and Film		245.16
	Medical supplies and vaccinations		40.15
	Personal and Belizean equipment		432.40
	Field and Scientific Equipment		1296.64
	Administration		168.71
	Website		90.00
Post expedition	Report printing and distribution	BUDGETED	1000.00
	Photographic development	BUDGETED	200.00
SUBTOTAL			**5498.26**
IN FIELD[7]	Food and Accomodation	NHM - includes batteries and transport to/from Cuevas, as well as food and accommodation for the duration of the field study	4673.34
	Research Permit	In LCRS Accomodation bill (£100)	
	In country travel		110
	Flights (Aviacxa)		1200
	visas		63
SUBTOTAL			**6046.34**
TOTAL			***11544.60***
TOTAL INCOME			***12450.00***

[7] Note that Food, accomodation and travel categories include expenditure of contingency funds during two enforced periods of departure

Remainder		***905.4***