

Department of Physics, Chemistry and Biology

Master Thesis

Habitat Utilization of the Endemic
Poison Dart Frog *Excidobates mysteriosus*
in North-western Peru

SANDRA MONSALVE PASAPERA

LiTH-IFM-A-Ex--11/2416--SE



Supervisor: Karl-Olof Bergman, Linköping University

Co-supervisor: Per Milberg, Linköping University

Examiner: Matthias Laska, Linköping University



Linköpings universitet

Department of Physics, Chemistry and Biology

Linköpings universitet

SE-581 83 Linköping, Sweden



Avdelning, Institution
Division, Department

IFM Biology

Datum
Date
2011-06-03

Språk

Language

Svenska/Swedish

Engelska/English

Rapporttyp

Report category

Licentiatavhandling

Examensarbete

C-uppsats

D-uppsats

Övrig rapport

ISBN

LiTH-IFM-A-Ex—11/2416--SE

ISRN

Serietitel och serienummer

ISSN

Title of series, numbering

LITH-IFM-A-Ex—11/2416--SE

URL för elektronisk version

Titel

Title

Habitat Utilization of the Endemic Poison Dart Frog *Excidobates mysteriosus* in North-western Peru

Författare

Author: Sandra Monsalve Pasapera

Sammanfattning

Abstract

The worldwide amphibian diversity is severely threatened, mainly due to deforestation. Lack of sufficient knowledge for species conservation is common. This is the first detailed study of the poison dart frog *Excidobates mysteriosus* including habitat requirements, occupancy patterns and behaviours. It is known only from one location in north-western Peru, living in Bromeliaceae plant species. The frogs and tadpoles were found in two unidentified Bromeliaceae species; Bromeliad Species 1 and Bromeliad Species 2. Bromeliad quality variables were evaluated for habitat requirements, behavioural recordings were performed ad libitum and catching-and-release with photographing of frogs for monitoring dispersals. Results show that for Bromeliad Species 1 significantly more frogs were present in bromeliads that were wider, received less sun exposure, had deeper water cavities and in bromeliads growing on lower altitudes. For Bromeliad Species 2 deeper water cavities had a significant effect on presence of frogs. In both bromeliad species higher presence of tadpoles were found in plants that were wider and had deeper water cavities. The density of bromeliads had no effect on frog or tadpole occupancy for neither bromeliad species. The dispersal, feeding and tadpole release behaviours are reported. Individual frogs were found to disperse 0-113m. The total number of encountered frogs was 1006 and 86 tadpoles, most of them found in three core areas. These areas should be targeted for conservation management and monitoring, diminishing the high deforestation pressure which saves old-growth forests and thereby as well the preferred wider bromeliads containing deeper water cavities and the biggest frog populations.

Nyckelord

Keyword

behaviour, bromeliads, conservation, Dendrobates, dispersal, *Excidobates*, *mysteriosus*, Peru

1 Abstract.....	1
2 Introduction.....	1
3 Materials and methods.....	2
3.1 Study area.....	2
3.2 Occupancy of tadpoles, juvenile and adult frogs.....	4
3.3 Habitat utilization.....	4
3.4 Behavioural recordings.....	5
3.5 Data analysis and statistics.....	5
4 Results.....	5
4.1 Number of tadpoles, juvenile and adult frogs.....	5
4.2 Bromeliad quality in relation to frog occupancy.....	6
4.3 Dispersal, feeding and breeding behaviour.....	10
5 Discussion.....	11
5.1 Importance of bromeliad quality, density and altitude.....	11
5.2 Dispersal, feeding and breeding behaviour.....	12
5.3 Conservation implications.....	13
6 Acknowledgments.....	14
7 References.....	14

1 Abstract

The worldwide amphibian diversity is severely threatened, mainly due to deforestation. Lack of sufficient knowledge for species conservation is common. This is the first detailed study of the poison dart frog *Excidobates mysteriosus* including habitat requirements, occupancy patterns and behaviours. It is known only from one location in north-western Peru, living in Bromeliaceae plant species. The frogs and tadpoles were found in two unidentified Bromeliaceae species; Bromeliad Species 1 and Bromeliad Species 2. Bromeliad quality variables were evaluated for habitat requirements, behavioural recordings were performed ad libitum and catching-and-release with photographing of frogs for monitoring dispersals. Results show that for Bromeliad Species 1 significantly more frogs were present in bromeliads that were wider, received less sun exposure, had deeper water cavities and in bromeliads growing on lower altitudes. For Bromeliad Species 2 deeper water cavities had a significant effect on presence of frogs. In both bromeliad species higher presence of tadpoles were found in plants that were wider and had deeper water cavities. The density of bromeliads had no effect on frog or tadpole occupancy for neither bromeliad species. The dispersal, feeding and tadpole release behaviours are reported. Individual frogs were found to disperse 0-113m. The total number of encountered frogs was 1006 and 86 tadpoles, most of them found in three core areas. These areas should be targeted for conservation management and monitoring, diminishing the high deforestation pressure which saves old-growth forests and thereby as well the preferred wider bromeliads containing deeper water cavities and the biggest frog populations.

Keywords: behaviour, bromeliads, conservation, Dendrobates, dispersal, *Excidobates, mysteriosus*, Peru

2 Introduction

Like most terrestrial elements of biodiversity, amphibian diversity is severely threatened (Sala et al. 2000), and the mountain regions have been especially hard hit (Young et al. 2001). The threats to the amphibians are various; deforestation, fertilisers, pesticides, acid precipitation, low pH, increased UV radiation, emerging diseases, introduced species, climate change, chytrid fungus (*Batrachochytrium dendrobatidis*), hybridization, human predation etc. (e.g. Duellman 1999 cited by Galindo-Leal et al. 2003; Beebee & Griffiths 2005). A global decline in amphibians was first recognized in 1989 (e.g. Vitt et al. 1990; Wake 1991) and the situation has not improved since then. In the end of the year 1998, 124 species of amphibians were categorized as threatened; in 2010 the number had increased more than 15 times to 1898 threatened species (IUCN 2010a). This is 29 % of the total number of amphibian species described in the IUCN Red List (IUCN 2010a), more than any other category of animals.

Latin America harbours a highly diverse amphibian fauna representing half of the world's total species richness (Duellman 1999 cited by Galindo-Leal et al. 2003). The amphibian declines are widespread throughout this area (Young et al. 2001). Peru harbour more than 500 species of amphibians and is regarded as a mega-diverse country (von May et al. 2008).

One of the 70 threatened and endemic amphibian species (IUCN 2010b) in Peru is the poison dart frog *Excidobates mysteriosus* (Marañón Poison Frog). The species is classified as endangered (EN) and the population trend is assessed to be decreasing (Icochea et al. 2010). The frogs are only known from a single location in the vicinity of the village Santa Rosa at

the foothills of Cordillera del Cóndor (Cajamarca Department), north-western Peru, at around 1000 meters above sea level (m.a.s.l.) (Icochea et al. 2010).

The knowledge about the frog species is scarce. Twomey and Brown (2008) performed a study on *E. mysteriosus* and used 13 presence points for a niche model, ending up with a possible distribution between 200-1500 m.a.s.l. They concluded that the species is terrestrial or scansorial and diurnal. Lötters et al. (2007) (cited by Twomey & Brown 2008) reported the clutch size to be 8-13 eggs, which develops into free swimming tadpoles that finally metamorphs into frogs. The only published note on behaviour found is from Twomey and Brown (2008) which wrote that the frogs are hesitant to jump and tend to “walk” instead.

One of the key aspects of frog ecology is the breeding behaviour. *E. mysteriosus* is said to use Bromeliaceae plant species for breeding similar to other neotropical poison dart frogs from the family Dendrobatidae. Many species use the small pools of water that accumulate for longer periods in plant parts in plants from the families *Bromeliaceae*; *Aechmea* and *Sarraceniaceae* (Walls 1994; Dejean et al. 1995; Dejean & Olmsted 1997). Mature plants have four types of cavities, where the most crucial cavities are the interfoliar cavities that are deep and narrow and accumulate rainwater, which remains well into the dry season because of low evaporation (Galindo-Leal et al. 2003). The tadpoles may grow and metamorph at the place of oviposition or being transported by adults to water pools (Wells 2007; Weygoldt 1980).

The utilization rate of individual bromeliads may be affected by different habitat factors (quality variables). Zotz and Thomas (1999) found that frogs were disproportionately encountered in large bromeliads. A study on the poison dart frog *Dendrobates pumilio* showed that increasing bromeliad density also increased the presence of adult frogs leading to more breeding, resulting in a higher presence of juveniles (Donnell 1989). In a study by de Silva et al. (2011) they found a bromeliad usage pattern, where higher presence of anuran frogs were found on bromeliads with higher sun exposure than on those who had lower sun exposure. No detailed studies have been performed on the behaviour including breeding or habitat utilization of *E. mysteriosus*. Crucial information for conservation of the species is therefore lacking.

The main aims of this paper were to study the basic ecology of *E. mysteriosus*, including habitat requirements, occupancy patterns, individual frog dispersal, feeding and tadpole release behaviours. This knowledge should facilitate efforts to preserve the species, including the development of guidelines for managing sites where it occurs.

3 Materials and methods

3.1 Study area

The study was conducted during the dry season, between 14th of June and 10th of November 2010, in the vicinity of the village Santa Rosa in the southern end of the Cordillera del Condor, northwestern Peru. The area is dominated by grazing grounds, coffee and banana plantations followed by agricultural fields for diverse crops (maize, cereals, root crops) leaving small areas with remnant forests. Rock walls are common elements in the landscape mosaic. Both remnant forests and rock walls are threatened by uncontrolled slash and burn agriculture, as well as the increasing deforestation. None of the areas are close to (<500m) running or standing water (bigger than the water cavities represented in the bromeliads), making the frogs strongly connected to the bromeliads for survival.

Around Santa Rosa there are six well known areas where *E. mysteriosus* (Fig.1) are or have been present in the recent years. These sites will be referred to as area A, B, C, D, E and F (Table 1) and are located between 1000-1600 m.a.s.l. The areas consist of remnant forests or rock walls with a presence of Bromeliaceae plant species.



Figure 1. The studied species *Excidobates mysteriosus*, left: adult with tadpoles on its back, center: juvenile and adult, right: juvenile.

Table 1

All investigated areas, possibly containing *Excidobates mysteriosus* with descriptive parameters.

Area	Size (ha)	Vegetation	Unidentified Bromeliad Species	Masl (mean)	Protected Reserve
A-area	9	Rock Wall, Remnant Forest	1, 2	1222	Yes
B-area	2.5	Remnant Forest	1	1010	No
C-area	1	Rock Wall	1	1320	Yes
D-area	3	Remnant Forest	1, 2	1000	No
E-area	8	Rock Wall	1	1627	Yes
F-area	2	Rock Wall	1	1302	No

The frogs were present in two different Bromeliaceae species, both still unidentified. The first species, named Bromeliad Species 1 had a mean width of 72 cm (range 25-187 cm) and a mean height of 42 cm (range 10-89 cm) (Fig.2). The Bromeliad Species 1 were recognized by their red and green coloured and wider leaves, compared to the Bromeliad Species 2, which was the second species (Fig.3). The Bromeliad Species 2 had a mean width of 142 cm (range 50-280 cm) and a mean height of 85 cm (range 30-150 cm). This species had thin, long and green leaves with white stripes on the leaves' undersides.



Figure 2. Unidentified Bromeliad Species 1



Figure 3. Unidentified Bromeliad Species 2

3.2 Occupancy of tadpoles, juvenile and adult frogs

To estimate the occupancy of tadpoles, juvenile and adult frogs, randomly selected bromeliad specimens with a minimum distance of 10 m from each other were checked in each site. Pellet and Schmidt (2005) showed that multiple (up to six) visits to each site were needed to establish presence or absence for all of the four amphibian species investigated, with 95 % confidence. Therefore, six visits were set as the minimum limit of visiting times to get a good estimation of the occupancy of each bromeliad. The presence was then determined to the different recordings of presence (for adults, juveniles and tadpoles together): presence ≥ 1 time, presence ≥ 3 times and presence all 6 times. Additionally recordings of lifestages: tadpole presence, juvenile presence and adult presence. The order of visits to each bromeliad was randomized. The visiting to bromeliads were performed during hours 05:00 am - 07:00 pm. Every individual bromeliad measured was marked with an individual ID number, by a permanent marker pen at first visit, to be recognized for the coming five visits. At all visits the bromeliads were searched for tadpoles, juveniles and adults. All encountered frogs that were inside or < 20 cm outside the bromeliads were directly related to the individually measured bromeliad.

In search for adult and juvenile frogs, firstly the bromeliad was only observed for detection; secondly all leaves of the bromeliad were searched through. The search for tadpoles during the day was done using only the sun light, or in darker weather conditions as well as at morning or night with a special handmade LED-lamp in the water cavities. Every cavity of water that was reachable was searched through.

The frogs were counted, captured, photographed and measured on the snout-vent length (SVL) and on the width in the widest part during resting position, to nearest millimeter. For all areas this was done only during the first visit to each bromeliad, and then during following visits, the individual frogs were only counted, not captured. But in the A-area this was a method performed at each visit, to be able to track individual dispersals of the frogs by photo recognition. Therefore, every encounter was recorded with date, time and location. After the first capture, photos were taken from distance to avoid disturbing and inducing additional stress if the individual was recognized as an earlier capture. Captured individuals were always released at the same spot of catchment. Individuals measuring ≤ 17 mm were classified as juveniles and individuals measuring ≥ 18 mm were classified as adults (modified after *Excidobates* measurements by Twomey & Brown 2008). Calling individuals were assumed to be males. Detected males were therefore grouped as “males” and the rest of the adult frogs grouped as “adults” containing unsexed females and males.

3.3 Habitat utilization

All bromeliads visited to investigate the occupancy of adult frogs, juveniles and tadpoles were measured to gather information of the importance of bromeliad characteristics for the frog species occupancy.

A number of variables for evaluating the quality of bromeliads and its surroundings were recorded: the size of the bromeliads, depth of the interfoliar water cavities in the bromeliads, sun exposure (%) received by the bromeliads, the bromeliad species, bromeliad density and finally the bromeliads' altitude.

The sizes of the bromeliads were measured as width (cm) with a regular field tape. At first visit to each bromeliad sun exposure was estimated by eye in percent taking into account shadings and location. The water cavities at the base of the bromeliads were measured in centimeters by dipping down a stick in the water cavity in its deepest part. The bromeliad density was measured by estimating or counting (depending on the location) all nearby bromeliads in a 5m radius from the individually measured bromeliads. The altitude (m.a.s.l.) of each bromeliad was monitored with a Garmin GPS 60CSx.

In total 281 bromeliads were sampled, ending up with a total of 1686 visits to these bromeliads.

3.4 Behavioural recordings

To gain information about the dispersal, activity, breeding and feeding behaviour of the *E. mysteriosus* behavioural recordings were performed (Table 2). Ad libitum sampling was used, making it possible to perform behavioural recordings simultaneously with the investigation of habitat quality variables and occupancy of frogs, since it is performed without systematic constraints. But also to be able to catch the behaviours of interest. Continuous recording was applied at every sampling session.

Table 2

*Ethogram for the observed behaviours in the *Excidobates mysteriosus*.*

<i>Behaviour</i>	<i>Description</i>
Eating	Successfully catching insects with their tongue.
Moving	Moving 1-3 legs, changing positioning, turning around etc. without walking.
Walking	Moving all four legs more than once.
Jumping	All four legs leaving ground in an intended jump.
Foraging	Making specific body movements, turning head and "lifting" chest and head, directing against the prey.
Tadpole release	Adults releasing tadpoles into water cavities, in detail in heading 4.3.

For recording individual frogs' dispersal all encounters of frogs were photographed, date and location noted. The activity was continuously recorded during field hours. The feeding behaviour was observed 20 times during different field visits. In addition, the tadpole release (breeding) behaviours were observed during six different times (by six different adults) in the two tadpole-areas (the A- and D-area). In the A-area all the three observations were done in the afternoon (~05:00 pm). In the D-area two observations were done in the forenoon (~10:00 am) and one in the afternoon (04:30 pm).

3.5 Data analysis and statistics

All data were analyzed using STATISTICA for windows version 9.1. Between the habitat quality variables standard correlation analysis (matrices) was used with correlation coefficients. The relationship between presence of frogs/tadpoles and the different habitat quality variables were analyzed with Generalized Linear Models (GLM; binomial and logit-link). For behavioural data descriptive statistics were applied.

4 Results

4.1 Number of tadpoles, juvenile and adult frogs

There was high variation in the number of individuals and occupancy rates among the six different areas (Table 3). The results show that there are three core areas with high numbers of the *E. mysteriosus*, namely the A-, B-, and D-area. Tadpoles could only be detected in two of the six areas (A- and D-area). In the E-area not one single frog or tadpole was encountered during the six visits. In total, 1006 frogs and 86 tadpoles were found, counting all areas. There were eight bromeliads that only had tadpoles (none juvenile or adult during all 6 visits), seven of them in the tadpole-rich D-area. In the A-area 32 tadpoles were found in 11 different bromeliads, giving a mean of 2.9 tadpoles per bromeliad that were used for tadpole release. In

the D-area 54 tadpoles were found in 22 bromeliads, giving a mean of 2.5 tadpoles per bromeliad that were used for tadpole release. At each visit the number of encountered frogs in the bromeliads ranged from 0-5 and the number of found tadpoles ranged from 0-11 per bromeliad.

Table 3

*Number of encountered adults, juveniles and tadpoles of *Excidobates mysteriosus* in all areas (inclusive frogs found outside the sampled bromeliads) and the number of frog occupied bromeliads per sampled area.*

	No. of adults	No. of juveniles	No. of tadpoles	Total no.	No. of searched bromeliads	No. of bromeliads with frogs ≥ 1 time	No. of empty bromeliads at all 6 visits	Proportion occupied %
A-area	368	115	32	515	81	36	45	45
B-area	104	85	0	189	46	13	33	28
C-area	7	7	0	14	6	3	3	50
D-area	188	120	54	362	97	61	36	68.5
E-area	0	0	0	0	30	0	30	0
F-area	7	5	0	12	21	3	18	14
Total no.	674	332	86	1092	281	118	167	

The mean sizes of frog individuals caught in the A-area were; juveniles (n=35) 14.9 mm SVL and 5.9 mm in width, males (n=12) 25.5 mm SVL and 11.3 mm in width and adults (n=129) (males and females) 24.5 mm SVL and 10.6 mm in width.

4.2 Bromeliad quality in relation to frog occupancy

The occupancy rate by frogs and tadpoles in Bromeliad Species 1 were 32.5% (62 of 191 plants) and in Bromeliad Species 2 the occupancy rate were 53% (48 of 90 plants).

In the Bromeliad Species 2, for all the different recordings of presences of frogs and tadpoles (i.e. presence ≥ 1 time, ≥ 3 times, all 6 times, tadpole-, juvenile- and adult presence), the bromeliads that had deeper water cavities had significantly higher presence of frogs and tadpoles (Table 4, Fig.4). Additionally, bromeliads with tadpoles present were significantly wider than bromeliads without tadpoles (Fig. 5). The rest of the bromeliad quality variables (sun exposure, density and m.a.s.l.) had no significant effect on the presences of adults, juveniles or tadpoles in the Bromeliad Species 2, except presence all 6 times, being significant for lower altitudes.

Table 4

Different recordings of presence and absence of frogs and tadpoles tested against habitat quality variables in Bromeliad Species 1 (n=191) and in Bromeliad Species 2 (n=90), numbers given are p-values from binomial GLM. Presence determined to different recordings, first three columns of p-values are presence of frogs and tadpoles together (presence ≥ 1 time, ≥ 3 times, all 6 times). Three last columns of p-values are presence of different life stages (tadpole, juvenile and adult). n= no. of bromeliads with the respective recorded presence.

Recordings of presence						
Brom Spec. 1 (n=191)	Presence ≥ 1 time (n=62)	Presence ≥ 3 times (n=53)	Presence all 6 times (n=9)	Tadpole presence (n=6)	Juvenile presence (n=55)	Adult presence (n=40)
Width	0.0004	0.00075	0.39	0.002	0.099	0.005
Sun Exposure	0.04	0.008	0.02	0.62	0.004	0.08
Water Depth	0.0006	0.0055	0.98	0.00065	0.53	0.002
Density	0.92	0.39	0.07	0.91	0.71	0.58
M.a.s.l.	0.0005	0.003	0.06	0.94	0.0004	0.001
Recordings of presence						
Brom Spec. 2 (n=90)	Presence ≥ 1 time (n=48)	Presence ≥ 3 times (n=33)	Presence all 6 times (n=4)	Tadpole presence (n=21)	Juvenile presence (n=42)	Adult presence (n=35)
Width	0.93	0.91	0.21	0.02	0.92	0.75
Sun Exposure	0.46	0.50	0.51	0.69	0.32	0.69
Water Depth	0.008	0.0001	0.01	0.02	0.02	0.0005
Density	0.27	0.87	0.28	0.87	0.77	0.65
M.a.s.l.	0.48	0.63	0.03	0.37	0.59	0.67

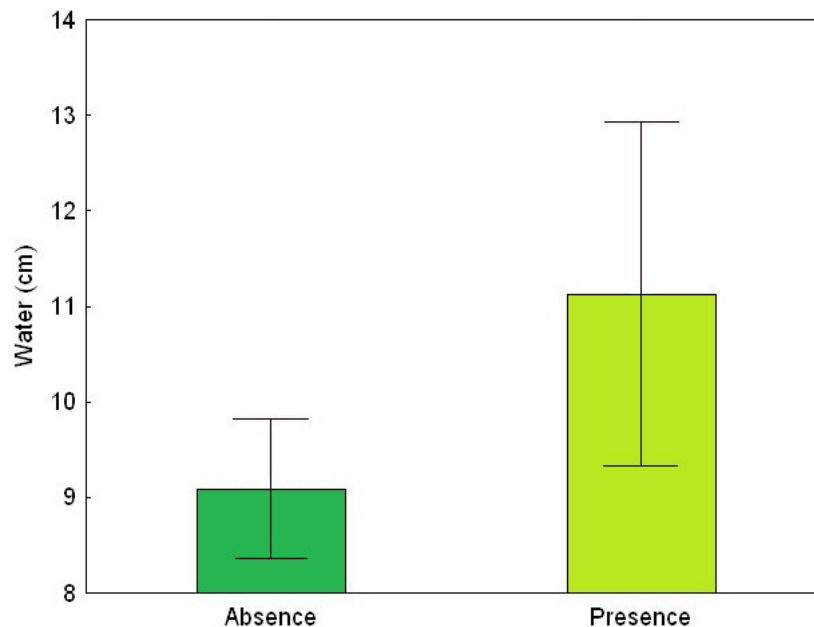


Figure 4. Mean (\pm SD) water cavity depth (cm) in Bromeliad Species 2 with absence of tadpoles (n=69) and presence of tadpoles (n=21).

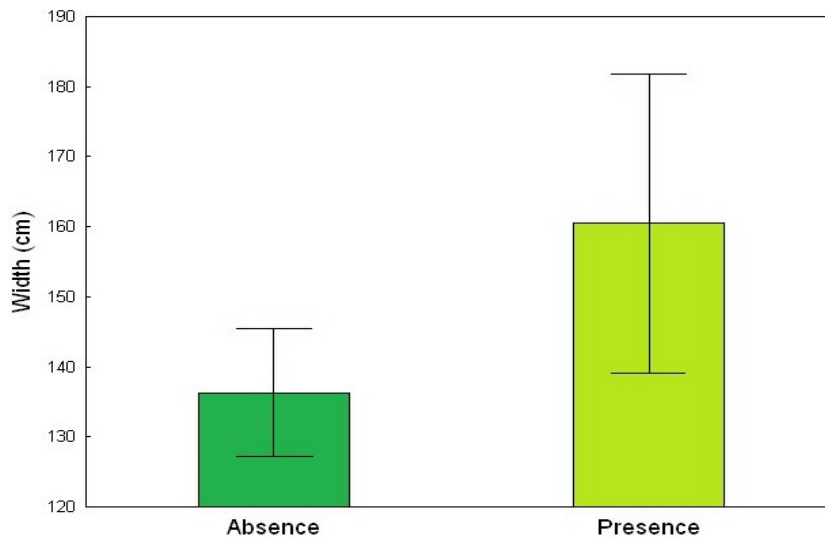


Figure 5. Mean (\pm SD) width (cm) in Bromeliad Species 2 with absence of tadpoles ($n=69$) and presence of tadpoles ($n=21$).

In the Bromeliad Species 1, significantly higher presence of frogs (presence ≥ 1 time and ≥ 3 times) were found on bromeliads that were wider, less sun exposed, containing deeper water cavities (Fig. 6-8) and growing at lower altitudes (Table 4). For presence of frogs all 6 times, there was a significant impact of lower sun exposure. Bromeliads with presence of tadpoles were significantly wider and had deeper water cavities than bromeliads without tadpoles. Moreover, bromeliads growing at lower altitudes had a significantly higher occupancy of both adults and juveniles. However, significantly more adults were found on bromeliads that were wider and contained deeper water cavities, while these factors had no significant impact on juveniles. On the opposite, juveniles were found to be more present on bromeliads having lower sun exposure compared to the presence of adults. Additionally, for all recorded presences of frogs (except tadpole presence and presence all 6 times), significantly higher presence of frogs were found in bromeliads growing at lower altitudes with a mean of 1153 m (range 895-1332 m) compared to bromeliads growing on higher altitudes with a mean of 1271 m (range 969-1723 m). Density of bromeliads had no significant effect on any level of frog or tadpole presence.

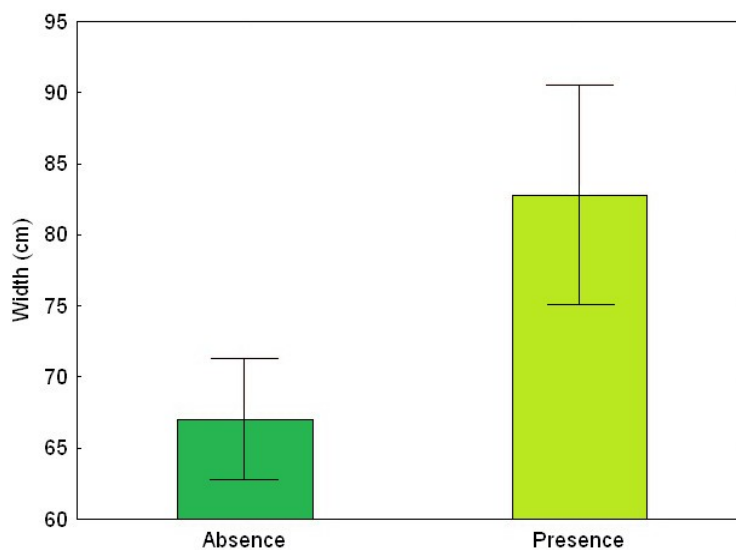


Figure 6. Mean (\pm SD) width (cm) in Bromeliad Species 1 with absence of frogs and/or tadpoles ($n=129$) and presence of frogs and/or tadpoles ($n=62$).

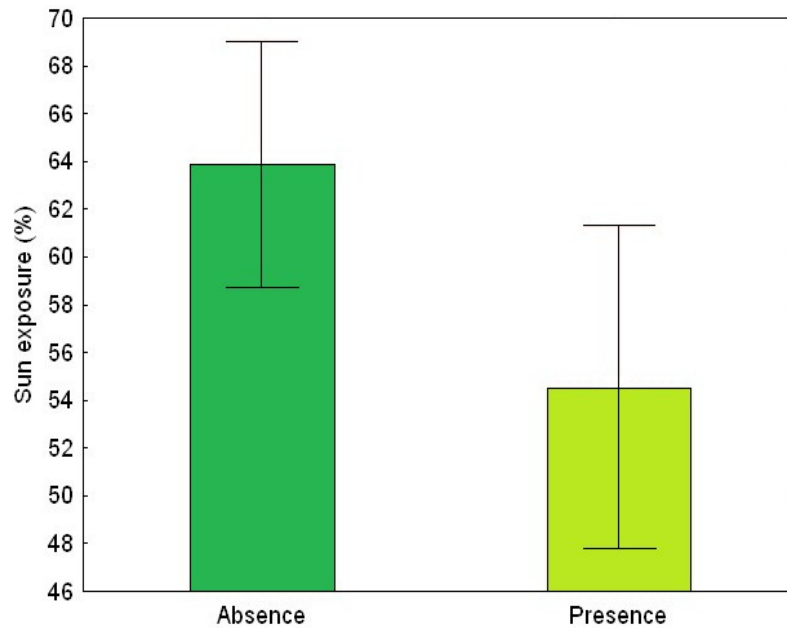


Figure 7. Mean (\pm SD) sun exposure (%) in Bromeliad Species 1 with absence of frogs and/or tadpoles (n=129) and presence of frogs and/or tadpoles (n=62).

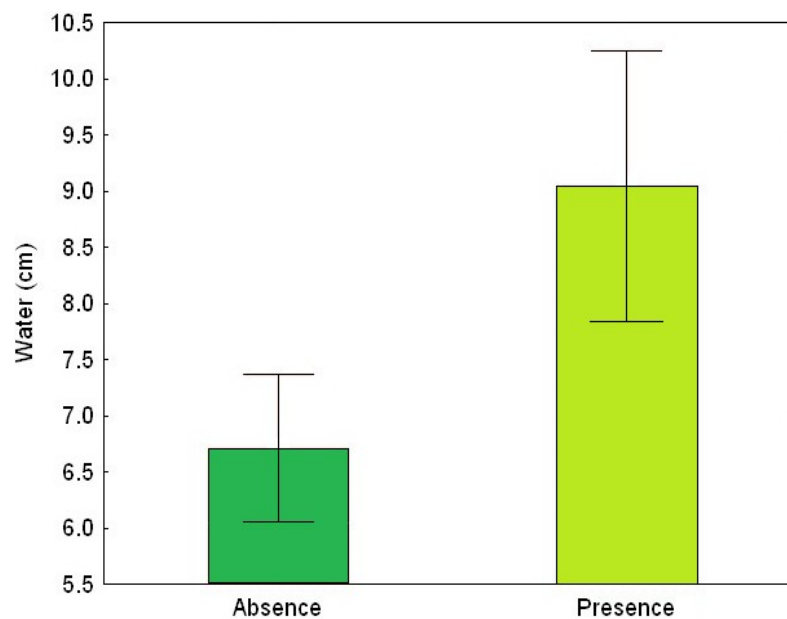


Figure 8. Mean (\pm SD) water cavity depth (cm) in Bromeliad Species 1 with absence of frogs and/or tadpoles (n=129) and presence of frogs and/or tadpoles (n=62).

For all bromeliad quality variables a correlation analysis was done (Table 5). For both bromeliad species' the water depth was positively correlated to the width, wider bromeliads containing deeper water cavities. The sun exposure and the bromeliads' altitude were as well positively correlated in both species, bromeliads at higher altitudes receiving higher sun exposure. Additionally for Bromeliad Species 1, correlations were found between sun exposure and water depth, sun exposure and density and m.a.s.l. and density.

Table 5.

Correlation matrices for bromeliad quality variables in both bromeliad species.

Bromeliad Species	Variables	Correlation coefficient	p-value
1	Width x Water Depth	0.59	<0.05
	Sun Exposure x Water Depth	0.25	<0.05
	Sun Exposure x Density	0.19	<0.05
	Sun Exposure x Masl	0.69	<0.05
	Masl x Density	0.23	<0.05
2	Width x Water Depth	0.23	<0.05
	Sun Exposure x Masl	0.25	<0.05

4.3 Dispersal, feeding and breeding behaviour

General notes were taken on the basic behaviours of the *E. mysteriosus*. Individual dispersal by the frogs were studied in detail while the activity, breeding and feeding behaviours were briefly observed.

For the dispersal, 187 encounters of adults and juveniles were photographed in the A-area. Of those 46 were recaptures by 25 frogs, which ranged from 2-6 recaptures per individual (mean was 2 recaptures). From the recaptures it was found that the frogs disperse in mean 20.5 m (range 0-113 m) between different bromeliads. 25 frogs were found at least once in the same bromeliad (0 m), 7 frogs were dispersing at least 2.5 m, 6 frogs were dispersing at least 5 m, 5 frogs were dispersing at least 7.5 m, 4 frogs at least 10 m and 2 frogs at least 17.5 m and a single frog at least 113 m (Fig. 9).

During direct observations on activity, the adults and juveniles when observed undisturbed, tended to walk more than jump when moving in more difficult terrain (as rock walls and trees), but could move fast and for longer distances by doing great jumps when disturbed or when moving on the forest floor. The frogs also seemed to dedicate large time on “moving” (see Ethogram, Table 2) inside and outside the bromeliads, mostly in shadowed spots.

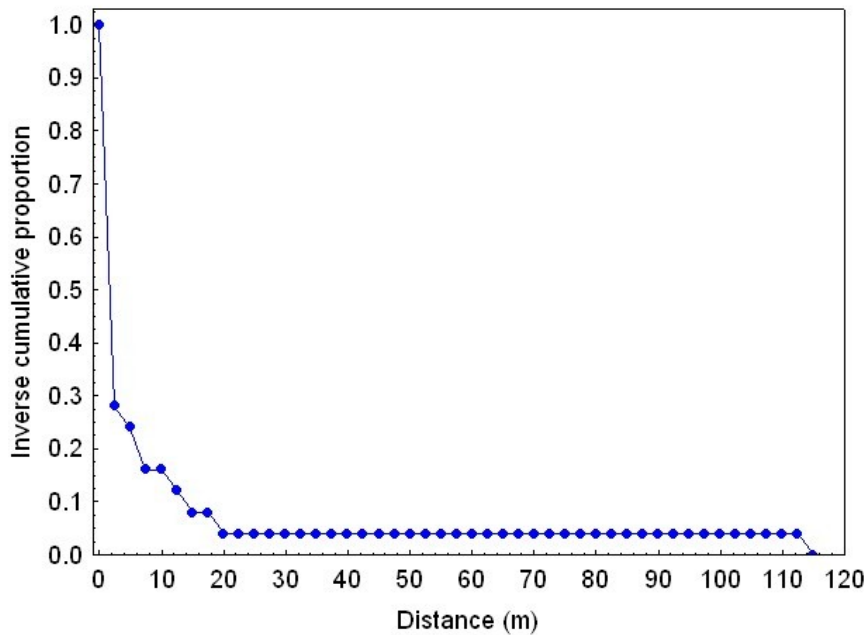


Figure 9. Inverse cumulative proportion distribution of the movements between bromeliads by 20 adult and 5 juvenile frogs.

In regard to breeding behaviour, interesting tadpole release behaviours were observed. When an adult was found carrying tadpoles it was observed to move between the bromeliad leaves, backing down and making a quick dip into the water, sitting still on the leaf, perhaps move a little bit, then go on to the next leaf to follow the same behaviour pattern there. When observing frogs without tadpoles they went head first into water cavities but all adults carrying tadpoles went back first. One adult also engaged in feeding while moving around between the leaves. When the adult decided to release a tadpole it entered a water cavity and became floating on the water surface, freely or with front legs as anchor on bromeliad leaf. While floating, the adult was gently caressing one of the tadpoles on its back with its hind legs (mean number of times 28 (range 25-32)) until it was released into the water. Only one tadpole was released in one water cavity. In the A-area the three adults were carrying 2, 3, 3 tadpoles respectively and those with three tadpoles did only release one tadpole the first evening, carrying on the tadpoles the next day. The one with two tadpoles released the tadpoles in one cavity each during the same evening. In the D-area, adults carrying tadpoles showed the same behaviour pattern as above. However, the carrying of tadpoles were higher than in A-area, observing adults carrying 4, 4 and 5 tadpoles respectively. One that had four tadpoles and the one that had five tadpoles were carrying one tadpole on the hind leg and the rest on their backs.

Adult and juvenile frogs were observed to be feeding on ants and small, winged flies both inside the bromeliad and outside on rock walls, forest floor and on other plants.

5 Discussion

The amphibians in Latin America are facing numerous threats (Young et al. 2001). Additionally, many species are endemic and endangered which generates a need for conservation managements. Although, for many threatened amphibians there is a lack of sufficient knowledge for species conservation planning. In this study, detailed data on habitat requirements, occupancy patterns and behaviours have been collected of the endangered and threatened poison dart frog *Excidobates mysteriosus*.

5.1 Importance of bromeliad quality, density and altitude

The present study has shown that *E. mysteriosus* has specific habitat requirements with regard to bromeliad quality and that bromeliads are crucial for its survival and reproduction, as the only stable and long-lasting water source. Among vertebrates, anuran amphibians (including the Dendrobatidae) have the highest number of species associated with bromeliads and possess a range of ecological, behavioural, and morphological specializations to live in these plants (da Silva et al. 2011), as was also shown by the present study.

There were significantly higher presence of frogs in wider Bromeliad Species 1 than on smaller, which is consistent with other studies. A study on Mexican arboreal frogs found a higher presence of frogs living in bromeliads larger than 70 cm (measured in height) (Galindo-Leal et al. 2003). Another study made on four earthworm species living in bromeliads showed that the bigger bromeliad (longer leaves) the higher presence of the worms (Fragoso & Rojas-Fernández 1996). In tropical forests the large bromeliads provide important microhabitats not only for amphibians but also for many other invertebrates and vertebrates, by holding water and moist, provide forage sites and protection (Dejean et al. 1995; Galindo-Leal et al. 2003). Larger bromeliads often shelter a higher species richness and abundance of invertebrates and vertebrates than smaller bromeliad species (Dejean et al. 1995). In the Bromeliad Species 2 however, width did not have a significant effect on frog presence (but on tadpole presence) which could be explained by that the sampled bromeliads had lower variation in width (overall being big) than had Bromeliad Species 1.

In general, deeper water cavities had a positive significant effect on the occupancy of *E. mysteriosus*. The need of water for amphibian survival makes them dependent on water availability. The depth of the bromeliads' water cavities were positively correlated with the width of the bromeliads, consistent with Fragoso and Rojas-Fernández (1996). Additionally, Zotz and Thomas (1999) report that larger bromeliads contain their water and humidity for longer periods than smaller bromeliads does in Panama. However, juveniles were not more frequent in bromeliads with deep water cavities in Bromeliad Species 1. This may be due to adult frogs using them for reproduction and therefore were more frequently present in bromeliads containing sufficient water for breeding whilst the juveniles were using them for feeding and therefore not dependent on how deep the water cavities are, only on water availability.

There were a higher presence of frogs in Bromeliad Species 1 receiving lower sun exposure (except tadpole- and adult presence), than in specimens with higher sun exposure. Higher sun exposure increases the UV-radiation, affecting the frogs negatively (e.g. Beebee & Griffiths 2005), by causing abnormalities in the skin affecting their possibility to breathe through their skin. However, Krügel and Richter (1995) performed a study on *Syncope antenori* (Microhylidae) in east-central Peru, where the presence of eggs and tadpoles were higher in bromeliads well exposed to sunlight (75%) compared to bromeliads in shade. In the present study, sun exposure was not found to have an effect on tadpole release nor on adult presence. Moreover, da Silva et al. (2011) found nine anurans using bromeliads in Brazil where bromeliads located at edges (exposed to sun) were more frequently occupied by anurans than bromeliads in the center of forests (in the shade). The difference to *E. mysteriosus* could be due to climate differences, since they are living in well-known dry habitats, making them less prone to “sunbathing” as species from rainforests tend to engage more in (da Silva et al. 2011). In the Bromeliad Species 2 sun exposure did not affect the frog presence, which could be explained by the Bromeliad Species 2 mainly growing in the D-area consisting of remnant dense forest and therefore had low variance of sun exposure.

In addition to the bromeliad quality variables the altitudes were also monitored for the individual bromeliads. Significantly higher presence of frogs were found on lower altitudes in Bromeliad Species 1 (except for presence all 6 times and tadpole presence) than on higher altitudes. However, this was not the case in Bromeliad Species 2. A possible explanation would be that the area where Bromeliad Species 1 grows are located at higher altitude than the area dominated by Bromeliad Species 2. That would be the probable reason for the lack of effect of altitude on occupancy in the Bromeliad Species 2 since they were already growing on an optimal altitude. The reason for the positive correlation in altitude and sun exposure could be due to the different vegetation types at the different altitudes. Higher up, bromeliads were mostly found on rock walls with low vegetation resulting in higher sun exposure compared to the lower altitudes mainly consisting of remnant forests with denser vegetation, where little sun perforate through the three canopy.

Another factor recorded was the density of bromeliads, which had no significant effect on the presence of *E. mysteriosus* in neither bromeliad species. It might be due to an overall low variation in bromeliad density in the areas, since they were mainly found in high numbers (clusters) and few were isolated. However, Donnelly (1989b) found that when increasing the bromeliad density the females and males increased in number, later on breeding increased as well as the number of juveniles in the poison dart frog *Dendrobates pumilio*.

5.2 Dispersal, feeding and breeding behaviour

The majority (72 %) of *E. mysteriosus* individuals stayed in the same bromeliad during the study of their dispersals. The mean dispersal distance of the territorial *Dendrobates pumilio* was found to be 20 m (Smith & Green 2005). The present study was performed during dry

months in Peru and the drought may make the frogs dependent on the bromeliads with water cavities more than during rainy months. However, it was discovered that the frogs could move up to 113 m which opens up possibilities of future habitat corridors, connecting suitable areas with wider bromeliads and plants containing deep water cavities for the frog species.

Caldwell (1997) stated that adult poison dart frogs primarily feed on ants, which is consistent with my observations.

There are numerous studies reporting on bromeliads being especially important in the reproduction, used for oviposition, tadpole development (Schiesari et al. 1996; Lannoo et al. 1987) and to complete metamorphosis (Donnelly 1989) in amphibians. Although, regarding the comparisons of tadpole releasing behaviour, not much articles have been found describing this in detail. The behaviour in *E. mysteriosus* does at least differ from one other Peruvian poison dart frog, who releases all their tadpoles at the same time in the same water cavity (pers. comm., Rainer Schulte, INIBICO, Peru). Nevertheless, the releasing of one tadpole per water cavity have been found in other poison dart frog species (Brown et al. 2008). The behaviour could be explained by space limitation in the water cavity, less competition of nutrients, decreased predation risk and avoiding of cannibalism (Brown et al. 2008). In terms of the breeding behaviour the positive correlation between depth of water cavities and bromeliad width are especially relevant since the adult frogs preferred to release their tadpoles in bromeliads that were bigger, which would then give tadpoles more water to develop in.

5.3 Conservation implications

The results show that the A-area had the most individuals, but the D-area had the highest occupancy rate in bromeliads. This might be due to the higher number of individuals encountered on ground in the A-area than in the D-area where most of the frogs were found inside the bromeliads. These two areas together with the B-area are core areas for the *E. mysteriosus* and should be the first targets for conservation efforts. The A-area is protected as reserve, which should be a priority for the other two as well.

But despite the protection as reserves slash-and-burn agricultural fires that run-away are common around the areas and most likely the biggest reason for possible extinction in the E-area and the great declines in the F-area. The A-area has also been affected by fires but are still harboring a large number of frogs. These fires need urgent controlling through conservation management of the areas.

Another big threat facing *E. mysteriosus* is deforestation. Both the frog-rich areas B and D are today threatened with total destruction giving way for plantations or grazing pastures. Species living in areas where the landscape is very fragmented have a higher risk of population isolation (Beebee & Griffiths 2005), which is happening in the areas right now due to the high deforestation pressure. If no conservation managements target these deforestations in the near future the possibility to create habitat corridors for the *E. mysteriosus* could get lost. Wider bromeliads (preferred by the *E. mysteriosus*) are older and since it takes years for them to grow big they are very sensitive for deforestation, forcing *E. mysteriosus* to use smaller bromeliads with higher risk of population declines. Galindo-Leal et al. (2003) found that bromeliads in Mexico were completely absent from young forests (10-40 years old). Various bromeliad species are old-growth forest specialists (Benzing 1980, cited by Galindo-Leal et al. 2003) and therefore sensitive to human activities eliminating forest structures. *Excidobates mysteriosus* is only found in old-growth forests and if the rest of the remnant forests are cut down the species will be confined to rock walls solely.

In conclusion, the endangered and endemic poison dart frog *Excidobates mysteriosus* was found with a higher presence on wider bromeliads than on smaller and with higher presence on bromeliads that had deeper water cavities than on bromeliads with shallower water

cavities. They were mostly found in the same bromeliad after recaptures. Adults and juveniles were eating small ants and flies. Finally adults preferred to release only one tadpole per bromeliad water cavity.

6 Acknowledgments

Many thanks to my great supervisor Dr. Karl-Olof Bergman for his enthusiasm and devotion to the project, Dr. Per Milberg for statistical consultation. Napoleón Monsalve Hoyos, Elí Monsalve Pasapera, Jhymmy Monteza Guevara, Jhonatan Monsalve Castillo and Manuel for invaluable field assistance. Family of Monsalve Hoyos Estela Castillo for their hospitality. Daniella Estela Castillo, Miroslava Blom for their outstanding support. Memories to my stalker and close friend Capitán. Thanks to Rainer Schulte giving me the opportunity to go to Peru for the thesis field work. Thanks to the three anonymous reviewers of the thesis. The study was financed by the Swedish International Development Cooperation Agency (Sida) as a Minor Field Study (MFS) and by the Linköping University, Linköping, Sweden.

7 References (Conservation Biology format)

- Beebe, T. J. C., and R. A. Griffiths. 2005. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* **125**: 271-285.
- Benzing, D. H. 1980. *The biology of Bromeliads*. Mad River Press Inc. Eureka.
- Bourne, G. R., A. C. Collins, A. M. Holder, and C. L. McCarthy. 2001. Vocal communication and reproductive behaviour of the frog *Colostethus beebei* in Guyana. *Journal of Herpetology*. **35**(supplement 2): 272-281.
- Brown, J.L., E. Twomey, V. Morales and K. Summers. 2008. Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. *Behaviour* **145**: 1139-1165.
- Caldwell, J. P. 1997. Pair bonding in spotted poison frogs. *Nature* **385**: 211.
- da Silva, H. R., A. L. G. de Carvalho, and G. B. Bittencourt- Silva. 2011. Selecting a Hiding Place: Anuran Diversity and the use of Bromeliads in a threatened Coastal Sand Dune Habitat in Brazil. *Biotropica* **43**(supplement 2): 218-227.
- Dejean, A., and J. Olmsted. 1997. Ecological studies on *Aechmea bracteata* (Swartz) (Bromeliaceae). *Journal of Natural History* **31**: 1313-1334.
- Dejean, A., J. Olmsted, and R. R. Snelling. 1995. Tree-epiphyte-ant relationships in the low inundated forest of Sian Ka'an Reserve, Quintana Roo, Mexico. *Biotropica* **27**:57-70.
- Donnelly, M.A. 1989a. Reproductive Phenology and Age Structure of *Dendrobates pumilio* in Northeastern Costa Rica. *Journal of Herpetology* **23**(supplement 4):362-367.
- Donnelly, M.A. 1989b. Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. *Ecological Monographs* **59**(supplement 3): 207-221.
- Duellman, W. E. 1999. Global distribution of amphibians: patterns, conservation, and the future challenges. Pages 1-30 in *Patterns of distribution of amphibians, a global perspective*. Duellman, W. E., editor. The Johns Hopkins University Press. Baltimore and London.
- Fragoso, C., and P. Rojas-Fernández. 1996. Earthworms inhabiting bromeliads in Mexican tropical rainforests: ecological and historical determinants. *Journal of tropical Ecology* **12**: 729-734.

- Galindo-Leal, C., J. R. Cedeño-Vázquez, R. Calderón, and J. Augustine. 2003. Arboreal frogs, tank bromeliads and disturbed seasonal tropical forest. *Contemporary Herpetology*. 12 September, No 1. ISSN: 1094-2246.
- Graves, B. M. 1999. Diel Activity Patterns of the Sympatric Poison Dart Frogs, *Dendrobates auratus* and *D. pumilio*, in Costa Rica. *Journal of Herpetology* **33**(supplement 3): 375-381.
- Icochea, J., K-H. Jungfer, S. Lötters, W. Arizabal, J. L. Martinez. 2004. *Excidobates mysteriosus*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org. Downloaded on 07 March 2011.
- IUCN 2010a. International Union for Conservation of Nature and Natural Resources. The IUCN Red List of Threatened Species. Available from: http://www.iucnredlist.org/documents/summarystatistics/2010_4RL_Stats_Table_1.pdf (accessed March 2011)
- IUCN 2010b. International Union for Conservation of Nature and Natural Resources. The IUCN Red List of Threatened Species. Available from: http://www.iucnredlist.org/documents/summarystatistics/2010_4RL_Stats_Table_8.pdf (accessed March 2011)
- Krügel, P., and S. Richter. 1995. *Syncope antenori*: A Bromeliad Breeding Frog with Free-swimming, Nonfeeding Tadpoles (Anura, Microhylidae). *Copeia* **1995**(supplement 4): 955-963.
- Lannoo, M. J., D. S. Townsend, and R. J. Wassersug. 1987. Larval life in the leaves: arboreal tadpoles types, with special attention to the morphology, ecology and behaviour of the *Osteophilus oophagopus* and *Osteophilus brunneus* (Hylidae) larvae. *Fieldiana Zoologica* **38**: 1-32.
- Lötters, S., K-H. Jungfer, F. W. Henkel, and W. Schmidt. 2007. *Poison Frogs: Biology, species & captive husbandry*. Chimaira, Frankfurt, Germany.
- Pellet, J., and B. R. Schmidt. 2005. Monitoring distribution using call surveys: estimating site occupancy, detection probabilities and inferring absence. *Biological Conservation* **123**: 27-35.
- Roitman, M. E. 1994. Male Territoriality and Female Mate Selection in the Dart-Poison Frog *Epipedobates trivittatus* (Dendrobatidae, Anura). *Copeia* **1**: 107-115.
- Sala, O. E., F. S. Chapin III, J. J. Armesto, R. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D.H. Wall. 2000. Global biodiversity scenarios for the year 2100. *Science* **287**: 1770-1774.
- Schiesari, L. C., B. Grillitsch, and C. Vogt. 1996. Comparative morphology of phytotelmonous and pond-dwelling larvae of four neotropical treefrog species (Anura, Hylidae, *Ostocephalus oophagus*, *O. taurinnus*, *Phrynohyas resinificatrix*, *P. venulosa*. *Alytes* **13**: 103-139.
- Smith, E., and H. Green. 2005. Dispersal rates of the territorial *Dendrobates pumilio* in the Amazonian Rainforest. *Journal of Herpetology* **4**: 613-615.
- Twomey, E., and J. L. Brown. 2008. Spotted poison frogs: Rediscovery of a lost species and a new genus (Anura: Dendrobatidae) from northwestern Peru. *Herpetologica* **64**(supplement 1): 121-137.
- Vitt, L. J., J. P. Caldwell, H. M. Wilbur, and D. C. Smith. 1990. Amphibians as harbingers of decay. *BioScience* **40**: 418.
- von May, R., A. Catenazzi, A. Angulo, J. L. Brown, J. Carrillo, G. Chávez, J. H. Córdova, A. Curi, A. Delgado, M. A. Enciso, R. Gutiérrez, E. Lehr, J. L. Martínez, M. Medina-Müller, A. Miranda, D. R. Neira, J. A. Ochoa, A. J. Quiroz, D. A. Rodríguez, L. O.

- Rodríguez, A. W. Salas, T. Seimon, A. Seimon, K. Siu-Ting, J. Suárez, C. Torres, and E. Twomey. 2008. Current state of conservation knowledge on threatened amphibian species in Peru. *Tropical Conservation Science* **1**(supplement 4): 376-396.
- Wake, P. R. 1991. Declining amphibian populations. *Science* **253**: 860.
- Walls, J. G. 1994. *Jewels of the Rainforests: Poison Frogs of the Family Dendrobatidae*. T. F. H. Publications, Neptune City, NJ.
- Wells, K. D. 2007. *The ecology & behaviour of amphibians*. The university of Chicago Press, London.
- Weygoldt, P. 1980. Complex Brood Care and Reproductive Behaviour in Captive Poison-Arrow Frogs, *Dendrobates pumilio* O. Schmidt. *Behavioural Ecology and Sociobiology*. **7**(supplement 4): 329-332.
- Young, B. E., K. R. Lips, J. K. Reaser, R. Ibáñez, A. W. Salas, J. R. Cedeño, L. A. Coloma, S. Ron, E. la Marca, J. R. Meyer, A. Muñoz, F. Bolaños, G. Chavez, and D. Romo. 2001. Population declines and priorities for amphibian conservation in latin America. *Conservation Biology*. **15**(supplement 5): 1213-1223.
- Zotz, G., and V. Thomas. 1999. How Much Water is in the Tank? Model Calculations for Two Epiphytic Bromeliads. *Annals of Botany* **83**: 183-192.