

WINDSOR RESEARCH CENTRE

Amphibian Handling Protocols

(with particular reference to CEPF-funded project, “An Action Plan to Save Threatened Biodiversity in Catadupa”)

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Direct handling of wildlife, with the specific focus of determining presence and prevalence of an infectious disease carries risks to each animal handled, to the community of focal species, and to the human researchers. Bio-security is of paramount concern and the following measures will be followed to reduce risk of disease transmission and spread:

The principal investigator will directly carry out or oversee all animal handling. Non-lethal superficial skin sampling will be used to test for presence, infection loadings of individuals and prevalence in the population of *Batrachochytrium dendrobatidis* (Bd) in the frog and toad populations of the Catadupa Key Biodiversity Area. We will follow protocols which have been developed in order to minimize the possibility of transmission of the pathogen among individual amphibians and amphibian communities and to avoid contamination of samples. During a sampling session, one person will be responsible for capture and handling, collecting the fungal sample, and taking morphometric measurements. A second person will be responsible for keeping sterile items, such as vials and swabs, protected from contamination, and recording all data. Both persons will wear latex or nitrile gloves. The person handling the amphibians will change gloves between every animal to reduce the possibility of transmitting Bd among handled animals and to prevent contamination across samples. While alcohol hand sterilizer will kill the pathogen, it also preserves the pathogen's DNA. Preserved DNA on the hands could contaminate subsequent samples, thus producing potentially false- positive results.

Capture: Only one person will capture amphibians during a sampling session. If the collecting process causes dirt to adhere to an animal's skin, it will not be washed – this could rinse free zoospores off the skin and reduce chances of detecting light infections and risk introducing amounts of the pathogen into the environment. The proper and accepted restraint method calls for the investigator to gently hold frogs and toads by the hind legs and let it rest in the hand; animals will not be cupped in the hand, as the warmer temperature of the hand may cause undue stress to the animal. Once positioned, a sterile swab will be wiped in a sweeping motion along each rear foot, the ventral surface of each thigh, and the ventral abdominal surface. Each area will be swabbed five times, for a total of 25 sweeps per individual. Each swab will be preserved in an individual ethanol-

filled vial, which will be properly labeled. Each individual also will be examined visually and scored for signs of Bd infection, including reddened or discoloured skin, excessive shedding of skin, and unusual posture.

After Bd samples are properly stored, the amphibian will be placed temporarily in a new plastic bag to enable body measurements and weighing while ensuring that equipment is not contaminated and infectious particles are isolated in the bag. Body measurements will be taken using dial calipers (NB, these can be sterilized, unlike digital calipers) and weight will be collected by placing the bagged-amphibian on the metal tray of an electronic balance (accuracy 0.01 g). All non-reusable equipment will be properly disposed under local bio-hazard guidelines and reusable equipment (e.g., calipers) will be sterilized after each sampling session. Alcohol and flame will always be available for decontamination during a sampling session if accidental contamination should occur.

All animals will be photographed with a digital camera for identification purposes and immediately released at the site of capture. No animal from this study will be restrained or otherwise temporarily held while waiting to be processed since only one individual will be sampled at a time. All effort will be made to identify each animal to species so that all individuals may be returned to the wild. However, individuals which prove so difficult to ID or are so different that the animal might prove to be potentially new to science will be collected as voucher specimens. In this case, up to five individuals may be collected throughout the length of the study and would be deposited at the Institute of Jamaica. In the case that collections are warranted, these will only occur after proper permits are secured. We do not foresee the need for animal immobilization, anesthesia, analgesic administration, or any other technique that should only be performed by a veterinarian. However, WRC has worked with a Jamaican veterinarian on other projects (e.g., radio-telemetry of snakes) and will consult him if warranted.

The following decontamination procedures will be adhered to in order to minimize the risk of spreading Bd and to avoid contamination of the field vehicle: Researchers and field assistants will wear non-porous, rubber, Wellington-type boots. Before leaving a study site, all organic (e.g., leaves) and inorganic (e.g., dirt) will be brushed, scrubbed, or removed from the vehicle, clothing and footwear. All footwear will be removed outside of the vehicle and placed in a plastic box located inside the vehicle and having a close-fitting lid.

On arrival at the field station, 6% sodium hypochlorite (household bleach) will be added to the plastic box to soak the footwear for a minimum of 10 minutes. Footwear and the box will subsequently be dried in direct UV sunlight for a minimum of three hours. The bleach solution will be disposed of as per product label instructions to avoid contamination of the environment.