

Chytridiomycosis in Dominica: An Assessment of Field Practices

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ABSTRACT

Chytridiomycosis is becoming an ever-present threat to the amphibian community. I studied the actions being taken about the spread of the fungus Bd in Dominica. I also followed the conservation efforts for the frogs of Dominica, with the mountain chicken, *Leptodactylus fallax* being of top priority on the island. I focused primarily on the field work procedures. This involved sampling of anurans on transects, including experiencing it side by side with the scientists who regularly monitor the frogs. I found that the measures in use on Dominica seem to be up to par with other areas of the world, but there are still crucial steps that need to be taken for amphibian conservation.

INTRODUCTION

The idea of worldwide amphibian population decline is becoming a hot issue that is receiving some heated debate. According to recent research, the main cause for this epidemic is the chytrid fungus. The fungus commonly referred to as chytrid is actually the saprobic fungus *Batrachochytrium dendrobatidis*, Bd, and it is creating havoc throughout the herpetological and scientific communities (Hyatt, et al. 2007). As far as we know, the only vertebrates that Bd affects are amphibians.

The only amphibians found on the island of Dominica are anurans. This includes *Eleutherodactylus martinicensis*, *E. johnstonei*, *E. amplinympha*, and the critically endangered mountain chicken, *Leptodactylus fallax*. The mountain chickens on Dominica do not actually live in montane regions, but rather within 330 m of sea level (Martin, et al. 2007). The common name is derived from the populations on Montserrat, which are not suffering from chytrid but other influences such as volcanic activity (Garcia, et al. 2007). I am investigating what is being done regarding the spread and diagnosis of Bd on Dominica, focusing on the field transect work.

METHODS

A fellow student, Ashley Brooks, and I went out on the night of 30 May 2008 accompanied by Stephen Durand and Randolph “Ronnie” Winston from the Forestry and Wildlife Division of Dominica. Shortly after 20:00 h, we arrived at Soufrière Sulphur Springs for our chytrid transect. After stepping out of the van, we scrubbed our shoes in a bucket solution of Virkon and gathered all of our materials for the transect: pen, datasheet, soil moisture meter, ruler, individual swabs, disposable latex gloves, and headlamps. Johnson et al. (2003) determined Virkon to be one of the disinfectant solutions to be 100% effective in killing Bd. There are two parts to walking transects here in Dominica. The first is a visual encounter of frogs, followed by an aural inspection of frog calls. The walking of this particular transect consists of following the uphill trail 250 ft from the beginning of the trail. To aid in transect location and identification, particular trees have been previously spray painted with an identifying marker, to keep consistency of transect points. The first part consists of walking uphill for an hour while checking 2.5 m on each side of the midline of the trail.

Once at the end of the transect, the group walks back down the trail for up to 30 minutes, each person listening and keeping track in their mind as to how many individual frog calls that

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were heard. At the very end of the aural inspection, each person is polled as to the number of frog calls heard. The average number is calculated and recorded on the datasheet as the numbers of calls heard during the aural inspection.

At the very beginning of the transect, the top of the datasheet is filled out with the transect number and name, date, weather, signs of human disturbance, range (Northern, Eastern, Southern, or Western), observers, and phase of the moon (Fig. 5 & 6). Upon starting both the visual and the aural encounter surveys, data for ambient humidity, ambient temperature, and soil moisture is taken and recorded on the datasheet. The start and stop signs are recorded as well. Signs of livestock, feral dogs, and/or feral cats are also noted on the sheet. This practice seems to stem from when it was originally believed that the feral cats might be the cause of death to the mountain chicken, *Leptodactylus fallax*.

An individual *Leptodactylus fallax* was overheard coming from a southerly direction from the trail. Once done with our transect, we drove up and around the corner to where the call could be coming from. We were close to the calling male, but we only stopped briefly to take an audio recording of the call of *L. fallax* using a microphone, tape recorder and headphones.

When a frog is spotted during the visual encounter survey, a new pair of latex gloves are put on by the handler for each frog seen in order to avoid possible contamination of Bd any further. Once caught, a small area on the ground is cleared of leaf litter and other debris. The utmost care is taken in handling the anurans to prevent damage to their delicate bodies. The frog is visually checked for any disease related markings, wounds, or abrasions. The species is also recorded. Frogs are then rubbed in a circular fashion on the back of the legs enough to get some material for Polymerase Chain Reaction (PCR) testing, using two separate swabs (Fig. 1). Each of the swabs are to be numbered as 1A, 1B; 2A, 2B; etc. This practice initially began when Dominica would send one sample to the Zoological Society of London for molecular diagnostic testing while keeping the second in country in case problems arise with the first sample sent. As of now, the island has their own Molecular Diagnostic Testing facility, located in the Botanical Gardens of Roseau, Dominica, with Trevorne Douglas serving as technician. The two samples from each frog are still taken and analyzed, but it is now a matter of an increase in the accuracy of taking multiple samples and performing multiple tests.



Figure 1: Swabbing legs of *E. martinicensis*



Figure 2: Measuring SVL of *E. martinicensis*

As the frog is held still, a ruler is used to measure the SVL, snout-vent length (cm), while being cautious to not touch the ruler to the frog at all, avoiding possible contamination (Fig. 2). Meanwhile, identifying features and markings are recorded. The behavioral health and habitat of the frog, including activeness, substrate and/or plant species where it was found on, and distance above or below the ground, is recorded. The number of frogs seen are recorded whether or not they are caught and/or swabbed. In addition, frogs too tiny to hold are usually discarded from the data and are not caught or swabbed.



Figure 3: Mating pair of *E. martinicensis*



Figure 4: Male *E. martinicensis* calling

The first frogs spotted were a mating pair of *Eleutherodactylus martinicensis* on a mango leaf (Fig. 3), but they were left alone and not swabbed. Randolph Winston and Stephen Durand caught two of the frogs that were swabbed. Both Ashley Brooks and I tried our hands at catching, and holding onto, frogs. It is a difficult task if one is not practiced in it, but I caught a calling male that was perched one meter above the ground on a mango plant stem and it was successfully swabbed. A total of 9 *E. martinicensis* were reported as seen for tonight's transect. Of those, swabs were collected for 3 of them. The average number of calls reported during the aural encounter survey was 39. After the transect was completed, we went back to the vehicle and scrubbed our shoes again with Virkon prior to departure.

RESULTS

I assisted in doing the PCR assays for the frogs we swabbed that night. I am happy to say that all of our frog samples tested negative for Bd.

The original epidemic was first noticed by civilians who saw mountain chickens exhibiting odd behavior such as sitting out in the open or in puddles of water. Shortly thereafter, usually within 48 hours, the frogs were found dead. When the chytrid epidemic began on the island in 2002, selected transects were walked once every month. As of now, there are two main transects used that are associated with the recent habitat of the mountain chicken, *Leptodactylus fallax*. Transects are walked once every two months in different areas.

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The measures in use on Dominica seem to be up to par with other areas of the world, but there are still crucial steps that need to be taken for amphibian conservation. Such steps include further community involvement and education in the schools.

Datasheet for monitoring Dominican amphibians on transects

Transect Number and Name: Soufriere Sulphur Springs
 Date: 30/5/08

Weather (tick box)

Clear	Cloudy	Overcast	Wind	Rain
		✓		

Human disturbance (tick one or more boxes)

Litter	Footprint	Transect markers	Burning/Fire	Report	Heard	Met
	✓					

Range: Southern Range Observers (list all names): Ashley Brooks, Diana Warrandorf, R.W, SD, A

Phase of the moon (tick one)

New	First quarter	Half	Three-quarters	Full
			✓	

Feral Dogs

Report	Heard	Seen

Feral Cats

Report	Heard	Seen

Livestock

Report	Heard	Seen

	Start Time	Stop Time	Ambient Relative Humidity (%) at Start	Ambient Temp (°C) at start	Soil Moisture
Visual Encounter Survey	19:17	20:34	66	27.0	2.0
Aural Encounter Survey	20:41	21:11	73	25.7	1.0

	Total Crapaud	Total <i>E.johnstonei</i>	Total <i>E.amplinympha</i>	Total <i>E.martinicensis</i>
Visual Encounter Survey	0	0	0	9
Aural Encounter Survey	1	0	0	39

General Comments
 Use this space for additional information (e.g. frogs seen or heard on the way to or from the transect)

Saw 6 Martinicensis - 5 escaped

Figure 5: Front page of datasheet for transects

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Transect Number and Name: *Sophies Sun / Peter Springs*

Date: *3/7/08*

For non-transect sites:
 GPS co-ordinates:
 Location (e.g. nearest village):
 Name of FWD staff:

Frog no.	Species code	SVL (cm)	Weight (g)	Sex M/F?	Health notes (i.e. healthy = tick box, or note abnormality e.g. lethargic)	Behaviour notes (e.g. in water, on land, calling, nesting)	Appearance notes (e.g. colour, spot shape, markings)	Swab FWD (A)	Swab ZSL (B)
1	MART	2.0	Nd	M	Healthy	Calling on a Mango plant - 2-3 m water above ground	Dark brown on back - Pow heel smelt	A1	B1
2	MART	2.2	Nd	M	Healthy	Calling on a Mango plant 2-3 m water above ground	Dark brown on back - double chevrons - pointed snout	A2	B2
3	MART	2.5	Nd	M	Healthy	Calling on a Mango plant 2-3 m above ground	Dark brown, white on back, black patch on vent, rounded snout	A3	B3
			Nd						
			Nd						

	Chytrid seen & not caught	<i>E. joblotovae</i> seen & not caught	<i>E. meridiensis</i> seen & not caught	<i>E. anolisophila</i> seen & not caught	Unidentified Eleutheroctylid frogs seen & not caught
Tally count	0	0	6	0	0

Figure 6: Back page of datasheet for transects

DISCUSSION

Since Bd is known to be a water-borne, saprobic fungus, it is interesting to point out that none of the four species of anurans on the island produce tadpoles in the water. *Leptodactylus fallax* is a foam nester and all of the genus *Eleutherodactylus* have direct development. It has been previously suggested that the fungus attacks the keratinized tissues of the tadpoles and then carries over to the adult anurans (Briggs, et al. 2005). Much is still not known about the mechanisms used by Bd to attack these tissues, though. It is widely believed that the fungus prevents gas exchange across the cutaneous membrane, as well.

Research from Berger, et al. (2005) suggests that Bd strains from multiple continents around the world are closely related genetic clones, further supporting the idea that the epidemic arose from a common source. This is an interesting theory that is still in debate, especially since older specimens from the 1970s and even earlier are being shown to be positive for Bd. More research should be done on these older strains in order to come to further conclusions.

Outside of governmental procedures, an audio recording of *Leptodactylus fallax* was done as part of the project. Its call had a 'whop' or 'whawp' sound. It is important to capture vocalizations along, with the frogs themselves, for future education and use in identification.

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This is particularly of concern if a species takes a long time to recover, but no one is around anymore who is able to recognize the calls. Sampling for identification of species through current molecular data may not contribute to phylogenetic accuracy, but can increase the accuracy of identification of these organisms for present and, hopefully, future generations. Further research in these areas should also help to solve problems in unsure identification of species commonly occurring due to intraspecific morphological variation.

I learned a very intriguing development during my correspondence with Arlington James of the Forestry & Wildlife Division of Dominica. Apparently when some live *Leptodactylus fallax* frogs were sent to the London Zoo for their Captive Breeding Programme in July, the PCR tests for Bd were negative each month. That is, until a few months later in November when positive tests were found for the fungus. It is still unknown as to the cause of this unexpected appearance of Bd in the captive bred frogs.

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Figure 7: Group picture, lowest to highest: Stephen Durand, Diana Warrendorf, Ashley Brooks, and Randolph Winston.

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