SAMPLING PROTOCOL FOR ASSESSING PREVALENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS*

INTRODUCTION

The B.C. Ministry of Environment Wildlife Health program is conducting a survey to assess prevalence of *Batrachochytrium dendrobatidis* (*Bd*) in amphibians in B.C. This survey will provide baseline information to develop management plans for this emerging threat to amphibians. The information from this survey will also be used in collaborative research with academia on ecological and environmental factors that influence *Bd* prevalence and *Bd* emergence.

In 2008, we will collect data on the prevalence of *Bd* using swab samples collected from juvenile and adult frogs, toads and some salamanders. We want to collect samples from all frogs and toads in B.C. with particular emphasis on species of conservation concern (Conservation Data Centre and COSEWIC listed species). We will also focus on widely-distributed species such as the Western Toad, Columbia Spotted Frog, and the Wood Frog for regional comparisons of *Bd* prevalence. *Bd* seems to affect salamanders to a lesser degree than frogs and toads. We will collect swab samples from Roughskin Newts which are known to carry *Bd,* Pacific Giant Salamander and Tiger Salamanders.

SITE SELECTION

A site is roughly defined **as an area within the maximum movement distance of a given amphibian species**, where the population can be expected to freely intermingle, exchanging parasites and pathogens. For not-at-risk species, **we would like a minimum of 10 and a maximum of 30 samples of each species at a site**. For rare and endangered species it may not be possible to meet the minimum number and we would like as many swabs as possible. You may collect different species at different sites within a region, choosing locations where each species is most abundant. If possible, all 30 samples should come from one site but multiple sites can be sampled for each species within a region.

The attached **Amphibian Hygiene Protocol** should be followed during fieldwork and sample collection.

ANIMAL HANDLING

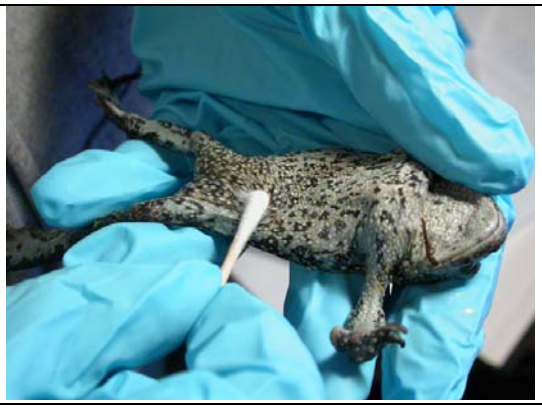
Animals should be captured and handled using fresh disposable gloves (non-powdered vinyl or nitrile preferred) for each animal and should be placed in individual containers (ziplock bags) prior to swabbing. Do not place multiple animals in the same container as a single infected animal can contaminate others. Dipnets used to capture animals can be rinsed in the pond water between animal captures.

The swab sample for the *Bd* analysis should be obtained **before** other procedures such as weighing and measuring. Callipers and weighpans should be wiped down with ethanol and rinsed with distilled water to decrease the risk of transferring *Bd* spores between animals. While 70% ethanol may prevent the spread of live *Bd* spores between animals, it does not destroy the *Bd* DNA. Rinsing with distilled water decreases the chances of residual DNA contamination and also prevents the residual ethanol from irritating sensitive amphibian skin tissue.

The attached Amphibian Hygiene Protocol recommends the use of bleach for decontamination between sites. Residual bleach even in minute concentrations can destroy DNA and irritate amphibian skin tissue. Therefore, all equipment has to be thoroughly rinsed with clean water and/or completely dried following decontamination procedures.

SWABBING PROCEDURE

Hold the animal in one hand using new gloves for each animal. Using the swab provided gently but firmly rub the tip of the swab against the abdomen, drink patch (underside of the pelvic region), all limbs, feet, webbing, and toes swabbing 3 – 5 times in each area. **Follow a standard sequence of swabbing** starting with the abdomen, drink patch, legs, webbing and toes. The feet, in particular the toe tips and tubercles, seem to have higher densities of *Bd* sporangia although the density is highly variable. Following a sequence ensures that each area is sampled in all the animals and assists in minimizing transfer of spores from high density areas to lower density areas on the animal body.



Photograph courtesy of Dr. Lauren J. Livo, Department of Integrative Physiology, University of Colorado.
To see a video of the swabbing procedure check out the video tab at the Amphibian Ark Website
http://www.amphibianark.org/frog_gallery.html
Field sampling techniques for living amphibians
And
How to swab a frog for chytrid
Or email Purnima.Govindarajulu@gov.bc.ca

The head of the swabs should be broken off into the 1.5 - 2 ml sterile, screw-top vials provided and stored in a cool and dry place. Protect from direct sunlight. Note that some of the video talk about preserving the swab tips in ethanol. This is not necessary for the protocol we will be using. The swabs can be stored in a dry tube for up to 18 months.

Each vial should be labelled according to the instructions below. All the vials from one site should be placed in one Ziploc bag (provided). Include the filled-in data form in the Ziploc bag. Label the Ziploc bag with the MOE Region, location name and code, and your name.

SAMPLE LABELLING

Use the permanent ink marker provided to write directly on the white label area on the vials. When you have finished labelling, please protect the labelling with a piece of scotch tape wrapped around the vial and over the label. This will prevent the writing from being rubbed off by accident.

The vials should be labelled according to the following format:

Sample Label

R01-PG01
BUBO 01
23/4/08

1. Region ID and Site ID

   Region ID (Map of MOE regions attached). In the example above, R01 indicates MOE Region 1. This is followed by the SiteID which starts with the initials of the name of the collector (in the example above, PG stands for Purnima Govindarajulu). This is followed by the site number. The sites are numbered sequentially in the order in which they are visited. On your subsequent returns to the site, please ensure that you retain the same Site ID and number. This Site ID should match that used in the data forms.
2. Species ID and Swab ID

   For Species ID use the RISC Code abbreviation given in the table below. For the Swab ID use a two digit number assigned to individual swabs, starting at 01 with each swab after that numbered sequentially.
3. Date (day/month/year)

Species ID Codes (RISC Codes) for use on sample labels

Scientific Name	English Name	RISC Code
Ambystoma tigrinum	Tiger Salamander	AMTI
Ascaphus montanus	Rocky Mountain Tailed Frog	ASMO
Ascaphus truei	Coastal Tailed Frog	ASTR
Bufo boreas	Western Toad	BUBO
Dicamptodon tenebrosus	Pacific Giant Salamander	DITE
Pseudacris maculata	Boreal Chorus Frog	PSMA
Pseudacris regilla	Pacific Chorus Frog	PSRE
Rana aurora	Red-legged Frog	RAAU
Rana catesbeiana	Bullfrog	RACA
Rana clamitans	Green Frog	RACL
Rana luteiventris	Columbia Spotted Frog	RALU
Rana pipiens	Northern Leopard Frog	RAPI
Rana pretiosa	Spotted Frog	RAPR
Rana sylvatica	Wood Frog	RASY
Spea intermontana	Great Basin Spadefoot	SPIN
Taricha granulosa	Roughskin Newt	TAGR

SHIPPING

Please store samples in a cool, dry place until ready for shipping. Ship all your samples together at the end of the collecting season. Ship samples in a well padded envelope or box to prevent vials from being crushed or broken. Samples can be mailed or couriered but please ensure there is some way of tracking the package in case they should go missing in transit. Please contact us if you need assistance with shipping samples.

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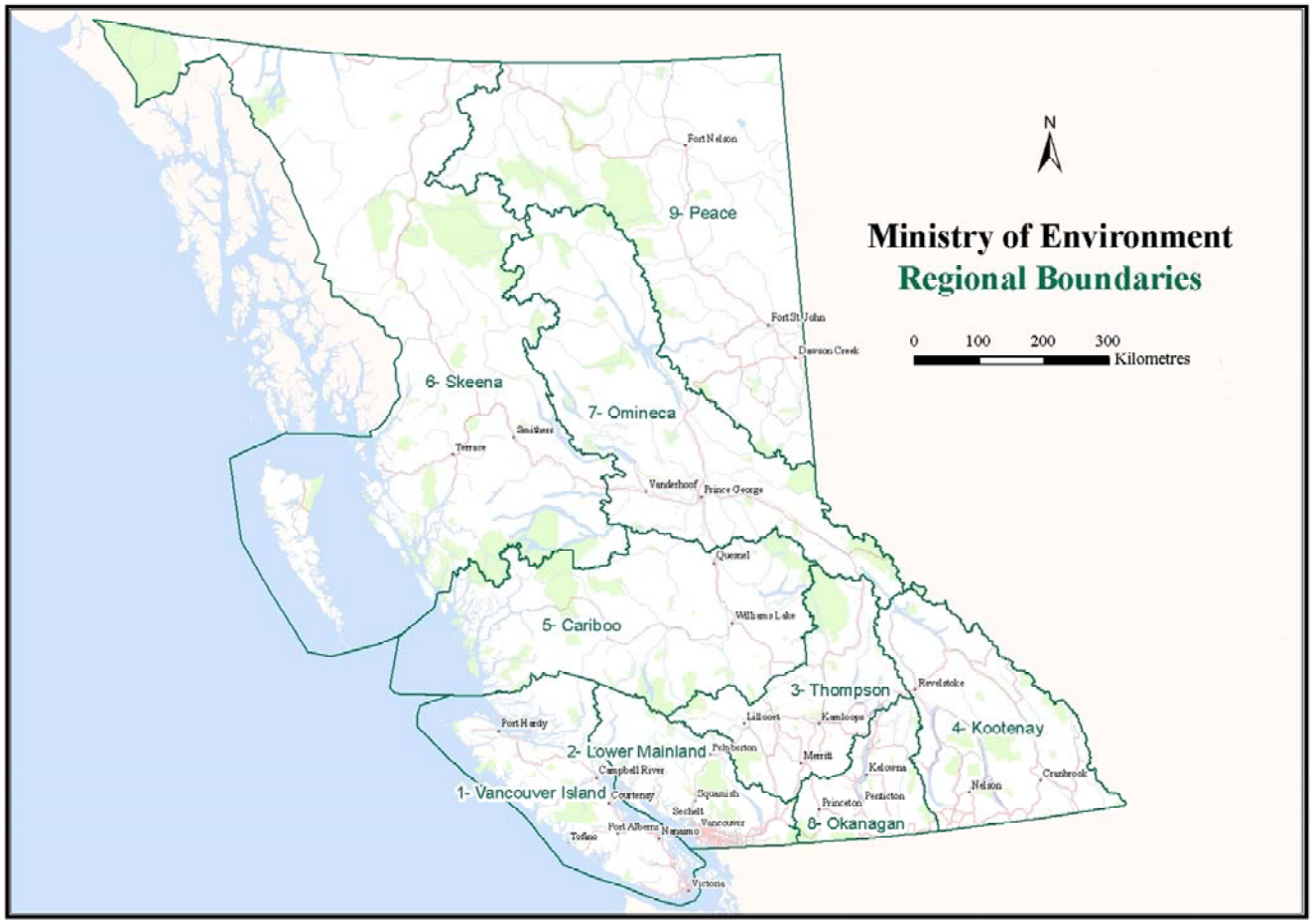
Thank you for our valuable assistance with this project, we couldn't do it without you!



DATA FORM – *BD* PREVALENCE ASSESSMENT IN B.C.

Name of Primary Contact: **Crew:**					
Address:					
Telephone No.:		**Email address:**			
LOCATION INFORMATION					
Location Name		**Region or nearest town**		**Date and time**	
Latitude/ UTMN		**Longitude/ UTME**		**UTM Zone Datum**	
SITE DESCRIPTION					
Site type (check box)	River	Stream	Lake	Isolated Pond	Terrestrial
Site Size	Width:	Length:	Depth:		
For terrestrial sites Indicate % cover	Forest	Shrub and bushes	Grassland	Urban/rural	Other (specify)
For wetland sites indicate substrate % cover (*best estimate)*	Fines (<0.5mm)	Muck (< 1mm)	Fine detritus (1–5 mm)	Coarse detritus (5-150 mm)	Sand (0.5-2mm))
	Small gravel (3-10 mm)	Large gravel (11-100 mm)	Cobble (101-300 mm)	Boulder (>300mm)	Bedrock (unbroken)
	Wood	Mineral soil / mud	Emergent vegetation	Submergent vegetation	Other (specify)
HUMAN USE ON SITE					
Human Presence (check box)	No evidence of human use	Some evidence of human use but no obvious impact		Evidence of heavy human use and impact	
Wetland use (check box)	Recreational fishing/boating	Swimming	Water supply	No obvious human use	Other (specify)
Upland use (100 m radius) (Indicate %)	Residential/ Urban(specify)	Agriculture/ Grazing (specify)	Logged forest	Undisturbed ecosystem	Other (specify)
BIOLOGICAL COMMUNITY					
List native fish, birds, water birds, reptiles, amphibians that you observe at the site.					
List introduced species that you observe, including bullfrogs, introduced fish or plants					

Details of Amphibian Swabs							
Date	**Swab Id** Match vial ID	**Species Code**	**Stage** *(adult, juvenile or larval)*	**If possible**		**Behaviour Appearance** (*Healthy? Normal?)*	**Notes** (*unusual collection methods, etc)*
				Sex	**SVL**		

Additional Comments

Thank you for collecting samples and completing the data form

Please enclose this data sheet in the Ziploc bag that contains the samples from this site.