A Guide to Saving Frogs:
Creating a Standard Protocol for El Valle Amphibian
Conservation Center Operations

Final Report
Michelle Reeves and Karen Wang
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McGill University
in affiliation with Smithsonian Tropical Research Institute
Contact Information

Home Institution
McGill University  
845 Sherbrooke Street West  
Montreal, Quebec  
H3A 2T5

Affiliate Institution
Smithsonian Tropical Research Institute  
Apartado Postal 0843-03092  
Panamá, República de Panamá  
Phone: +507 212-8222  
email: ibanezr@si.edu

Host Institution
El Valle Amphibian Conservation Center (EVACC)  
El Nispero Zoo, El Valle de Anton  
Coclé, República de Panamá

Supervisors
Edgardo J. Griffith and Heidi L. Ross  
Phone: (+507) 66768094  
(+507) 66768038

Mailing address: G0284 Panama Express, 8619NW,  
68 ST, Miami,  
Florida, USA  
33166-5977

E-mail: rossheid@yahoo.com
A Thank You note should be sent to these coordinates addressed to Edgardo Griffith and Heidi Ross.
Host Institution: El Valle Amphibian Conservation Center

El Valle Amphibian Conservation Center is in El Nispero Zoo of El Valle de Antón in the province of Coclé. It is located 126 km from Panama City.

Location of El Valle de Antón, Coclé. (Source: http://www.antonvalleyhotel.com/location.htm)

Opened in 2007, El Valle Amphibian Conservation Center is one of two captive breeding facilities for endangered amphibian species in Panama. Situated within the El Nispero Zoo, the center is home to amphibians of roughly 33 different species, 12 of which are “priority species,” recognized as such because of their endangered status. They house some of the last remaining golden frog individuals and are attempting a captive breeding program with this species, which is now presumably extinct in the wild. This breeding program is becoming quite successful, as they have recently hatched 42 golden frog juveniles. The publicly accessible exhibition of the center is the only place in Panama where this national icon can still be seen. Apart from the exhibition, the center also has a quarantine lab and a main building divided into a laboratory and three separate rooms for different species of amphibians.
Edgardo Griffith is a graduate of the University of Panama in biology, and has always had a passion for amphibians. The conservation project started in 2006, when the deadly (to amphibians) chytrid fungus hit El Valle de Antón one year earlier than expected; it was being run out of a hotel room at the Hotel Campestre in El Valle. Construction finished on the current building in El Nispero in 2007, and the first frogs were transferred. The building was constructed with help from The Houston Zoo and other partners.

EVACC is funded in part by the Houston Zoo, Minera Panama and receives donations from a consortium of zoos in America. It is also affiliated with the Smithsonian Tropical Research Institute, which employs three people working at EVACC, and Canadian engineering company SNC-Lavellin.

EVACC has its own website in both Spanish and English (amphibianrescue.org) that was visited by over 25,000 unique viewers in 2011. The conservation center accepts volunteer applications and donations through their website, which plays a crucial role in international outreach.

The field site that serves as the source for most of the center’s amphibians is the Minera Panama mine site, near Penonomé. Many animals are threatened by habitat loss due to the construction of the mine.
Location of El Nispero Zoo, and therefore of EVACC, is circled in red on this map of El Valle de Antón. (Source: Antonsvalley.com)

**Number of equivalent full days spent on the project in Panama: 38**

Tour of the conservation center: January 10, 11

Literature review on chytridiomycosis: January 17, 18

Literature review on *Rhabdias spp*: January 28, 29, 31, February 1-4, 21, 22

Writing permit applications for ANAM and IACUC: February 5, 6, 8

Mining safety session: February 7

Work on informal presentation: February 28

Informal presentation: March 1

Interviews for standard operating protocol: March 18 – 23, April 11, 12, 13, 18
Editing standard operating protocol: April 14-17, 19-24

**Number of equivalent full days spent in the “field” (in El Valle): 24**

Tour of the conservation center: January 10, 11

Literature review on chytridiomycosis: January 17, 18

Literature review on *Rhabdias spp*: January 28, 29, 31, February 1-4, 21, 22

Writing permit applications for ANAM and IACUC: February 5, 6, 8

Mining safety session: February 7

Interviews for standard operating protocol: March 18 – 23, April 11, 12, 13, 18

**Budget Breakdown**

Number of trips to El Valle: 8

Price for round trip bus ticket to El Valle: 8.50$

Price of food for an average day in El Valle: 10$/day

Cost of materials for project 23$

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Total funds spent on this internship: 448$
Background information

A Standard Operating Protocol (SOP) is a set of written instructions that documents a routine or repetitive activity (EPA 2007). One was never written for EVACC due to a lack of time and personnel. However, every conservation center should have the methods for all of their protocols in written form, to be used as a reference for new and current employees. As interns, we were given the task of collecting all the relevant information to put together a comprehensive user’s manual to the center. In order to do this, we first had to understand the inner workings of the center for ourselves. We did extensive research on the main diseases plaguing amphibians as well as on amphibian husbandry theory and practices.

There has been a spread of *Batrachochytrium dendrobatidis* (Bd) in wild populations of amphibians in Panama. When amphibians are brought in to the conservation center, there are a specific set of procedures that must be followed to ensure the detection, isolation, and treatment of diseases. Employees much follow hygienic and safety protocols in order to ensure the continued maintenance of captive bred populations.

The Fight Against Chytridiomycosis

Native to South Africa, the *Batrachochytrium dendrobatidis* fungus has been running rampant through the Neotropics since the 1980s (Lips 2005). It was spread throughout the world by *Bufo bufoniformis*, also native to South Africa, being shipped all over the world as a means to carry out pregnancy tests (Griffith, pers. comm 2013). When these amphibians were no longer needed for these tests due to technological advances, they were widely released into various environments where they quickly became invasive. This lead to the Bd fungus being spread throughout the
world at a steady rate. The complete mechanism of dispersal is not fully understood yet. However, there are relatively accurate ways of predicting when it will arrive in an area. Today, the fungus is present on all continents except Antarctica.

Its spread through Central America was particularly fast, resulting in its arrival in El Valle one year earlier than expected. The dates of its arrivals are pictured below:

Figure 1. Lips et al. 2006. PNAS 103(9):3165-3170.
Batrachochytrium dendrobatidis is the only fungus in its phylum that is known to cause disease in vertebrates (Berger et al 2005). It infects the skin of amphibians, where it releases toxins (Berger et al 2005). This infects the keratin layer and causes eventual inflammation of the pelvic patch, which reduces the skin’s ability to absorb moisture (Berger et al 2005). In tadpoles, the mouthparts are the only part of the body that is keratinized. If the fungus attacks tadpoles, it causes oral deformities before and after metamorphosis (Griffith pers. comm. 2013).

The chytrid fungus’ life cycle includes two main stages (Berger et al 2005). The first is mobile: the round, flagellated zoospores are transmitted through water. Once an amphibian absorbs the zoospore, it becomes a developing organism. This organism produces a zoosporangium, which in turn releases zoospores. The entire life cycle takes 4 to 5 days in vitro at 22 degrees Celsius, and it is assumed that this time frame is identical in the wild (Berger et al 2005).

The spread of this deadly fungus is the reason the conservation center was founded in the first place. Therefore, it was important that our final product - EVACC’s standard operating protocol - featured an extensive explanation of its origins, symptoms, diagnosis and treatment. Importance
was also given to the several measures of biosecurity that are in place at the center to prevent further spread of the fungus.

**Preventing and Treating *Rhabdias spp.***

The second largest threat at the conservation center is the parasitic nematode from the *Rhabdias* genus. It uses frogs and toads as a host in its direct life cycle (Gendron *et al* 2003). It is of great concern to the conservation scientists at EVACC because there have been a high number of incidences of *Rhabdias* eggs or larvae found in frog fecal samples of various species. *Rhabdias* has a direct life cycle, which means it only requires one species to propagate. The threat of infection may be amplified in a conservation center because of the stress of captivity combined with an enclosed environment and a high density of potential hosts. Adults can survive a small amount of *Rhabdias* infection, but tadpoles may be susceptible to more severe consequences such as deformed or absent limbs after metamorphosis. It is crucial that staff members adhere to a standard cleaning and maintenance procedure to minimize the risk of spreading the parasite. Our initial project involved investigating a detection method for *Rhabdias spp.* in tadpoles, and later a treatment method. However, the necessary permits for this experiment proved too time-consuming to obtain. Currently at the center, tadpoles are being treated with a prophylactic, arbitrary dose of Levamisole. The experiments to find out more about the nematode in tadpoles are still a priority for EVACC.
Diagram of the *Rhabdias spp.* life cycle (source: Gendron et al. 2003)

**Reason for project**

It is common practice for conservation centers to have standard operating procedures. Written guidelines are of utmost importance to ensure the clarity of rules and practices at the center. With the increasing amount of staff at El Valle Amphibian Conservation Center, there has been a greater need for a standard operating procedure to serve as a reference manual for employees. An SOP allows regularly carried out activities to be done correctly and consistently. It is a means of keeping the valuable work done at EVACC efficient and continually improving. In previous years, a new staff member has taken weeks to train, due to the intricate nature of the work. With a complete and comprehensive Standard Operating Protocol, new employees can read up on and study even before their first day. Furthermore, supervisors can refer to it when training new employees to ensure that all sections are covered. The SOP will provide a strong basis of
knowledge and can be used as a reference by anyone at the center. It will cover all activities carried out at the center; everything from how to change a water filter, to how to raise cockroaches, to how often to feed each species will be discussed in detail. A table of contents is presented below.

Tabla de contenidos

Advertencias de Salud y Seguridad
  Salud
  Seguridad

Higiene de empleados

Limpieza
  De la exposición
  Del centro
  Limpieza de tanques

Mantenimiento de registros

Mantenimiento de Tanques
  Tanques de vidrio
  Tanques de acrílico

Luces UV
  Gluconato de calcio

Nutrición
  Protocolo de nutrición
  Moscas y otro insectos
    Drosophila hydei
    Drosophila melanogaster
    Collembolas
    Grillos
    Cucarachas
    Superworms
    Mealworm
  Dieta específica de cada especie
    Atelopus
    Hylidae
    Hemifractus fasciatus
    Otras especies
Tabla de nutrición

Parasitología y Enfermedades
   El hongo quitrido
   Rhabdias spp.
   Hipocalcemia
   Tratamiento preventivo de salud
   Tratamiento de apoyo
   Formulario médico de EVACC

Cuarentena
   Protocolo de cuarentena
   Bioseguridad

Apareamiento y Crianza
   Especies que se reproducen en corrientes de quebrada
      Atelopus spp.
   Especies que se reproducen en charcos o agua estancada
      Atelignis lemur
      Sachatamia ilex
   Especies que se reproducen en huecos de árboles
      Anotheca spinosa
      E. midiaria
      E. laborum
   Especies marsupiales con bolsa
      Gastrotheca cornuta
      G. nicefori
   Especies marsupiales sin bolsa
      Hemifractus fasciatus

Filtración y Calidad de Agua
   Fuentes de agua
   Reemplazo y mantenimiento de filtros de carbón activado
   Que hacer en caso de perdida de presión de agua
   Notas importantes

Exposición

Protocolo de Emergencia

Glosario
There are many factors that can increase an animal’s stress when held in captivity, which in turn can reduce its resistance to disease (Coborn 1992). Breeding amphibians in a conservation center can pose even greater threats due to the high concentration of amphibians in a confined space (Wright and Whitaker 2001).

**Methodology**

I. Study site

All firsthand information was collected from El Valle Amphibian Conservation Center in El Valle de Anton, Coclé. The literature search was conducted in the Smithsonian Tropical Research Institute Library in Panama City.

II. Literature search

A literature search was conducted regarding the parasite *Rhabdias spp.* and the fungus *Bd.* Amphibian medicine textbooks in the EVACC collection were consulted. Finally, amphibian husbandry and parasite guides were borrowed from the STRI library. Notes from these sources were recorded and compiled to produce a preliminary knowledge base for future reference.

The following 6 actions were taken to ensure a thorough investigation on *Rhabdias* spp:

1. Investigate if there is any literature about how to detect (diagnosis) *Rhabdias* (or other similar nematodes) in captive amphibians.
2. Investigate life cycle of amphibian *Rhabdias* spp.
3. Look up literature on aquatic amphibians and nematodes as well as tadpoles and nematodes.
4. Taxonomic descriptions of different *Rhabdias* species (dichotomous key)
III. Project design

This project was a result of many attempts to create a project that would benefit the conservation center. The first few internship days were dedicated to familiarization of the conservation center and its needs. Initially, the literature search on *Rhabdias* was conducted in order to develop a project about the detection and treatment of this parasitic nematode in tadpoles. However, we lacked the proper permits to do scientific experiments on vertebrates. Following this, samples of an unidentified white worm were taken from tadpole tanks and sent to the STRI parasitology lab at NAOS. The second project idea was to create an inventory of all the parasites in the center. However, this project proved to be too difficult due to the high level of parasitology experience required for identification. Finally, it was decided that the center would greatly benefit from a written document about the standard operating procedures at the center.

IV. Transcription of the protocol

Interviews were conducted with both of the directors of EVACC, Edgardo Griffith and Heidi Ross, in person for approximately two hours per day over a week. During this time, they outlined and demonstrated procedures that were performed at the center. Notes on the procedures were transcribed electronically during the interview. Secondly, an interview was conducted with Dalina, the microbiologist at the center, concerning microscope work to detect parasites.

All the species in the center and the exhibition were recorded in a list. The information gathered from the interviews was then edited and formatted into a Standard Operating Protocol.
document. Charts were created to summarize the nutrition information and to provide a sample schedule for medical treatment.

V. Diagrams and illustrations

EVACC directors were consulted about the sections of the protocol that required supplementation with visual aids such as tables, graphs and illustrations. Photographs of the center were taken on April 19 with the help of staff members who offered to handle the amphibians while we took photographs. Illustrations of insects and the 12 priority amphibian species were drawn by hand using pen and then scanned at STRI. The digital copies were then incorporated into the Standard Operating Protocol document.

VI. Finalization of product

The final document is entirely in Spanish, since its users are EVACC employees, and therefore are usually Panamanian nationals. A minimum of technical jargon was used to facilitate comprehension and accessibility for all. Any words that might require further clarification were included at the end of the document in a glossary.

Printed copies of the Standard Operating Protocol were produced and bound at Happy Copy. Two copies of the final Standard Operating Protocol will be given to EVACC in a binder format, so as to allow for modifications and additions in the future.

This project was carried out according to the Code of Ethics of McGill University. No animals were harmed during the photographing of the center. All material produced is solely for the use of the center and its staff members.
Results

In this section, we outline the main points we learned in each section of the Standard Operating Protocol.

Protocols for Staff

Gloves and dust masks prevent transmission of parasites, pathogens, or toxins to both amphibians and humans alike. As a further precaution, staff members are discouraged from entering the facility if they are feeling sick. Containers with hazardous materials are always labelled and stored appropriately. The chemicals that are used in the center include: chlorine, vinegar, Lysol, soaps, formalin, and alcohol. Daily, weekly, and monthly cleaning tasks are outlined in the SOP.

Amphibians

There are four groups of species found at the center: Atelopus, Hylidae, Hemifractus, and “varied”. The Atelopus group includes: A. zeteki, A. varius, A. limosus, Colostethus panamensis, and Silverstoneia flotator. The Hylidae group consists of: Gastrotheca cornuta, Anotheca spinosa, Phyllomedusa venusta, Gastrotheca nicefors, Ecnomiohyla rabborum, Trachycephalus typhonius, Ecnomiohyla miliaria, Hylomantis lemur, and Hylocirtus spp. The Hemifractus group only contains one species, Hemifractus fasciatus. Finally, the “varied” group contains: Strabomantis bufoniformis, Craugaster punctarioles, Crugaster tabasarae, and Agalychnis lemur.

The 12 priority species at the center are: A. limosus, A. zeteki, G. cornuta, H. fasciatus, P. museosus, H. lemur, C. punctarioles, C. tabasarae, A. spinosa, S. bufoniformis, A. glyphus, and A.
varius. These 12 species were illustrated and incorporated into the Standard Operating Protocol (Appendix 1).

The species in the exhibition are: *Rhinella marinus, Trachycephalus venulosa, Leptodactylus savagei, Centrolene prosoblepon, Incilius coniferus, Rhaebo haematiticus, Agalychnis calliotryas, Dendropsophus ebraccatus, Dendrobates auratus, Craugaster megacephalus, and Smilisca phaesta.*

**Reproduction**

Due to the fact that different species of amphibians have different habitats, there are special considerations that must be accounted for when creating an environment for breeding. First, *Atelopus spp.* reproduces in strong water currents and require an environment that mimics this kind of environment. *Atelignis lemur* and *Sachatamia ilex* breed in standing water. *Anoteca spinosa, E. midiaria,* and *E. laborum* reproduce in holes found in trees. *Gastrotheca cornuta* and *G. nicefori* are marsupial species where the female has a sack for its young. *Hemifractus fasciatus,* on the other hand, is a marsupial species where the female does not have a sack. The care and handling of each of these species depends on their preferred habitat for reproduction.

**Medical treatments**

The detection of parasites requires the collection of fecal samples from the amphibian. Feces are then examined under the microscope for presence of parasite larvae. There is a scoring system to determine if the specimen is infected by *Rhabdias:* if the random sample of feces contains at least 1 larva in one’s field of vision at a low potential (10x objective), the amphibian must be treated for infection.
If *Rhabdias* is detected in fecal samples, the specimen must be subjected to medical treatment. Options include Drontal Plus, which is given as an oral dose (dosage is given according to weight of animal) and repeated in 2-3 weeks. Alternatively, a topical application of Levamisole can be applied to the individual. This treatment must be repeated every 2 weeks for a total of 2 to 3 dosages. One hour after each application, the amphibian must be washed with fresh water and closely monitored for possible signs of paralysis. In the case where the specimen is too small to be treated directly with the above methods, the third option is to treat with Fenbendazole (also known as Panacur). These fine granules are administered for five consecutive days and the treatment is repeated after 2-3 weeks.

Spastic movements, stiffness, cramps are signs of hypocalcaemia. The treatment for this is to administer calcium gluconate immediately and every 4-6 hours for 24 hours.

Chytrid fungus (Bd) requires a ten-day treatment. The quarantine period, however, lasts for a minimum of 30 days in order to monitor the progression of the illness. Ideally, the arrangement would be an “all in, all out” process whereby all infected amphibians are admitted into and released from quarantine at the same time.

Given that many of these treatments require strict adherence to a schedule, EVACC directors have expressed the need for a chart that details exactly when each specimen should receive treatment. Appendix 3 is an example of the type of chart that will be used at the center to keep track of medicinal treatments.
Nutrition

Each species has their own customized diet that consists of one or a combination of the following insects: fruit flies (*Drosophila melanogaster* and *Drosophila hydei*), springtails (*Collembola*), crickets (*H. domesticus*), cockroaches (*Blaberus discoidalis*), superworms (*Zophobas morio*), mealworms (*Tenebrio molitor*), kattydid (*Tettigoniidae*), redworms (*Eisenia foetida*), and geckos (*Gekkonidae spp.*).

See Appendix 2 for the breakdown of nutritional requirements for each species in the center.

The *Atelopus* group consumes fruit flies and juvenile crickets. The *Hylidae* group consumes adult crickets, kattydids, cockroach larvae, and superworms. *Hemifractus fasciatus* consumes red worms and geckos. Finally, the varied gro group consumes mostly gut-loaded adult crickets.

There is a separate room for breeding insects. Kattydids are not bred at the center because it is simpler to purchase large quantities from a local provider who catches them in the wild. Fruit flies reproduce quickly; 30-40 new colonies are created per week. Colonies are kept in clear plastic containers and covered with a double layer of coffee filters for aeration. Springtails, or *collembolas*, are used to feed tadpoles. Crickets are raised in Rubbermaid bins and the progeny are harvested in two stages, juvenile and adult, depending on the needs of the amphibians. They must maintain a gut-load of a minimum of 8% calcium for 24-48 hours before they are fed to the amphibians. A new addition to the protocol is to provide crickets with a gut-load of vitamin A. cockroach, superworm and mealworm colonies are also raised in the insect room.
**Equipment**

The water used in EVACC facilities comes from an underground well that is mechanically pumped at a pressure of 40-80 psi. This water is sent through carbon filters before being pressurized and split into two directions. One source is directed to another set of carbon filters and then to a reverse osmosis (RO) membrane filtration system. The water is then stored in a reservoir to be used to maintain amphibian tanks. The other source is directed toward an on-demand delivery pump that has a pressure of 40-50 psi. This water is used or water heaters, laundry, and sources of water used for washing tanks, floors, gloves, etc.

The SOP also has a section detailing the steps to setting up a new amphibian tank. There are glass tanks of various sizes: 75 gallons, 55 gallons, 20 gallons (wide), 20 gallons (tall), and 10 gallons. There are also acrylic tanks that are used to house amphibians temporarily. Plants and rocks that are to be used to create a habitat in the tank must first be disinfected.

**Discussion**

**Protocols for Staff**

Amphibians have thin skin, which makes them vulnerable to infection (Wright and Whitaker 2000). Therefore it is important to take precautions to prevent physical contamination of the skin during handling. This section of the SOP is especially important during the training of new employees because it ensures that each staff member follows appropriate guidelines to minimize risks to health and spread of infection.
Amphibians at the center

The species listed as “priority” are of high importance for the captive breeding program. These 12 species are designated as priority based on IUCN categorization that takes into account both the distribution of the species and the population status in the wild (IUCN 2012). These particular species have a small distribution: many are only found in Panama. In addition, their population level is declining, leading to a need for conservation centers to preserve captive bred populations. Illustrations of the 12 priority species are intended to assist in rapid identification and serve as a reference for new staff members. Ink drawings were chosen instead of photographs in order to provide clear depictions of the species without any background “noise” that would normally be present in a photograph.

The species in the exhibition are not necessarily a conservation priority. The main goal of the exhibition is to serve as a showcase for the amphibians in Panama. Panamanian citizens can learn about the amphibians of their country, while foreign visitors can discover new species that they would not encounter in their own country.

Reproduction

The conservation center faces many challenges when it comes to reproduction because different species require different environmental conditions for breeding to be successful. Even if conditions are perfect for mating, there is still no guarantee that the tadpoles will develop properly and survive to become adults. For example, many offspring of species that reproduce in tree holes are cannibalistic and eat their own siblings for nutrition. These natural adaptations pose a threat to the breeding program’s success. Careful monitoring will reduce the risk of development problems and maximize the number of offspring that grow to become reproductive adults. Selecting the
proper individuals to breed can also pose a challenge, as cannibalism often arises after copulation. EVACC employees must ensure that the male is not less than 50% smaller than the female, so as to avoid cannibalism.

Medical treatments

Parasites and pathogens are continuously evolving and adapting to thrive in their environment. New threats to captive-bred amphibians need urgent attention. There are many procedures that must be followed at a conservation center to ensure the proper care and maintenance of amphibian populations.

Medical treatments require a standard regime to ensure proper care of the amphibians. There are important cautionary measurements that must be taken when treating amphibians with levamisole. Levamisole is an imidazothiazole anthelmintic that is effective against nematode parasites. It is equally effective against nematodes in the lungs as well as the gastrointestinal tract. It works by interfering with nematode carbohydrate metabolism, leading to paralysis of the worm. However, its side effects include respiratory distress and paralysis for the amphibian (Klingenberg 2007).

There have been many studies conducted on treatment for *Batrachochytrium dendrobatidis*, for example: chemical disinfectants, heat, and dessication (Johnson et al. 2003). However, these experiments were done *in vitro*. The goal of the conservation center is to treat live amphibians for the fungus, which poses as an even greater challenge.

The EVACC directors described the types of charts they would like to incorporate into the regular proceedings of the center in order to keep organized records. A sample chart outlining a medicinal treatment schedule (Appendix 3) was drafted in Spanish and included in the Standard
Operating Protocol. Through sample tables like these, the Standard Operating Protocol can serve to maximize the efficiency of record keeping and ensure that there is good communication between staff members.

_Nutrition_

Amphibians in the wild have diets that vary depending on what is available. Over many years of breeding them in captivity, the directors of EVACC have created a standard nutrition program for the amphibians in the center.

Minimization of the amphibians’ exposure to chemicals is important to maintaining proper growth and development. The center uses a specific brand of coffee filters (Duran) and paper towels (Holiday) for maintaining fruit fly colonies and lining cages, respectively. These brands cater to the center’s needs because the products do not contain bleach, or were not bleached in the process of manufacturing.

Vitamin D deficiency is a major concern for animals in captivity. For this reason, amphibians are exposed to ultraviolet B light for a few hours each day. Exposure to UVB needs to be regulated because there are detrimental effects associated with too much ultraviolet radiation, including: tissue damage, vitamin A and D degradation, and possibly death (Adkins et al. 2003). UVB light is necessary for proper growth and development because it is responsible for the cutaneous conversion of 7-dehydrocholesterol to pre-vitamin D3, which in turn is important for absorption of calcium (Adkins et al. 2003). Failure to provide adequate UV lighting can lead to chronic stress and immunosuppression. There is a delicate balance between over and underexposure to ultraviolet light. Furthermore, insects are fed a high-calcium diet to ensure that
the amphibians consume enough calcium to remain healthy. This is an effective way to ensure that amphibians receive enough calcium without having to treat them directly.

*Equipment*

Reverse osmosis filtration is an effective way to filter water for use with amphibians in the center. It is a purification technology that uses a semi-permeable membrane and pressure is applied to overcome osmotic pressure (Crittenden 2005). EVACC provides amphibians with filtered water to prevent sicknesses associated with contamination.

The SOP serves as a guide for situations in which the directors may not be available. Having a printed copy of the procedures will allow any staff member to deal with unusual circumstances in the correct manner. In terms of setting up equipment, the SOP provides guidelines to ensure that all tanks are set up and maintained in the same manner.

*Limitations*

This was the first time that EVACC has received interns from the McGill Panama Field Studies Semester program, so perhaps this led to unrealistic expectations about our first project. The major obstacles to conducting scientific experiments with *Rhabdias spp.* were the time frame and the number of permits required. We did however learn an important lesson about the reality of conservation work during the early stages of this project. We now understand the bureaucracy behind conservation work, and that though it is a noble mission, it is time-consuming and often thankless work. If more people were interested in dedicating their lives to conservation, like Edgardo and Heidi Griffith have, perhaps these hurdles would become less significant, or at least easier to overcome.
The supervisors were kind enough to set aside time in their busy schedules to talk with us. However, there were days when there were too many things to do at the center or other unpredictable circumstances. On these days, we reviewed and edited our previous interview transcripts.

**Recommendations**

Recommendations for future students who wish to work with EVACC would be to begin applications for IACUC (Institutional Animal Care and Use Committee) and ANAM (Autoridad Nacional del Ambiente) as soon as possible. Scientific experiments on vertebrates can only proceed if the proper permits are granted. Furthermore, if students wish to accompany Edgardo to the field sites, they should keep in mind that they must undergo an application process with the mining company, Minera Panama, which includes a safety session and background check that may or may not lead to access to the field site. In our case we were not granted access despite having attended the safety session and providing proper documentation. A quality research project could be created if access to the mine is granted in the future.

One of the goals of EVACC is to educate the public about the importance of conserving amphibians. The exhibition is well equipped to educate visitors about the various species on display: each tank has a plaque that provides information about the amphibian. However, one suggestion for a future project would be to conduct interviews before and after people enter the exhibit in order to gauge how much information they actually gathered. The results of this research could provide helpful feedback on how to improve the exhibition portion of EVACC.
Conclusion

The purpose of this internship was to contribute to the conservation center in a way that benefits its staff and to ameliorate the standard of care for the amphibians. This project has been helpful not only in providing a written copy of the procedures at the center, but also in creating awareness of the lack of clarity in certain procedures. For example, during the interviews it became clear that EVACC needed to spend time developing an emergency protocol in case of fire. In addition, the supervisors realized that communication in the center could be made more efficient with the use of whiteboards and cleaning schedules. This thorough examination of the procedures carried out at the center have helped its directors generate more ideas for a better and smoother way to conduct daily activities.

Working with the El Valle Amphibian Conservation Center has been an incredible experience. As biology students, this internship has been a valuable opportunity to learn from a conservation center and has left us with organizational, time-management and communication skills that we can readily apply in our future careers.

Acknowledgements

We would firstly like to thank the entire staff of El Valle Amphibian Conservation Center, especially Edgardo Griffith and Heidi Ross, for welcoming us into their place of work and their home. They were gracious hosts and represent a hard-working organization doing great work. We are also grateful to STRI, notably Roberto Ibañez, for acting as our advisor for this internship. Thank you to Victor Frankel for answering our questions and always being available to help.
Last but absolutely not least, thank you to Catherine Potvin and to everyone involved in the PFSS program for making it a valuable and unforgettable experience professionally, academically, and personally. We are very grateful for this opportunity.

Works Cited


Appendix 1

Drawings of twelve priority species at EVACC

*Atelopus glyphus*
Atelopus limosus

Atelopus varius
Atelopus zeteki

Anotheca spinosa
Hylomantis lemur

Strabomantis bufoniformis
Craugaster punctarides

Craugaster tabasarae
Pristimantis muscosus
Appendix 2

Graph of relative number of amphibian species that eat each species of prey
## Appendix 3

### Nutrition table

<table>
<thead>
<tr>
<th>Especies</th>
<th>Dieta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grupo Atelopus</td>
<td></td>
</tr>
<tr>
<td><em>Atelopus zeteki</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td><em>A. varius</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td><em>A. limosus</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td><em>A. glyphus</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td><em>Colostethus panamensis</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td><em>Silverstoneia flotator</em></td>
<td>moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td>Grupo Hylidae</td>
<td></td>
</tr>
<tr>
<td><em>Gastrotheca cornuta</em></td>
<td>Katydids + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Anotheca spinosa</em></td>
<td>Katydids + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Phyllomedusa venusta</em></td>
<td>Katydids + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Gastrotheca nicefori</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Ecnomiohyla rabborum</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Trachycephalus typhonius</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Ecnomiohyla miliaria</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Hylomantis lemur</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td><em>Hyloscirtus spp.</em></td>
<td>Tetigonidos + grillos, larvas de cucarachas gigantes y superworms</td>
</tr>
<tr>
<td>Grupo Hemifractus</td>
<td></td>
</tr>
<tr>
<td><em>Hemifractus fasciatus</em></td>
<td>Lombrices de tierra y geckos</td>
</tr>
<tr>
<td>Grupo variado</td>
<td></td>
</tr>
<tr>
<td><em>Strabomantis bufoniformis</em></td>
<td>Grillos</td>
</tr>
<tr>
<td><em>Craugaster punctariolus</em></td>
<td>Grillos</td>
</tr>
<tr>
<td><em>Craugaster tabasarae</em></td>
<td>Grillos</td>
</tr>
<tr>
<td>Grupo de exposición</td>
<td>Agalychnis lemur</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Rhinella marinus</td>
<td>Katydids (lunes), grillos con vitamina A (miércoles y jueves), grillos con calcio (sábado)</td>
</tr>
<tr>
<td>Trachycephalus venulosa</td>
<td>Katydids (lunes), grillos con vitamina A (miércoles y jueves), grillos con calcio (sábado)</td>
</tr>
<tr>
<td>Leptodactylus savagei</td>
<td>Katydids (lunes), grillos con vitamina A (miércoles y jueves), grillos con calcio (sábado)</td>
</tr>
<tr>
<td>Centrolene prosoblepon</td>
<td>Moscas de fruta (cubierto con vitaminas o calcio), grillos con vitamina A (jueves)</td>
</tr>
<tr>
<td>Incilius coniferus</td>
<td>Katydids (lunes), grillos con vitamina A (miércoles y jueves), grillos con calcio (sábado)</td>
</tr>
<tr>
<td>Rhaebo haematiticus</td>
<td>Katydids (lunes), grillos con vitamina A (miércoles y jueves), grillos con calcio (sábado)</td>
</tr>
<tr>
<td>Agalychnis calliotryas</td>
<td>Moscas de fruta (cubierto con vitaminas o calcio), grillos con vitamina A (jueves)</td>
</tr>
<tr>
<td>Dendropsophus ebraccatus</td>
<td>Moscas de fruta (cubierto con vitaminas o calcio), grillos con vitamina A (jueves)</td>
</tr>
<tr>
<td>Dendrobates auratus</td>
<td>Moscas de fruta y grillos juveniles</td>
</tr>
<tr>
<td>Craugaster megacephalus</td>
<td>Grillos</td>
</tr>
<tr>
<td>Smilisca phaesta</td>
<td>Grillos</td>
</tr>
</tbody>
</table>
### Appendix 4

**Sample medical treatment scheduling form**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosage and Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. glyphus 243-3</td>
<td>Enrofloxacin 11.35 mg/ml, 1 gota una vez al día sobre la piel</td>
</tr>
<tr>
<td>G. cornuta F1006/27-1</td>
<td>Enrofloxacin 11.35 mg/ml, 0.01 ml una vez al día sobre la piel, Dexamethasone 4 mg/ml, 3.7 microlitro una vez al día sobre la piel, Calcium gluconate 10%, 0.01 ml una vez al día sobre la piel</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Species</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 noviembre</td>
<td>A. glyphus</td>
<td>Enrofloxacin _ (aquí se escribe los iniciales del empleado que ha dado las medicinas)</td>
</tr>
<tr>
<td></td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dexamethasone ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td>21 noviembre</td>
<td>A. glyphus</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td>22 noviembre</td>
<td>A. glyphus</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td>23 noviembre</td>
<td>A. glyphus</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td>24 noviembre</td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td>25 noviembre</td>
<td>G. cornuta</td>
<td>Enrofloxacin ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcio ___</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tubo de alimentación pequeña cantidad Emeraid carnívoro dieta ___</td>
</tr>
</tbody>
</table>

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Tubo de alimentación pequeña cantidad Emeraid carnívoro dieta ___