Husbandry Manual
Panamanian Golden Frog
Atelopus zeteki

Second Edition by Vicky Poole, National Aquarium in Baltimore

BACKGROUND HISTORY

A pathogenic fungus capable of causing sporadic deaths in some amphibian populations and 100% mortality in others, *Batrachochytrium dendrobatidis*, has been isolated from the skin of dead and dying amphibians on every amphibian-inhabited continent. Chytrid fungi typically live in water or soil, although some are parasites of plants and insects. They reproduce asexually and have spores that 'swim' through the water. Only the amphibian chytrid fungus is known to infect vertebrate species. Individual frogs are thought to contract the disease when their skin comes into contact with water that contains spores from infected animals. Chytrid causes death in post-metamorphic frogs by causing focal hyperkeratosis (thickening) and erosion of epidermis in frogs (excessive skin shedding and/or red areas, generally the first clinical sign) and infects the keratinized mouthparts of tadpoles causing erosion (potential starvation). Some amphibians, including two commercially important and widely traded species, American bullfrogs (*Rana catesbeiana*) and African clawed frogs (*Xenopus leavis*), have proven to be carriers of the *B. dendrobatidis* without becoming susceptable to the disease chytridiomycosis.

In Central America, chytridiomycosis (or “chytrid”) was first observed in montane central Costa Rica, where it may have been the cause for extinction of the golden toad, *Bufo periglenes*. It has since become evident that this epizootic is advancing southeast through the mid- to high-elevation mountain forests of Central America, decimating entire populations of amphibians, including *Atelopus senex*, and *A. chiriquensis*. As of 2005, the disease front is documented as far east as eastern El Cope (K. Zippel, pers. comm.). The current species being affected by the disease is the Panamanian Golden Frog (PGF), *Atelopus zeteki*. Other factors impacting PGFs include collecting for the local zoos and hotels as well as the illegal international pet trade [listings include CITES Appendix I status since 1975; and USFWS Endangered Species Act (ESA) - Endangered species since 1976], deforestation, and habitat alteration by logging and farming that also leads to sedimentation of their pristine waters.

In response to this crisis, a group of concerned biologists convened and formed Project Golden Frog/Proyecto Rana Dorada (PGF/PRD), a conservation consortium involving numerous Panamanian and US institutions. Specific initiatives of PGF/PRD have included field studies, captive management, education, and financial support (See Appendix I for current contact information).

As a precautionary measure against extinction, *ex situ* populations of golden frogs are being maintained. Over two dozen founder pairs of have successfully spawned producing thousands of offspring. To ensure genetic viability, permits were obtained to export specimens collected from unprotected remnant populations outside of two national parks where they occur. The numbers collected are actually less than those removed yearly to replace dying animals at the hotels and a local zoo that display golden frogs for tourism.

Should the chytrid epizootic decimate *in situ* populations of PGFs, PGF/PRD would propose that tadpoles be reintroduced into the national parks, but only of independent research concludes that the chytrid fungus is no longer a threat. The rationale for releasing tadpoles versus adults is that captive reared adults presumably will not possess the skin toxins necessary for survival against endemic predators and or/may have difficulty in properly homing after reintroductions.

The PGF husbandry and captive program is modeled after the 1992 *Atelopus varius* (Fortuna, Panama) breeding at National Aquarium in Baltimore (NAIB). A female of a collected pair went into amplexus and spawned in the shipping bag during transport back to the US. Robin Saunders successfully reared approximately two dozen *A. varius* froglets at NAIB.

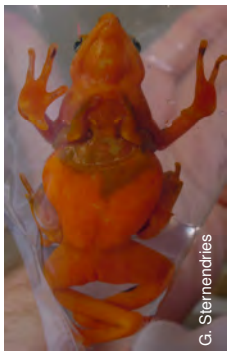
SPECIES DESCRIPTION

Rana dorada or the "golden frog" is culturally significant to the people of Panama. They are as revered as the bald eagle is in the US, with a history dating back to the Mayan civilization. Golden or pottery replications, called huacas ("wa-cas") were symbols of good fortune and the frogs are still considered lucky to Panamanians. PGFs are used to promote hotels and restaurants, and are such a part of the culture that they show up on their lottery tickets.

Atelopid frogs are unique members of the family Bufonidae (toads). Endemic to the cloud forests, the Panamanian golden frog, *Atelopus zeteki*, was originally described by Dunn in 1933 as a subspecies of *Atelopus varius*. It has been recognized as a distinct species based on a unique skin toxin, zetekitoxin, and bioacoustical differences. In addition to vocalizing, PGFs communicate by semaphoring, a hand-waving phenomenon that is theorized to have developed so that the frogs could locate conspecifics for breeding near the deafening sounds of waterfalls, where their gentle vocalizations are inaudible. Male frogs will perch on rocks in or along the banks of streams and waterfalls, defending their territory by semaphoring as a warning or wrestling other males that come too close while awaiting the females' return to the breeding areas.

Mature golden frogs can be sexed easily as the females are significantly larger in size for most populations (wild Locality **A** females range 55-63 mm while males range 39-48 mm, for example). Females full of eggs are easily identified by examining their ventral surface for light-colored abdomen. Males also have obvious nuptial pads (darkened and enlarged during breeding season) on their hands that they used to increase pressure on the female during amplexus.

The female's eggs appear light in color.



Male amplexing gravid female.

Depending on their locality, PGFs will differ in appearance. Their colors range from brilliant gold, egg-yolk or pale yellow, to a greenish-yellow. Their individual black patterns may vary from large solid chevrons, large random blotches, lots of small spots, few markings at all somewhat isolated to sides and limbs, to no markings whatsoever. Compared to other atelopids, PGFs have smooth skin.

There are two general types of habitats that golden frogs occupy in Panama, and unique sizes that vary with locality:

- There are the wet forest PGFs that are larger and more dispersed in and along the streams (up to 3m above the ground). The habitat typically includes waterfalls and large boulders covered with moss that they utilize as visible territories. These animals have been found sleeping on big leaves at night.
- There are also frogs that inhabit dry forest streams and are more likely to be seen on the forest floor (no higher than 1.5 m). They are smaller, ~2/3 as large as the other frogs, but they exist in much greater densities.

For more explanation on this see *genetic conversation in the section below.

In 2002, PGF skin samples were collected in the field for toxicology analysis in order to compare to that of captive-bred offspring, performed by Dr. John Daly of the National Institutes of Health (NIH). It appears that all Atelopids secrete digitalis-like substances, bufadienolides, synthesized even when raised in captivity. These steroids, which are also found in plants, are potent inhibitors of the Na^+ pump. The frogs also possess the unique zetekitoxin. Dr. Daly believes that zetekiton, like all other tetrotoxins requires a symbiotic microorganism and

will not be present in captive raised PGFs, similar to his findings with captive bred *A. varius*. Samples have been sent to Dra. Yotsu-Yamashita in Japan for further analysis.

NOTE: Type Locality: El Valle de Anton, Provincia Cocle, Panama
Holotype: MCZ 16018, Harvard University, MA.

COLLECTION NUMBERS, DATES, AND SITES

Prior to any involvement from US zoos, the only known captive specimens were a few wild-caught golden frogs exhibited at two hotels and one small local zoo in Panama. These animals have never reproduced, mortality rates are extremely high, and their legality is highly questionable. Although the local Panamanians were concerned about the future of golden frogs in the wild, none of the facilities in Panama at that time were equipped or skilled to handle the challenge of a captive breeding effort.

In 1999, the Republic of Panama issued scientific collecting permits for a total of 20.20.100 *Atelopus* (*varius*) *zeteki* to PGF/PRD's founding researchers, Erik Lindquist, Roberto Ibáñez, Anthony Wisnieski, and Kevin Zippel, in order to conserve genetic variability and maintain viable captive populations of *Atelopus zeteki* due to the impending chytrid crisis. CITES/ESA Importation permits for those specimens and all of their offspring were sought and have been maintained through the Baltimore Zoo since 2000 (original No. 00US027256-9). Intentionally, the ownership of the animals belongs to the permit holder (in this case to The Baltimore Zoo, now called The Maryland Zoo in Baltimore) and not to the Republic of Panama, as has happened with other species of animals. The imported animals and their offspring are then placed on loan by the Maryland Zoo in Baltimore **to other AZA institutions only** (per the USFWS) with a Memorandum of Understanding (MOU). This restriction is intended to prevent the protected species from entering the pet trade via captive zoo breeding, potentially creating a situation in which wild-caught illegal specimens could be "laundered" under the guise of coming from legal "zoo stock," as has happened with other species of amphibians. Once this situation begins, it is impossible to stop and the remaining wild populations become even more at risk of being collected and traded illegally.

As of January 2006, the following collecting trips and importations have resulted in the only known legal wild-caught golden frogs in captivity in the world (*AZA facilities can contact the author or studbook holder to decipher the following population locations in order to protect the localities from illegal collection*):

- January 17, 2001: 7.7.0 *Atelopus zeteki* from Locality **A**, Panama are collected/imported as amplectant pairs and sent to the Baltimore Zoo (3.3) and Detroit Zoo(4.4).
- January 25, 2002: 3.3.12 *Atelopus zeteki* from Locality **A**, Panama and 1.1.25 golden frogs** from Locality **C**, Panama are collected/imported and sent to the Baltimore Zoo (2.2.12 from Locality **A**) and Detroit Zoo (1.1 from Locality **A** & 1.1.25 from Locality **C**).
- December 19, 2003: 9.9.0 *Atelopus zeteki* from Locality **B**, Panama and 0.0.22 golden frogs** from Locality **C**, Panama are collected/imported and the two groups are sent to the Baltimore Zoo and Detroit Zoo, respectively.

Renewals of the permits will allow for the balance of 0.0.41 *Atelopus zeteki* to be collected/imported from Locality **A** at this point to minimize chytrid risk.

*In 2002, as part of a grant from the St. Louis Zoo, genetic material was collected from several Panamanian localities with golden frogs to determine the relatedness of various PGF populations and the prioritization of our conservation efforts (in prep.). Rough analysis of the three populations were are currently maintaining in captivity has shown the following:

- Locality **A** animals' phenotype is a large golden frog with the genotype of *A. zeteki*
- Locality **B** animals' phenotype is a small yellow/green frog with the genotype of *A. zeteki*
- Locality **C** animals' phenotype is a large golden frog with the genotype of *A. varius*.

In light of this, the Cleveland Zoo solicited permits to collect and import *A. varius* from Locality **C** to increase the potential founder numbers for this population as *A. varius* (*varius*) is not listed on CITES or the USFWS ESA. In April 2005, 6.6.0 golden frogs from Locality **C**, Panama were collected/imported by the Cleveland Zoo.

Since January of 2001, the Baltimore Zoo, Detroit Zoo, and Cleveland Metroparks Zoo have been successful in the captive hatching of eggs laid by amplectant pairs from Panama, and tadpoles were reared through metamorphosis. Other captive population statistics are listed in Appendix II.

NOTE: Since it is a priority for long-term maintenance of our captive program to maximize the gene pool, **it is important for institutions not to mix populations of frogs**. In order to control this we recommend that each AZA institution only work with one population of frogs at a time. This will limit the chance for mistakes. All of these frogs should be reported and maintained in the PGF studbook and managed together due to limited space and resources for golden frogs of either species. Institutions participating in the program will be prohibited from producing hybrids.

SHIPPING PGFs

Use disposable plastic containers (i.e., deli cups, Gladware®/shareware, Rubbermaid®, or Tupperware®) so that frogs are not crushed during transport. Line containers with damp toilet paper or paper towels (avoid natural materials that could cause delays in customs or with airlines due to agricultural restrictions). Pack securely within an International Air Transport Association (IATA) approved cardboard box/Styrofoam container, with small perforations for air exchange. Avoid shipping during summer and winter, when the temperatures can be severe.

During our initial importations, PGF/PRD was fortunate to obtain written permission from the airline in advance to carry-on our specimens in the airplane cabin along with us to avoid any potentially dangerous cargo-shipping issues.

For international shipments, be sure to notify federal wildlife agencies due to their legal status and preschedule inspections at customs to minimize delays. Make sure that copies of all permits and MOUs relating to the PGFs accompany all shipments.

Specimens should be taken immediately to isolated quarantine facilities upon arrival at the final destination.

QUARANTINE

Accessioning frogs: It is preferred that adult PGFs be accessioned as individuals, especially if only a few are received. If they are accessioned as a group, it is preferred that each group number represent a single bloodline (sibling group), so that there is minimal confusion for genetic pairing recommendations.

In order to identify frogs as individuals, it is ideal to photograph and record patterns digitally. It is recommended that juvenile frogs be photographed periodically as they grow since their patterns may change as they mature. Adult frogs' pattern are fairly stable and patterns are generally unique. As a final measure, elastomer marking imbedded on a frog's inner thigh may work as an identification method, and was utilized by the Detroit Zoo.

Receiving from the wild: Due to the potential of transferring chytrid into an existing healthy collection, it is important that wild-caught PGFs undergo a minimum 30-day quarantine in an area isolated from rest of the exhibit collection, preferably in another building. Animals should be screened for ecto- and endoparasites. As wild-caught *Atelopus* have been shown to die when housed in sterile enclosures and over-medicated, it is preferred that the animals be placed in a naturalistic enclosure (see the HOUSING section below for details),

parasite loads be monitored, and not medicated unless it becomes a health issue. Be aware that some specimens may appear thin with protruding pelvic girdles, but that may be normal for the individual. Specimens that are considered 'poor-doers' should be isolated and medicated as necessary.

It is also important during the first few days in quarantine that the frogs coming from the wild undergo chytrid treatment prophylactically. Safe treatment for post-metamorphic frogs exposed to chytrid or coming from a known chytrid (+) environment involves using recommended itraconozole baths (Nichols and Lamirande, 2000). Animals should be soaked for 5 minutes at a depth of 1cm in a 0.01% suspension of itraconazole (prepared from a 1% suspension of itraconozole using 0.6% saline as the dilutant) for 11 consecutive days (Sporanox®, Janssen Pharmaceutica, Inc, Titusville, NJ, USA).

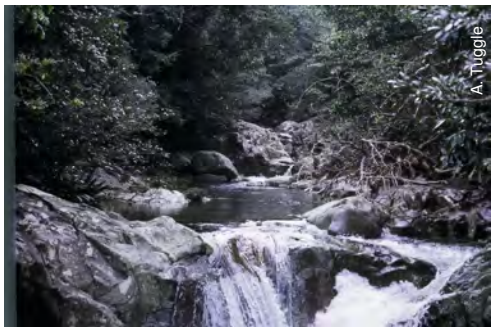
Good husbandry practices are necessary when dealing with animals housed in a quarantine situation. Every attempt should be made to minimize the potential for contamination between enclosures. Keepers should wash their hands or utilize a different pair of latex gloves between cages; equipment should not be shared between cages and should be disinfected using chemical or thermal sterilization (see Appendix III for recommended protocols). As the amphibian chytrid fungus is extremely sensitive to temperatures above 29 C (84.2°F), and *B. dendrobatidis* is killed at 32 C (89.6°F), there is no zoonotic risk since it cannot survive on human skin. Also, complete drying will kill the amphibian chytrid fungus.

Transfer between AZA facilities: As quarantine protocols may vary between institutions, it is recommended that all captive-bred PGFs undergo a minimum 30-day quarantine with three successive negative fecals (float and direct) in an area isolated from the rest of the exhibit collection. This will allow time for the PGFs to manifest any disease issues due to post-shipping stress and minimize disease transfers to your permanent collection. If the animals have come from an institution with chytrid is in the collection, the procedure for itraconozole baths and equipment disinfection protocols should be employed (see section above).

FACILITIES & CARE

ENVIRONMENTAL PARAMETERS

Wild golden frogs reside in high elevation cloud and rain forest habitat with clear waterfalls and streams.



In situ A. zeteki *stream – our ideal!*

Our recommendations for air and water parameters are based upon PGF/PRD environmental assessments of PGF habitat in Panama (see Appendix IV & V for original data):

- Air Temperature: 68-73° F daily, without seasonal variation
- Water Temperature: 69-72° F, without seasonal variation
- Humidity: 75-100% with little seasonal variation
- Water Quality
    - Dissolved Oxygen: High range 8.3 – 9.0 mg/L (influenced by water turbulence)
    - pH: Neutral range 6.5-7.5
    - General Hardness: Soft water 0-1 degrees
    - Ammonia, Nitrite, & Nitrate: 0 ppm

WATER AND FILTRATION

Biofiltration is necessary to provide water quality required for PGFs (i.e., no detectable nitrogenous wastes and high oxygen levels) in captivity. External canister filters work well and should flow at between 250-350 gph. A robust biological filterbed, either through an under-gravel or external canister, will reduce ammonia from animal wastes. Live plants and water changes several times per week (up to 100% preferred for tanks without tadpoles) are needed to help reduce nitrates.

As some institutions have experienced health issues associated with elevated phosphate levels, consider using phosphate sponges or absorbing resins as part of the filtration system.

Water chemistry analyses showed golden frog stream water is surprisingly soft and pure. General Hardness (GH, the measure of calcium and magnesium) of golden frog stream water was very low, only 0-1 degrees. By comparison, hard tap water can be 1-20 degrees. Conductivity, the measure of electrically charged particles in the water in golden frog habitat ranges from 44-86 microsiemens, compared to near 0 for Reverse Osmosis (RO) water and 200-400 microsiemens for tap water.

Depending on source water at each location, aging tap water, adding an in-line carbon filter, using reconstituted reverse osmosis (RO) water, or a dechlorinated tap water RO system may be necessary to be able to house and breed PGFs. A recipe for reconstituting RO water with a pH buffer created by Kevin Zippel is listed in Appendix VI, although commercial additives are available. It is strongly recommended that institutions monitor water quality parameters as part of routine husbandry.

Water temperature should be maintained between 69-72ºF. Cool water temperatures plus high turbulence help maintain high dissolved oxygen levels, which decrease as water temperature warms. A thermostatically-controlled chiller or submersible heater may be necessary to maintain desired water temperatures depending on ambient air temperatures.

TEMPERATURE, LIGHT, AND HUMIDITY

Ambient air temperatures should be kept cool, varying between 68-75ºF on a daily basis. Some institutions have been able to maintain PGFs successfully at slightly warmer ambient temperatures, ranging from 78-82ºF, but it is recommended that enclosures in these environments be misted more frequently.

As PGFs are fond of basking directly in rays of sunlight penetrating the canopy, a full-spectrum or plain incandescent basking spot light (60-100W) works well and can provide isolated thermoclines (up to 100ºF). In addition, provide full-spectrum lighting (UV A & UV B) as you would for basking lizards ([1]Vita-lights®, [1]Powertwist®, [2]Verilux®, [3]Reptisun™, [3]PowerSun UV™, [4]GE® Chroma 502™, black lights, compact fluorescents, etc.) on a 12:12 lighting cycle year round

([1]Duro-Test® Lighting, Philadelphia, PA; [2]Verilux®, Inc., Stamford, CT; [3]Zoo Med Laboratories, San Luis Obispo, CA; [4]General Electric Co. ®, Cleveland, OH).

Relative humidity stays around 100% annually in the cloud and rainforests of Panama. Humidity levels can be maintained with automated misting systems. Wide angle, high flow nozzles will aid in simulating the rainy season.

Automated equipment such as light timers and programmable temperature control valves for both air and water make husbandry easier. Back-up generators to power filtration and temperature monitoring systems in case of power failures help with peace of mind, especially for such an environmentally restricted, valuable species. We also recommend logging environmental data, if possible, especially during breeding attempts as one parameter may be the difference between failure and success.

HOUSING

The housing recommended for golden frogs is large, naturalistic enclosures modeled after rocky streambeds of the frogs' native habitat, but the exact specifications will obviously vary depending on the space and resources of each institution. Minimum space allotted for a pair of adult *Atelopus* should be no less than a 15 gallon aquarium to provide sufficient microhabitats, although up to a total of 6 adult frogs could be housed in a 15 gallon tank.

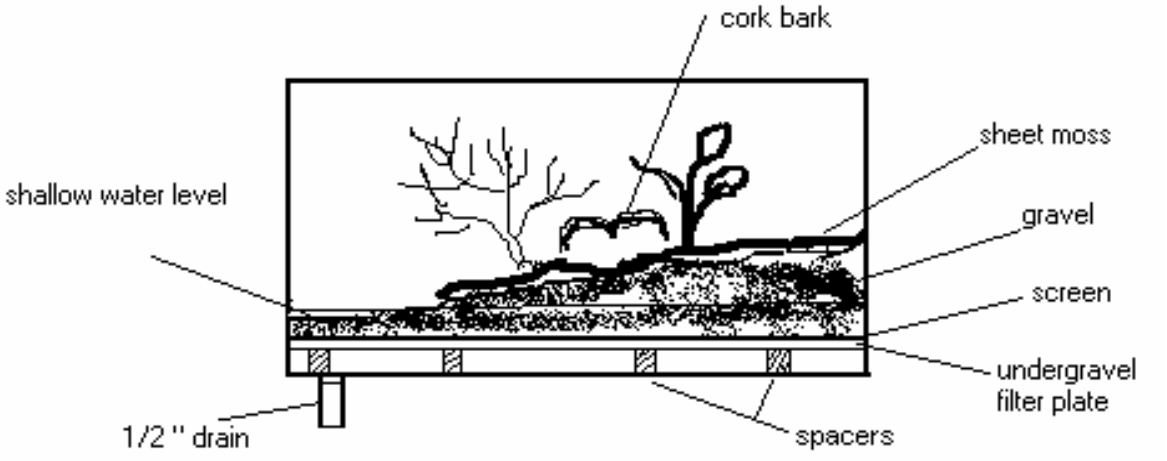
Custom glass enclosures with front-opening access work well for housing PGFs and screen windows/tops allow for ventilation. As golden frogs climb and newly metamorphosed froglets are quite tiny, make sure that all openings are secure and have a tolerance of less than 1/16" (2mm). If lids/doors are lose fitting, consider adding self-adhesive foam weather-stripping to tighten any gaps (available in various thicknesses).

Standard "breeding tanks" should include rocky streambeds flowing down to a large pool (2-3" deep, as more than that may be too deep and tired amplectant females could drown) with lots of large rocks or artificial structures to provide dark egg-laying sites during breeding season and provide territory sites. An inexpensive dark glass plate was used at the Detroit Zoo as an alternative to using large heavy boulders to provide egg laying sites. Use a basic flow-through tank set-up with an elevated undergravel filter plate and a bed of gravel including raised "land areas" covered with moss where animals can get away from flowing water and rest. Be sure to seal the screen mesh to the tank at the edges to prevent tadpoles from getting underneath the filter plate and into the filter.

Natural plants help create microclimates, refugia, and visual barriers for frogs. There was little to no submerged vegetation observed at golden frogs streams, but its addition to captive enclosures will help maintain good water quality. Emergent and terrestrial vegetation are also important for maintaining high humidity and providing sleeping sites. We found one male golden frog sleeping 2 feet above the ground on an arrow-leaf next to the stream, and this behavior is apparently not uncommon for certain species in the genus.

Filters should be used to recirculate water (exterior canister filters work well) and provide biofiltration. Using a 1" hole-saw diamond bit, a drain can be drilled into the bottom, front corner of the tank, fitted with a bulkhead fitting and ½" tubing going to a canister filter, and another hole drilled at the opposite back wall corner for returning. Water returned to the cage can be directed over descending rock-piles to create small waterfalls and splash pools.

For juveniles and adults that are not paired for breeding, an enclosure without a flowing stream can be used, although a shallow pool of clean water should be provided, not unlike a standard dendrobatid enclosure (see image below). Aquariums are plumbed with floor drains and include false bottoms, (i.e., undergravel filter plates). Gravel is placed on top of a false bottom (undergravel filter plate) on a sloped grade, which is then covered with sheet moss. Enclosures are then equipped with cork bark, hiding huts, live plants, and a shallow pool (just above surface of gravel).



SOCIAL GROUPINGS

Males are highly territorial and shouldn't be housed together long term in order to minimize stress. There are reported cases of captive males defending territories in cages by pinning down passing males (wrestling) and semaphoring. Males have been observed to displace other males and begin calling from the new location.

Females can be housed in groups up to 8 animals in a 20 gallon aquaria without stress. When PGFs are paired for breeding, it works well to house females together in groups during the off-season, and introduce them to the males within the "breeding tanks." After oviposition, separate the pair and return the adults to their respective tanks.

Juveniles can be housed in dense groups during rearing (up to 50 froglets in 20 gallon aquaria) and separated by sex and size as they mature. Take into consideration space required for potentially separating out territorial males into individual enclosures when considering the number of PGFs each institution can hold long-term.

NOTE: Several institutions have attempted housing PGFs in mixed species exhibits with no interactions or related problems reported to date. The most diverse attempt is the Houston Zoo in which 3 *Dendrobates auratus*, 3 *D. azureus*, and 1 *Gastrotheca marsupiata* all share the 72"Lx30"Wx18"H exhibit with 8 PGFs. 1.1 *Abronia graminea* lizards have also been housed successfully with 17.0 PGFs at The Philadelphia Zoo in a 3'x 2'x 4' exhibit.

ROUTINE MAINTENANCE

The enclosures described above reduce the labor involved in maintenance as substrate can be easily rinsed through with fresh water. A keeper's routine should involve the following:

- **Misting:** 1-2 times daily with fresh water; either an automated misting system or manual misting is sufficient.
- **Cleaning cages:**
    - Rinse enclosure thoroughly and change water completely 2-3 times per week to remove nitrogenous wastes.
    - Rinse filter media weekly (don't change media completely unless too clogged to filter due to the beneficial bioload).
    - Dismantling entire cage, disinfecting, and replacing moss substrate and organic decorations every 4-6 months.

FOOD

Stomach contents of wild adults indicate an assorted diet, including a wide variety of arthropods. Even wild-caught captive adult frogs readily feed on standard captive insects: they readily take flightless fruit flies (*Drosophilia melanogaster* or the larger *D. hydei*), gut-loaded 2-week crickets (*Acheta domesticus*), larval and adult flour beetles (*Tribolium* sp.), and they relish termites (Isoptera). Springtails (*Collembola* sp.) or porch/field sweepings can be collected as alternative supplements to the standard fare. Some institutions have also reported offering waxworms (*Galleria mellonella*) every few weeks. Variety may be important for providing all necessary nutrients and producing animals robust enough to handle breeding. Food items offered should never exceed the size of a 2 week cricket (1/2" in length). Although the offered food can be broadcast throughout the enclosure, the volume offered per frog should be roughly 1/4 tsp. per frog 3-5 times per week.

As a standard practice, every feeding should be dusted with a vitamin-mineral supplement as it can be brushed off most food items in a short amount of time once placed into an enclosure and all food is not consumed immediately. It is also important to gut-load crickets for at least 24 hours prior to feeding with commercially available cricket diet (Zeigler®, etc.) and fresh vegetables high in calcium (sweet potatoes

and endive, for example) [Zeigler Brothers Inc.®, Gardners, PA]. Examples of various vitamin-mineral supplements used by facilities breeding PGFs:

- Mix of [1]Nekton® Rep™, [1]Nekton® MSA™, and [2]Rep-Cal® D™ each 1/3 by weight (Baltimore Zoo)
- [3]Walkabout Farms Insectivore and Pinhead Supplement (NAIB)
- [2]Herptivite™ and ground calcium carbonate (Denver Zoo)
- [4]Reptimin® (Cleveland Metroparks Zoo).

([1]Gunter-Enderle Enterprises Inc. NEKTON®-PRODUKTE, Clearwater, FL; [2]Rep-Cal® Research Labs, Los Gatos, CA; [3]Rock Solid Herpetoculture; [4]Tetra/Spectrum Brands, Inc., Blacksburg, VA)

HEALTH CARE

MEDICAL CARE

Beyond the health assessments performed when new animals arrive into quarantine, a proactive health care plan is important in all captive animal collections. Routine health care performed by veterinary specialists and husbandry staff should include the following:

- Weighing valuable specimens periodically for baseline information and health monitoring. Baseline *Atelopus* weights-

Avg. adult male:	Avg. adult female:
8-12g for Locality **A** & **C** populations	10-15g for Locality **A** & **C** populations
3-5g for Locality **B** population	4-7g for Locality **B** population

- Bi-annual testing to monitor parasite loads.
- Worming: We recommend worming wild individuals only to reduce excessive loads in order to maintain natural gut flora species in the event that reintroduction becomes necessary or a symbiotic relationship exists with gut flora. Captive-produced offspring will not have the same parasites as wild specimens, so worming can be performed with the approach that neither animal nor enclosure will ever remain parasite free, however load levels can be reduced.
- Isolation and treatment of thin, ill, or injured specimens
- Any animals that dies in captivity should be sent for thorough histopathology from a qualified pathology lab as part of a necropsy (if you need a suggestion, Northwest ZooPath Laboratory of Monroe, WA is proficient with amphibian pathology).

REPORTED MEDICAL ISSUES IN CAPTIVITY

Since this is the first opportunity for most AZA institutions to work with an *Atelopus* sp., it is expected that there would be many losses at holding institutions as they work out the husbandry of these unusual frogs. There have been various degrees of success and failure with F1s at the holding institutions. Some have lost all of their frogs, while others have lost only a few, and some have lost none. There have been some common medical issues at multiple institutions housing golden frogs over the past years and the brief presentation of these issues is just a starting point for conversations with your facilities' health care and husbandry staff.

- **Chytrid** has been reported in three collections containing PGFs. In all cases it has come from other species in same area of building. It has been an issue in captive collections, especially in walk-through public exhibits since disease control is practically impossible. With the development of rapid-testing techniques, chytrid testing can be done in most diagnostic labs these days. If a specimen in your collection is chytrid (+), isolate the animal (or entire enclosure) and begin the procedure for itraconozole baths on the animal and disinfection protocols on all equipment, enclosures, and cage materials (see Quarantine section and Appendix III for details on treatment and disinfection).
- **Trauma** is a risk with any species. In addition to typical nose rubs cleared with topical medicated ointment, there was an incident of a bleeding eye possibly due to predation from a cockroach

(Houston Zoo) in a PGF exhibit. One case of hyphema (blood under the lens of one eye) from an unknown trauma was also reported. The vet was able to seal the eye shut with liquid surgical glue for a few weeks while the eye healed fine. The glue was shed off with the skin every few days, and more glue was reapplied.

- **Vitamin A deficiencies** may have been the cause for some captive offspring being unable to catch and hold onto their food items. Commonly referred to as "short-tongue syndrome," it is a squamous metaplasia (loss of mucous glands) of the tongue that was first identified in Wyoming toads. Treatment involves increasing the Vitamin A supplement levels (hepatic retinol).
- **Cutaneous nematodiasis** was diagnosed from dorsal skin lesions in six specimens in separate enclosures at NAIB in 2004-2005. The nematode has not yet been speciated nor origin identified. The disease has only been seen in *A. zeteki* within the collection. Animal were diagnosed using cytology and/or histology. The initial three cases were found dead or died rapidly. However, antibiotics, deworming, and wound treatment cleared three other cases. The lesions initially presented as a pale blanching on the dorsal aspect of the frogs and the skin was easily removed demonstrating an open ulceration. Preventative husbandry management includes more frequent tank break-downs and routine deworming. Animals with skin nematodes did not necessarily have evidence of intestinal nematodes.
- Skin sensitivity to an irritating substance, toxin, or virus that seems to cause **excessive skin sloughing & redness in animals that test chytrid (-)**. These golden frogs have had necrosis in the glands on their skin (interstitial cells). Recommended treatment regimes should include isolating the infected frogs, keeping the environment as clean as possible to minimize secondary bacterial infections, and soaking with Amphibian Ringers Solution (ARS).
- **"Baggy pants" syndrome** refers to the fluid filled area around thighs and vent of frogs, and is common in both male and female PGFs housed in stream systems and high humidity environments. It is <u>not actually a medical concern</u> as it occurs in wild specimens found in breeding streams, as well as captive *Atelopus*. When PGFs were first brought into captive situations, it was treated by draining the excess fluid off of the vent area, but reoccurred quickly. In collections where the frogs were moved to a drier environment, the condition improved, which may be important to maintain kidney health.

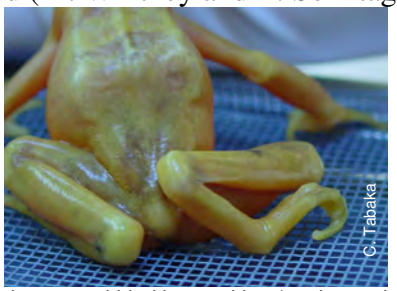
"Baggy Pants" edema in a wild specimen.

- A few specimens were slightly lethargic and legitimately had **edema** associated with kidney failure (full body edema, not localized in vent area), but this was not as common as just having baggy pants. A possible cause of this in otherwise apparently healthy animals is the use of improperly reconstituted RO water or other poor water quality issues, causing the pure or low-level ionized water to move into and dilute the highly ionized tissues within the animal's body via osmosis. The body's attempts to remove the excess water in the tissues puts a lot of strain on the kidneys causing failure and potentially death if the water quality issue isn't corrected.
- Several institutions have reported cases of paralysis in golden frogs. Typically, frogs are found with **full hind limb extension and whole body rigidity (tetany)**. The following list of potential factors are probable:
    - Upper thermal limit exceeded: The animals were possibly stressed when an air conditioning unit failed at one facility. All recovered when cooled appropriately.
    - Dietary Imbalance – Calcium or Vitamin A? Confirm that the Ca:P balance in the diet and supplements are adequate, or else kidney failure may occur over time. Central Park Zoo had

reported calcified kidneys from necropsied PGFs (cases associated with tetany and edema) as the calcium had precipitated into the kidneys due to an imbalance. Dietary or environmental causes have not been concluded. See other issues with calcium below.

- Toxicity (chemical or pathogenic):
  - *Case 1*: 24 golden frog offspring awaiting shipping were temporarily maintained overnight in a crowded 5-gallon isolette due to an unexpected delay with the airlines. All animals appeared fine at 24 hours, but 5 PGFs were found in a rigid state, with the hind limbs fully extended at 28 hours. The animals were immediately placed under running filtered fresh water from a hose and rinsed well over a 1-2 hour time period, in which all recovered fully. Potentially toxic steroids, bufadienolides (and possibly, but doubtfully zetekitoxin) secreted from the skin of captive raised *Atelopus* caused a reaction (Daly, pers. comm.). Ingestion of plants containing bufadienolides, including *Kalanchoe* sp., foxglove, oleander, lily-of-the-valley, and various species of milkweed has been responsible for cardiac and neurological symptoms, including tetany in canines.
  - *Case 2*: Overdosing PGFs with a 10x the recommended dose of itraconazole due to a dilution mistake caused frogs to go into a catatonic state within 3 minutes. The animals recovered a few hours later.
  - *Case 3*: Exposure to unexpected chemicals or toxins produced from fungal/viral pathogens on cage materials and/or skin may also be a potential cause of paralysis. The Baltimore Zoo experienced an epidemic of skin sloughing, accompanied by tetany in some cases. Fungal dermatitis was cultured from sacrificed specimens, and it is believed that toxins released by the fungus were responsible for the tetany. Recommended supportive care for the frogs along with complete steam sterilization of cages and enclosure materials stopped the outbreak.
- Water quality issues – In collections where these episodes of tetany occur, many factors, including water quality, have been investigated. The Detroit Zoo noticed an increase in phosphorus in their city source water (1-2ppm) which is used for corrosion control to bind lead in cities with old pipes. Detroit Zoo switched water source to reconstituted RO water, began adding commercially available phosphate binding crystals/resins to the water, and ceased gut loading crickets with phosphorus-rich Zeigler's cricket diet, which stopped the cases from occurring, and when these procedures were discontinued, the symptoms returned (DZI staff is further studying these effects to identify the causative agent and degrees of impact). It is believed that these animals are experiencing tetany due to a phosphate replacing calcium in the metabolic pathway, as can occur in captive fish. It is recommended that phosphate sponges or resins are used to eliminate excess.

Staff at the Detroit Zoo noticed a **pre-tetany posturing**, characterized by animals that *hold their back legs closer to the body than normal with their hind legs, feet, and knees slightly elevated often resulting in hind feet off the ground, ankles that overlap and rest on the lower back* as opposed to ankles just touching and resting on the ground in healthy animals. The toes may be curled a bit, but not always. Pre-tetanous animals also have difficulty self-righting when flipped onto their backs, however healthy animals have been shown to have difficulty with this when repeated multiple times or stressed (M. Whitney and E. Sonntag, pers. comm).



Curly toe and unnatural hind leg position (resting on lower back)

Full hind limb extension associated with tetany.

Recommended care for cases of paralysis includes isolating the animal, immediately soaking with a buffered Amphibian Ringers Solution (ARS), and having a veterinarian treat with an oral calcium supplement. Also using antibiotics if it is due to pathogenic toxicity. To prevent outbreaks, be careful to sterilize cage materials prior to use; keep full-spectrum lighting levels high; and test water quality regularly, including phosphorus and bacteria.

- **Metabolic Bone Disease** was evident in the curved long bones and rubbery limbs documented in juvenile or recent metamorphs at three facilities. It is due to insufficient calcium absorption, which can be improved by increasing full-spectrum lighting and improving the volume and frequency of vitamin dusting/gut loading food items to increase calcium absorbed in the diet.

Radiograph – curved leg bones.

Deformity from MBD (knees straight out).

REPRODUCTION

Captive PGF breedings are based on their natural *in situ* reproductive cycles and behavior. From field research, it appears that eggless female frogs move into the forests in the late dry or early rainy season (February-March). Females full of eggs typically return to the streams in the late rainy season and early dry season (November-December). Males tend to stay near the streams year-round awaiting the females return, hence the need for established territories. Male PGFs can be observed challenging intruding males for prime sites along breeding streams. Some males may amplex returning females in the forest, but all females encountered at the stream are amplexed. There is a gender bias in favor of males and the majority of males are therefore single.

Amplexus can last from a few days up to 2 months, with males probably not feeding during this time and females can become exhausted carrying around the extra weight of the male. The amplectant pair search for a suitable underwater, darkened rock crevice to lay eggs. Preferred sites have good water circulation to aerate the eggs that are arranged in strings. The 200-900 white eggs are light-sensitive, thus the need to lay them underneath a darkened rock. Hatching begins in 2-6 days and the tadpoles graze on diatoms (golden-brown algae) on the surface of rocks while adhering to the rocks in the swiftly moving water by utilizing their suctorial ventral disc. They have been observed climbing up rock surfaces into elevated pools while feeding.

The tadpoles metamorphose into green and black froglets within 120-240 days. Although a few have been found associated with the streams, it is believed that the juveniles also move into the forests for foraging.

A recent captive-bred Atelopus zeteki *metamorph.*

POPULATION MANAGEMENT

The primary goal of our breeding endeavors has been to breed unrepresented potential founders at our **Primary Breeding Facilities** (facilities that received wild-caught animals). Per our Small Population

Management (SPMag) Advisor, it is recommended to breed unrepresented wild caught specimens only to each other to maximize genetic potential in the overall captive population, minimizing overall gene loss. Further, each F1 bloodline will be bred to another F1 bloodline of the same population (Locality **A**, **B**, or **C**) and not bred back to a wild-caught animal for the same reason.

The next step was to place offspring from one unique bloodline at other AZA institutions for housing (**Phase I Facility**). Once an institution had become proficient at PGF husbandry and was able to successfully breed and raise metamorphs, that facility would be designated to receive a second (or more) bloodline(s) from the same population for designated valuable breedings (**Phase II Facility**).

Long-term captive management plan is to maintain 30-50 frogs from each bloodline. The Population Management Plan (PMP) is still in process as of this writing.

If you have questions about which animals should be bred, contact the Studbook/Population Manager (see Appendix I). Basic recommendations are as follows:

- Phase I Facilities: If all of your animals are from the same bloodline (sibling group), you should practice breeding PGFs in order to become more proficient at cycling and rearing PGFs. It is not as easy as some other anuran species, so do not become discouraged if the first attempt is not successful. These offspring will be undesirables and may displace other more valuable offspring from a desirable breeding, so euthanasia will be necessary to eliminate, or at least reduce their numbers. Be prepared to house offspring indefinitely if allowed to survive.
- Phase II Facilities: If you have two different bloodlines, then it is recommended that pairs represent each bloodline to maximize genetic diversity. Offspring from desired pairings may be sent on to other facilities, but be prepared to maintain desired numbers indefinitely.
- Over-represented females or egg-heavy females can be paired randomly and allowed to dump eggs, which should be discarded. As the female F1s began reaching sexual maturity (2-3 years), we lost a few animals that were not able to spawn successfully. It is recommending that institutions set these animals up in breeding situations and dispose of any eggs that are laid or practice rearing techniques and cull as a metamorph.

CAPTIVE REPRODUCTION METHODS

PREPARATIONS

- Preparation for the breeding season begins 1-2 months in advance by setting up clean "breeding tanks" with suitable underwater egg-laying sites and allowing the tank to condition (i.e., establishing healthy water quality and natural algae growth). You will need one tank per recommended breeding pair for ease of assigning sire/dam and since these are the enclosures in which the eggs and tadpoles are to be raised.
- In preparation for rearing offspring, establish 5-20 springtail (*Collembola* sp.) colonies several months in advance for feeding new metamorphs since fruit flies and pinheads are too large. Also consider increasing the number of fruit fly jars (flightless *Drosophila melanogaster*) your facility maintains by 6-12 jars weekly per breeding pair for when the offspring are large enough.
- Find out your institution's policies and procedures on culling (euthanasia for population management purposes). Euthanasia as a management tool has been approved by the USFWS. Due to the number of eggs laid and froglets produced, it may be necessary to eliminate individuals at the tadpole or metamorph stage. Consult your medical department in advance, if needed. An overdose of the anesthesia tricaine methanesulfonate (Finquel MS222) has been used successfully on PGFs for this purpose (Argent Chemical Laboratories, Inc., Redmond, WA)
- Acquire food for the tadpoles - Sera-micron® and/or ground Hikari Algae Wafers® and/or consider setting up a diatom culture (methods described by Edi Sonntag in Appendix VII).

CYCLING

- In captivity, pairs may go into amplexus at anytime of the year, however it is recommended to **target November-December for pair introductions**. For ease, rotate introduced pairs into stream breeding tanks from smaller holding enclosures.
- Pairs tend to go into amplexus almost immediately (1-2 days) after being introduced. Pairs that fail to amplex immediately tend to never spawn and should be re-paired if possible, to take advantage of their breeding condition.
- Monitor the pairs closely during amplexus for healthy body condition. Separate and feed heavily for a few days if it appears that either is wasting.
- It has been shown that it is not necessary to manipulate any environmental parameters (temperature, lighting, humidity, etc.) for PGFs to breed in captivity.

SPAWNING/EGGS

- Spawning tends to take place overnight. Successfully introduced pairs spawn typically within a 15-30 days of pairing. On average, wild-amplexed frogs took 2-17 days to spawn after being placed into the breeding tanks (if the pair spawned at all), however it is unknown how long they were amplexed prior to collection.
- If you find the pair separated, it is most likely that they have spawned under a rock. Do not move rocks to locate eggs or you risk crushing them. Be patient!
- **Do not remove the eggs to another tank for rearing**. It is preferred to leave them in the well-cycled breeding tank.
- Once the adults have spawned, remove them from the reproduction enclosure so that they do not disturb eggs or eat metamorphs once they develop.
- Typically, a single strand of 200-400 white (or off-white) eggs are laid that take 3-7days to hatch. Infertile eggs show no signs of development and may appear fuzzy (dispose of them after all other eggs hatch).

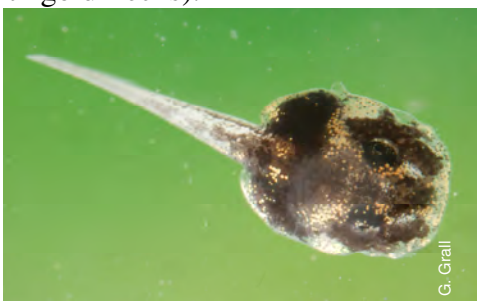
Strings of white Atelopus eggs at the Detroit Zoo.

- The eggs are <u>light sensitive</u>, so shield from direct light if exposed and <u>avoid photography</u>!
- To provide a larger volume to stabilize water quality and to increase the surface area for tadpoles to graze upon, consider raising the water level, if possible, once the adults have been removed from the breeding tank.
- During routine maintenance (water changes, etc…), be very careful not to disturb eggs.
- Even if you are not prepared to rear all of the offspring or if the breeding is just for the experience, avoid disposing of a portion of the eggs at this point. Hatch success varies, especially for novice breeding efforts, so it is recommended that you wait to dispose tadpoles if a portion of the breeding is to be culled. As your confidence in successfully rearing multiple clutches of PGFs increases, it may be easier to dispose eggs rather than tadpoles or froglets (consult the studbook holder or Vicky Poole before making this step). [Be aware that you may be risking your overall chance of breeding success when disposing eggs, as you may inadvertently select infertile eggs or eggs that are not as healthy as others. Eggs may be removed and placed into a strong bleach solution for disposal.]

LARVAL HUSBANDRY/METAMORPHOSIS

- Tadpoles are entirely white for the first few days after hatching, and then they develop pigmentation (black with gold flecks).

Dorsal view



Ventral view

- They graze on rock surfaces and tank walls, consuming diatoms (golden-brown algae) if available. They should also be offered supplemental foods daily since the diatoms in most tanks are not sufficient. As they are grazers, it is necessary to adhere offered food to the surface of rocks, Petri dishes, tank walls, etc., placed in a location that the tadpoles will encounter it. A paste of the preferred tadpole foods, [1]Sera-micron® and/or ground [2]Hikari® Algae Wafers™, can be made and smeared onto tank rocks or the tank surface directly using a syringe, or onto a Petri dish, rock, or other solid surface, left to dry, and placed with the tank once dry (overnight works well). [[1]Sera Laboratory, Heinsberg, Germany; [2]Hikari® Sales USA, Hayward, CA]
- In our initial year we even collected rocks from a clean, local stream to provide adequate food diversity. This proved to be unnecessary, yet can be a source for diatoms if needed.
- Maintain water temperatures between 72-74ºF as tadpoles housed below 70ºF do not feed well and waste away.
- Good water quality is very important for tadpoles, and water changes/filter rinses should be maintained. Be careful of the small tadpoles and try to remove as much uneaten food as possible. If there is uneaten food left in the gravel on a regular basis, reduce the amount offered to encourage the tadpoles to forage within the gravel and improve water quality.
- If the Dissolved Oxygen (DO) is lower than 5mg/L, it may be necessary to add an airstone or two to increase the oxygen level in the water.
- If there is an unexpected loss of tadpoles, check water quality, especially phosphates. A phosphate binding resin may be necessary to prevent the loss of additional tadpoles.
- If you are not prepared to rear all of the offspring or if the breeding is just for the experience, it is a good idea to dispose of a portion of the tadpoles at this point (up to 50%). Do be aware that you may be risking your overall chance of breeding success as you may inadvertently select individuals that are not as healthy as others. Selected tadpoles should be removed from the breeding tank and euthanized by an overdose of tricaine methanesulfonate (Finquel MS222) [Argent Chemical Laboratories, Inc., Redmond, WA].
- It will take roughly 75-100 days to see the first metamorph and as long as 150-265 days until the final tadpole metamorphs.
- SVL upon metamorphosis: ¼” (6mm)

JUVENILES

- Juvenile care can be difficult based on the need for successful springtail colonies or alternate small food items. It is helpful to heavily seed the moss substrate within the breeding tank with springtail colonies in advance (while there are still tadpoles in the tank) as a constant source of food.
- Offer *ad libitum* springtails, fruit flies, or pinheads daily (volume offered increasing with population density and as the size of the frogs increase). Dust all pinheads and fruit flies with the same vitamin supplements as offered to adult PGFs in order to help minimize MBD issues.
- As the frogs grow, they can be offered larger food items.

- Enclosures housing juveniles should be misted more often than the adults to prevent desiccation of small froglets. It is recommended at least twice daily with fresh dechlorinated water. Relative Humidity should be between 80-100% and air temperatures should be maintained between 72-75°F.
- Mist and rinse more frequently to keep cages more humid and cleaner.
- Caution should be used when opening tanks as froglets climb glass sides of tanks and may congregate in the upper corners of tanks.
- NOTE: It is acceptable to accession juveniles into ISIS as a group, as long as each breeding is designated by its own group number in order to track parentage.
- If animals are to be euthanized, it is recommended to wait until the offspring are at least 6 months post metamorphosis due to the difficulties of getting PGF froglets to feed sufficiently and survive this problematic time period. Euthanizing frogs immediately after metamorphosis may instill a false confidence in an institution's abilities to rear PGFs through to adulthood.
- Juvenile and subadult animals can be housed together in mixed sex groups for the first one or two years, however be aware that they may need to be separated by sex as they grow and become sexually mature.

SURPLUS SPECIMENS, CAPACITY BUILDING, AND REINTRODUCTIONS

PGF/PRD's primary goal is to produce genetically valuable specimens and place them at other AZA institutions, however there has will always be a surplus of offspring. It will be necessary to maintain a certain number of every PGF bloodline in order to have as great a genetic diversity as possible so that valuable genes are not lost, especially once the species goes extinct in the wild.

The USFWS has granted the permission to use euthanasia as a population management tool, so excessive numbers of offspring do not need to be produced once a facility is comfortable with their PGF reproduction methods.

Some animals' offspring may be placed at other institutions in accordance with recommendations made by the Studbook Keeper/Population Manager (see Appendix I for contact information).

One of PGF/PRD's original goals was to relieve collection pressure for Panamanian "zoos" and hotel displays by providing over-represented specimens to captive situations in Panama. Creating breeding and holding centers within range countries is referred to as "capacity building," and is a valuable conservation tool due to the decreased costs and limiting health risks. The ideal situation is to empower the range country to be able to sustainably manage their own species. However, providing long-term stable and skilled husbandry staff and population management within range country will be the challenge.

Our current efforts to establish one or more captive centers and possibly a preserve in Panama will soon reach fruition. The Houston Zoo has partnered with the El Nispero Zoo in El Valle to build, staff, train, and support the El Valle Amphibian Conservation Center (EVACC), and is also soliciting a similar partnership with the Summit Zoo in Panama City. Other initiatives are being pursued by the Denver Zoo and the Cleveland Metroparks Zoo. **Institutions can help by offering *financial support, construction assistance, equipment, and/or staff training and funding* to the EVACC (through PGF/PRD) or for another center in Panama**.

There is also the development of another amphibian initiative within Panama. The Amphibian Recovery and Conservation Coalition (ARCC), initiated by Zoo Atlanta and the Atlanta Botanical Gardens, focuses on Panamanian amphibian species *other than PGFs* at risk to extinction due to the coming chytrid epizootic. Comparable to PGF/PRD, they are also working with the EVACC to house animals from their rescue efforts.

One last, yet long-term possibility for surplus PGFs, would be the return of golden frogs to the wild should all *in situ* populations become extinct and the chytrid fungus no longer a threat to survival. Animals would

be released following strict health guidelines, of course. The likelihood of this occurring is low, yet always a possibility when a species is brought into captivity as a last measure for survival.

MISCELLANOUS

- How do I get frogs for my institution?

We are always looking for facilities to house and exhibit golden frogs; however**, if you are not affiliated with an institution accredited by the Association of Zoos and Aquariums (AZA), then you will not be able to receive frogs** due to their conservation status and the restrictions on our permits. This is to protect the frogs by not providing a conduit for them to make it into the pet trade, thereby increasing collection pressure on wild animals.

If you are an AZA facility, please consider how much space you can allot for the frogs and contact the PGF studbook/PMP holder or Vicky Poole (vpoole@aqua.org) for other husbandry questions. Contact information is available in Appendix I.

- ATAG Recommendations

Prior to PGF/PRD's efforts, the Amphibian Taxon Advisory Group (ATAG) 1999 Regional Collection Plan lists Atelopus zeteki as a Priority 4 – Phase-in Population. Now that golden frogs have been imported into AZA institutions, the taxon has been upgraded to a Priority 1 – PMP. PGF has been a priority project on the ATAG's action plan.

- PGF/PRD's Field Recommendations to Minimize the Spread of Chytrid

As pathogens can last a long time on field clothing and equipment, we are quite diligent about scrubbing off all soil and disinfecting all field equipment used in this project with 10% bleach or 10% ammonia solution, and completely drying equipment between uses. In addition, we always work in an east to west pattern to prevent the spread of chytrid. For more field disinfection options, see Appendix III.

For more information on chytrid and other amphibian disease links, we recommend the following:
http://dendroworld.co.uk/BDGarchive/chytrid_fungus.html - treatment of chytrid fungus
http://coloherp.org/cb-news/Vol-28/cbn-0111/Chytridiomycosis.html - another article on chytrid treatment
http://www.jcu.edu.au/school/phtm/PHTM/frogs/adms/scope.htm - strategies to control amphibian diseases
http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm - up to date global distribution of chytrid
http://www.jcu.edu.au/school/phtm/PHTM/frogs/chpr1/longcore.htm - description of *B.d.*
http://www.cdc.gov/ncidod/EID/vol10no12/03-0804.htm - origin of the amphibian chytrid fungus

- Website and PGF/PRD Listserve

Like all modern organizations, we have developed a website, which is maintained through the Denver Zoo. The site is bilingual (English/Spanish) in order to share information with the range countries of *Atelopus* frogs and the addresses are as follows:

www.projectgoldenfrog.org
www.proyectoranadorada.org
www.ranadorada.org

Also, it is recommended that supervisors, staff working with golden frogs, and appropriate researchers join the AZA zeteki listerve in order to stay current with husbandry information and field efforts. Please contact Pete Johantgen at the Columbus Zoo to join (Pete.Johantgen@columbuszoo.org).

- Field Opportunities

If you or a staff member at your institution is interested in participating in PGF/PRD field efforts, please contact Kevin Zippel or Rick Haeffner for upcoming trip information.

- Fundraising
  Fundraising efforts for PGF/PRD's activities have been in place since it's inception as Financial Support was one of our four main goals, along with Field Studies, Captive Management, and Education. Most of the efforts to date have been funded by grants. The balance of personnel costs and time been has covered by individuals or their institutions. Current fundraising efforts include grants applied for by PGF/PRD directors or coordinators, donations, and t-shirt/cap sales. Donations can be made on-line at the www.projectgoldenfrog.org website or made payable to "Project Golden Frog" and sent to the Denver Zoological Gardens (address listed in Appendix I).
- Museum specimens
  As captive-bred, surplus PGFs were culled, we preserved specimens (entire or skinned) by various methods including 10% buffered formalin, ethanol, or methanol to make them available to researchers and museum collections. To date, PGF/PRD has provided preserved specimens to the following institutions: American Museum of Natural History (NY), The Field Museum (Chicago, IL), Museum of Zoology (Ann Arbor, MI), Museum of Comparative Zoology (Cambridge, MA), and The National Museum of Natural History (DC). Surplus preserved specimens with permits can be made available to accredited museum collections only by contacting Erik Lindquist (address listed in Appendix I).

Second Edition by Vicky Poole, National Aquarium in Baltimore
First edition by Kevin Zippel, PhD, National Amphibian Conservation Center, Detroit Zoological Institution

Thanks to the folks at 11 institutions that took time in their busy schedules to fill out and return the husbandry surveys I sent out in 2005, and those who've answered my inquiries and given input since. My appreciation also goes out to Eli Bryant-Cavazos, Meredith Whitney, Bill Flannagan, Edi Sonntag, Kathy Duffy, Chris Tabaka, Leigh Clayton, and John Daly for answering all of the last minute questions that needed their valuable input and to the PGF photographers for the use of their pictures. Thanks to Karen St. John and Holli Friedland for editing advice. My undying gratitude belongs to Rick W. A. Haeffner for stepping up and helping out with PGF/PRD when all the pieces were falling apart. And finally, special thanks to Kevin Zippel for all of his help, advice, data, general comments, and friendship while allowing me to bastardize his original edition of this document. ☺
-V

APPENDIX I – Project Golden Frog/Proyecto Rana Dorada Contacts

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	VACANT - **Veterinary Advisor**
	VACANT - **Education Liaison**

APPENDIX II – Captive Population statistics as of March 2006.

For a rough idea of the number of specimens…

- Number of living wild-caught in US institutions –
    - 9.8 Locality **B** at MZB and National Zoo
    - 3.4 Locality **A** at DZI (2.0) and MZB (1.4)
    - 10.6 Locality **C** at DZI and Cleveland Zoo
- Number of total founders/potential founders collected to date:
    - 12.15.5 collected at Locality **A**
    - 9.9 collected at Locality **B**
    - 14.15.27 collected at Locality **C**
- Number of bloodlines represented: 2121 living offspring representing 28 bloodlines
- Number of F1 offspring currently –
    - 417 Locality **B** F1s from 6 bloodlines at 2 institutions
    - 889 Locality **A** F1s from 19 bloodlines at 27 institutions
    - 815 Locality **C** F1s from 3 bloodlines at 2+ institutions
- Number of institutions holding PGFs (of any population) – 27+ (2161 specimens total) per MZB
- List of institutions producing offspring as of 2006 (inbred or not):
    - MZB
    - Detroit Zoo
    - Denver Zoo
    - San Diego
    - Cleveland Zoo
    - NAIB
    - National Zoo
- Number of new institutions awaiting offspring – 11 (8 new to PGF/PRD)

APPENDIX III – Recommended Protocols for Disinfection of Chytrid on Surfaces

Table 1: Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis* and ranaviruses in field studies. Where concentrations and time are given, these are minimum shown to be effective. For *B. dendrobatidis* based on Berger (2001) and Johnson et al (2003) and for ranaviruses on Langdon (1989) and Miocevic et al (1993).

Purpose	Disinfection	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (e.g. scales)	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
	Vircon	1 mg/ml	1 min	*B. dendrobatidis* Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	*B. dendrobatidis*
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1:1000 dilution	0.5 min	*B. dendrobatidis*
	Complete drying		3 hours or greater	*B. dendrobatidis*
	Heat	60 C	5 min	*B. dendrobatidis*
			15 min	Ranaviruses
	Heat	37 C	4 hours	*B. dendrobatidis*
	Sterilizing UV light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1:1000 dilution	1 min	*B. dendrobatidis*
	Complete drying		3 hours or greater	*B. dendrobatidis*
Disinfecting cloth (e.g. bags, clothes)	Hot wash	60 C or greater	5 min	*B. dendrobatidis*
			15 min	Ranaviruses

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HYGIENE PROTOCOL FOR HANDLING AMPHIBIANS IN FIELD STUDIES

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8 October 2004

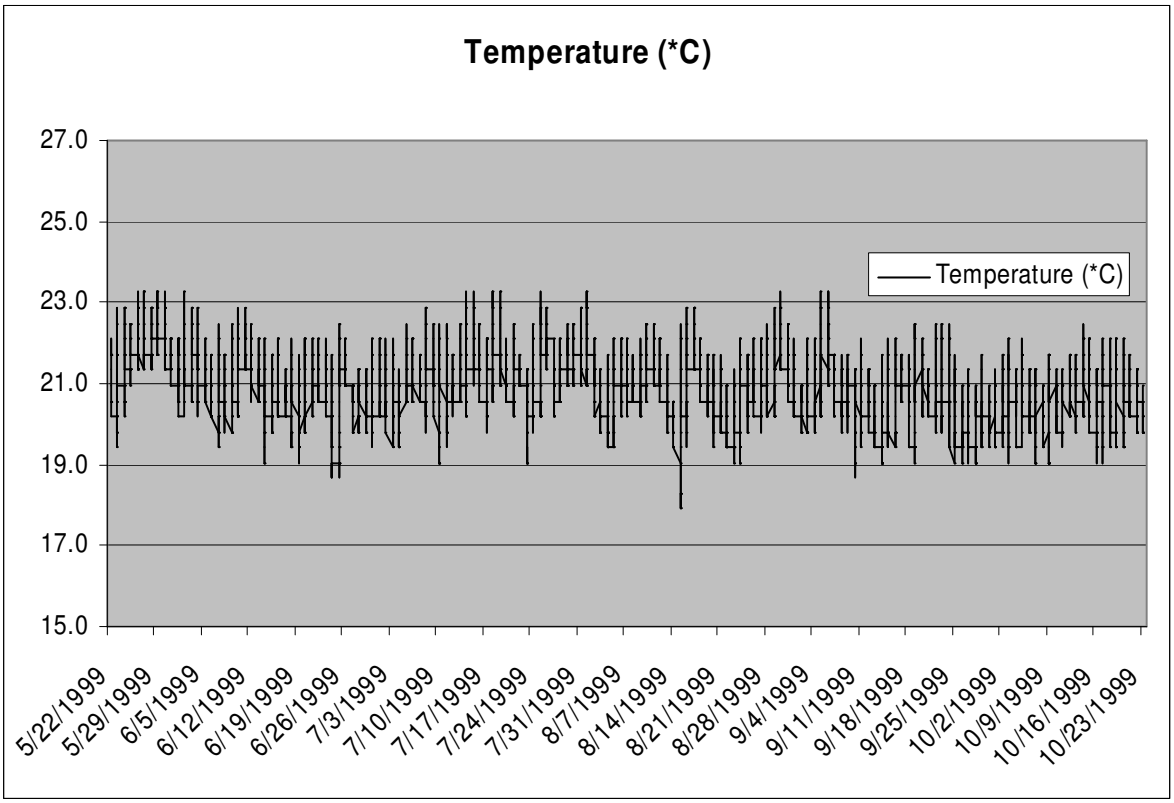
APPENDIX IV – Water Quality Data Collected in PGF Habitat

Locality	**A**	**D**	**D**	**E**	**E**	**E**
Elevation (above sea level)	2550'	2200'	2200'	2000'	2000'	2000'
Site Number	**Site 1**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 3**
Date	10/20/99	10/23/99	10/24/99	10/19/06	10/19/06	10/19/06
Time	12:00 PM	10:30 AM	11:30 AM	1:00 PM	2:30 PM	4:30 PM
Temp	22.5 C	21.1 C	21.1 C	21.6 C	21.8 C	21.7 C
pH	7.6	7.5	8.5	6.8	6.5	6.9
Dissolved Oxygen - DO (mg/L)	9.06	8.6	8.6	8.41	8.37-8.68	8.79
Ammonia	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Nitrite	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Nitrate	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Conductivity (microsiemens)	59	86	62	49	47	
General Hardness - GH	0-1 degree	1 degree	2 degree	0-1 degree	0-1 degree	0-1 degree
Carbonate Hardness - KH	1 degree	0-2 degree	0-2 degree	0-1 degree	0-1 degree	0-1 degree
Iron	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Phosphate	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm

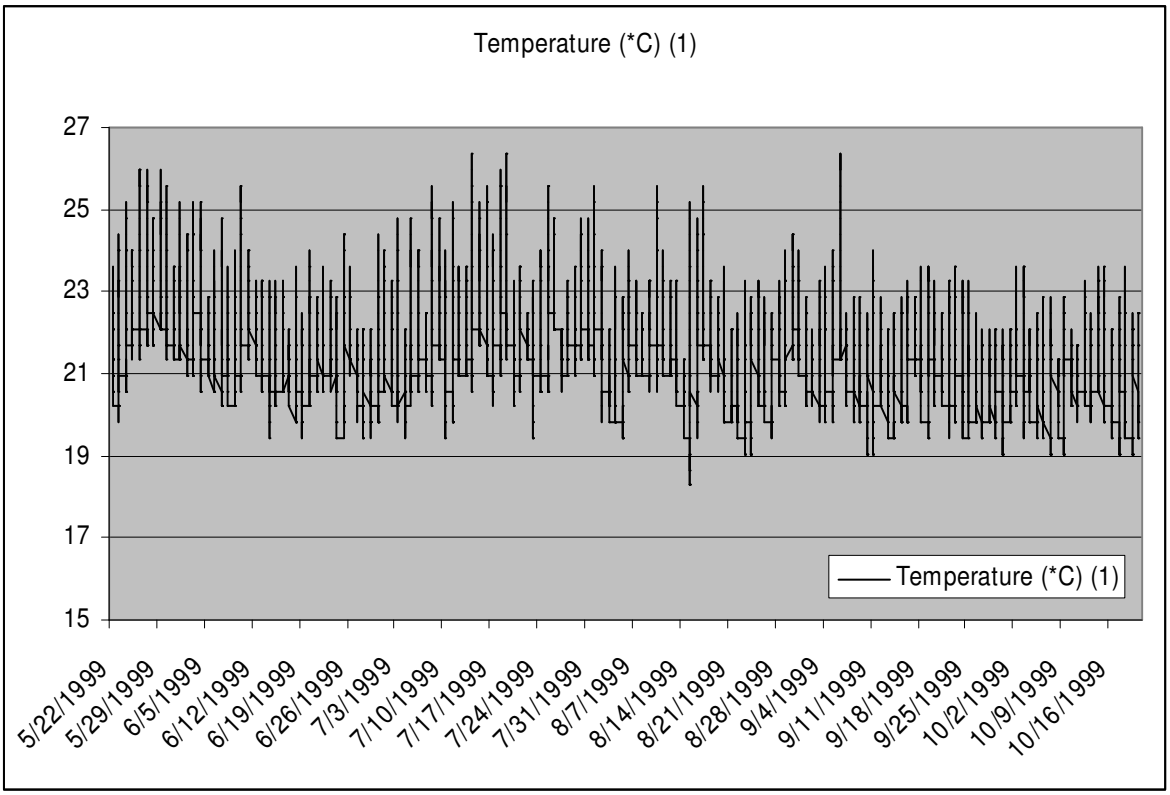
AZA facilities can contact the author or studbook holder to decipher the following population locations in order to protect the localities from illegal collection

APPENDIX V – Environmental Data Collected in PGF Habitat

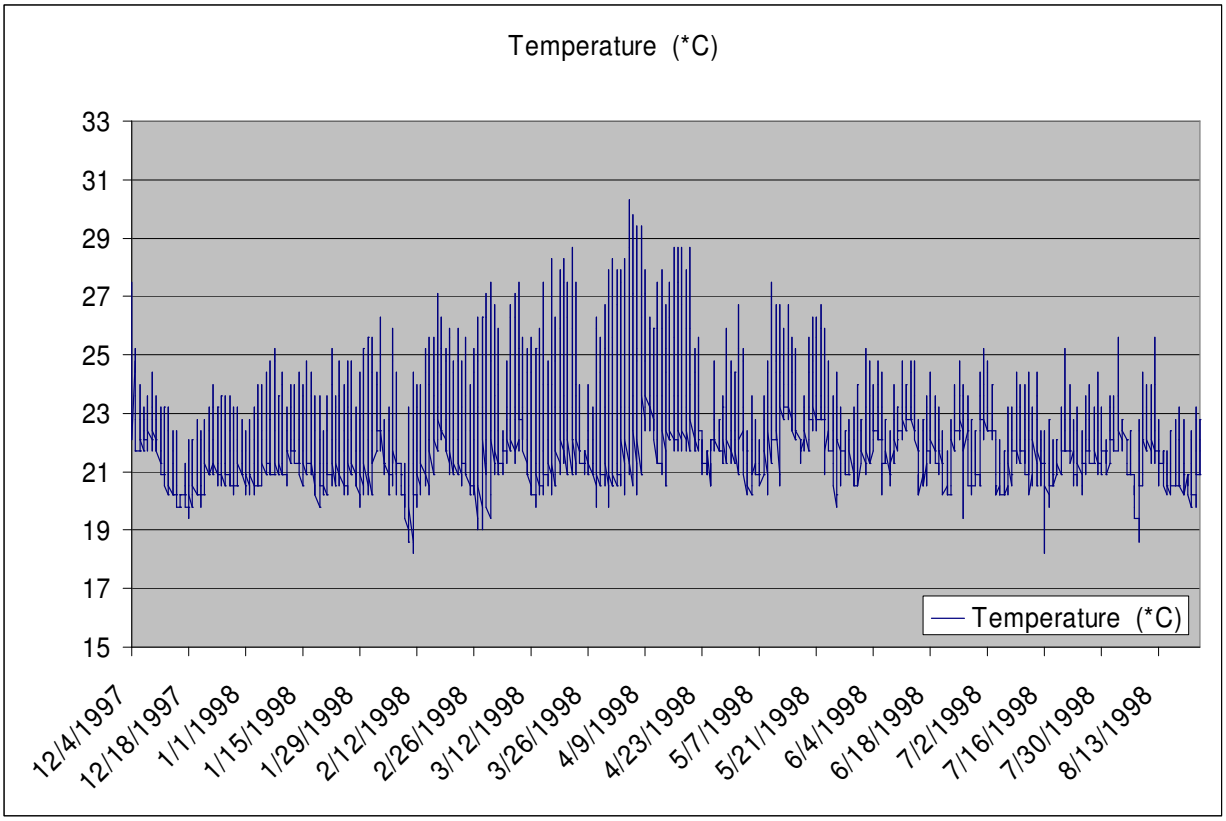
WATER **SITE: Locality D, Panama** (5/22/99 – 10/23/99)



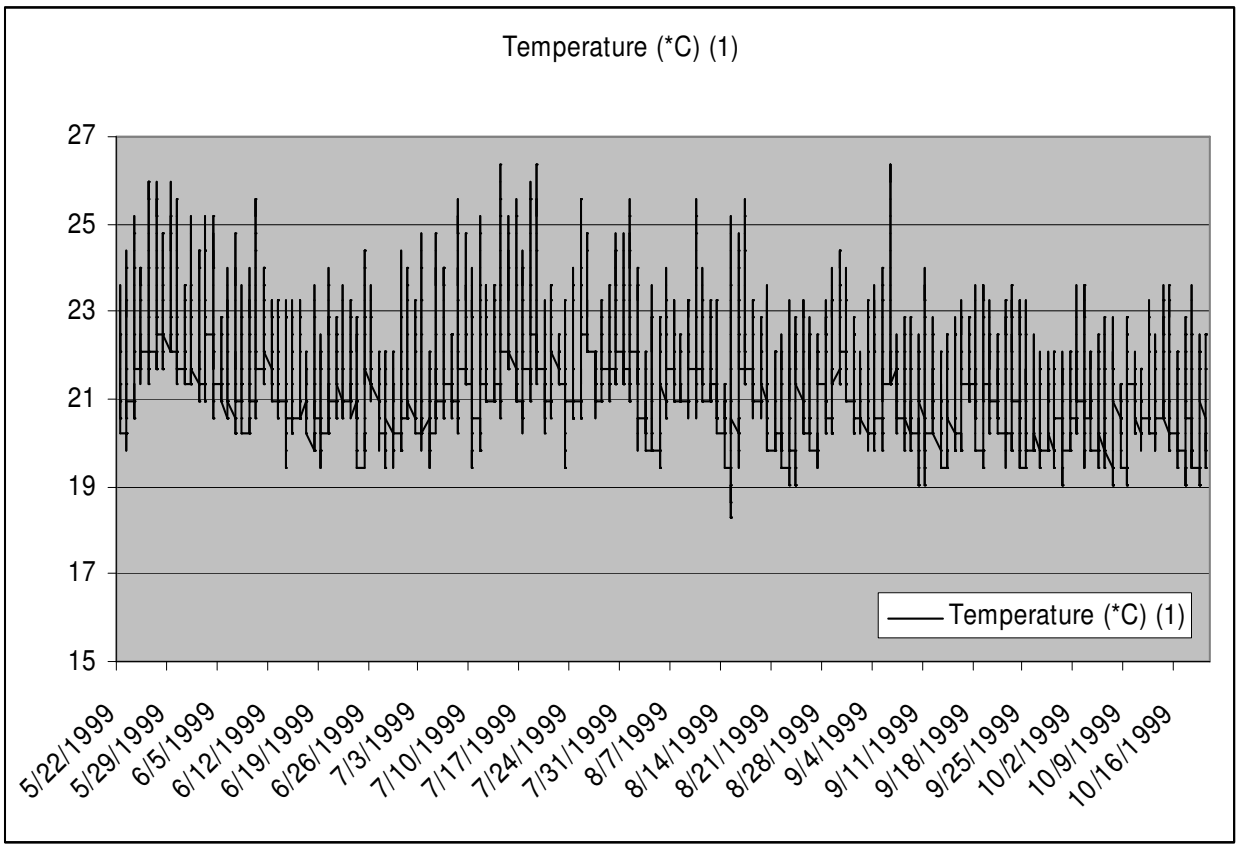
AIR **SITE: Locality D, Panama** (5/22/99 – 10/20/99)



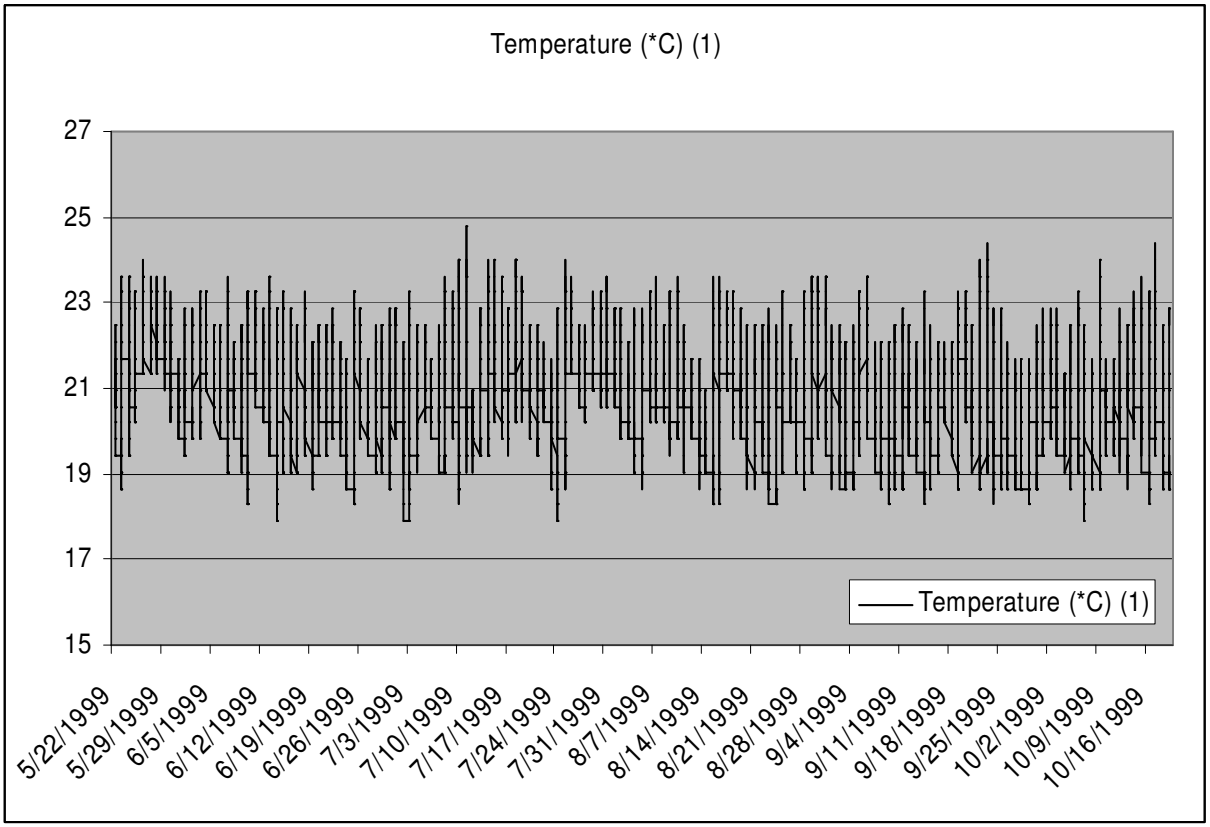
AIR **SITE: Locality D, Panama** (12/4/97-8/31/98 El Nino Year)



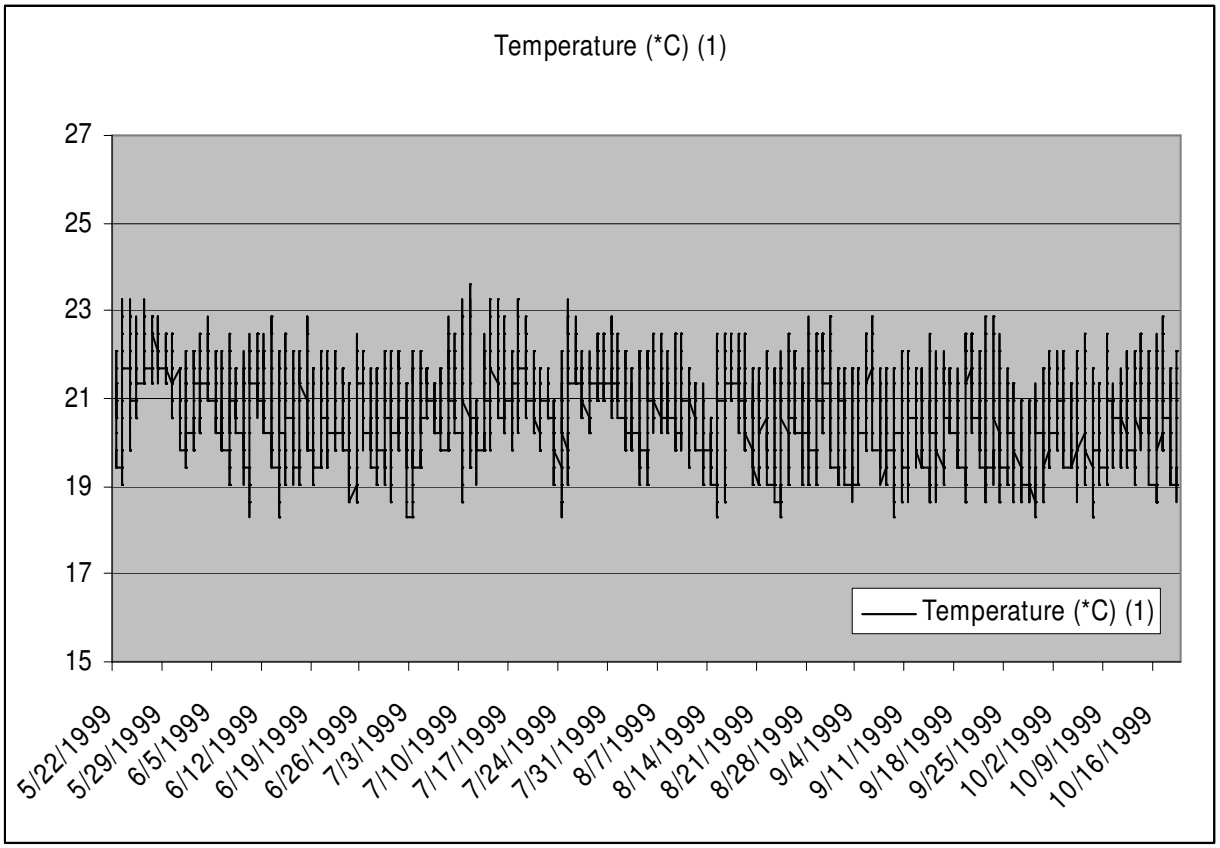
AIR **SITE: Locality A, Panama** (5/22/99 – 10/20/99)



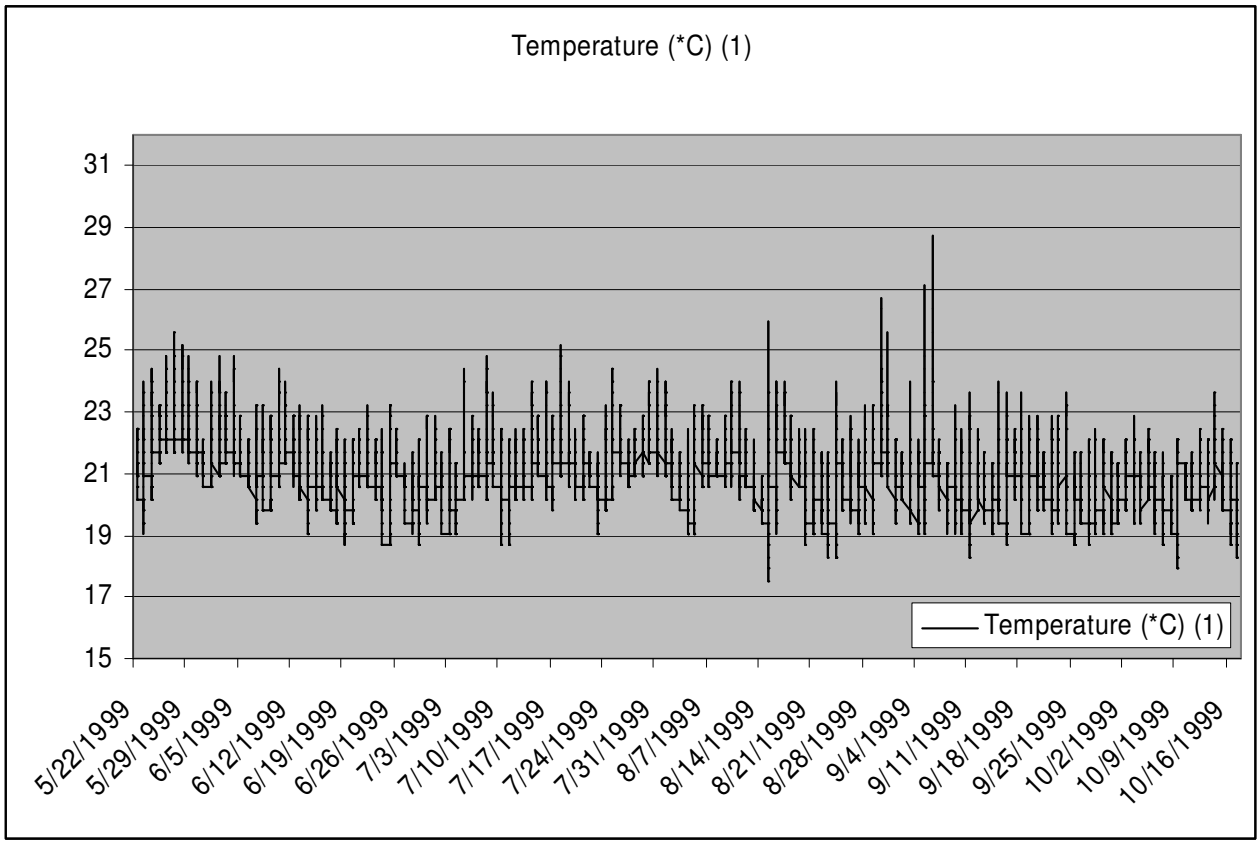
AIR **SITE: #1 Locality E, Panama** (5/22/99 – 10/20/99)



AIR **SITE: #2 Locality E, Panama** (5/22/99 – 10/20/99)



AIR **SITE: #1 Locality F, Panama** (5/22/99 – 10/20/99)



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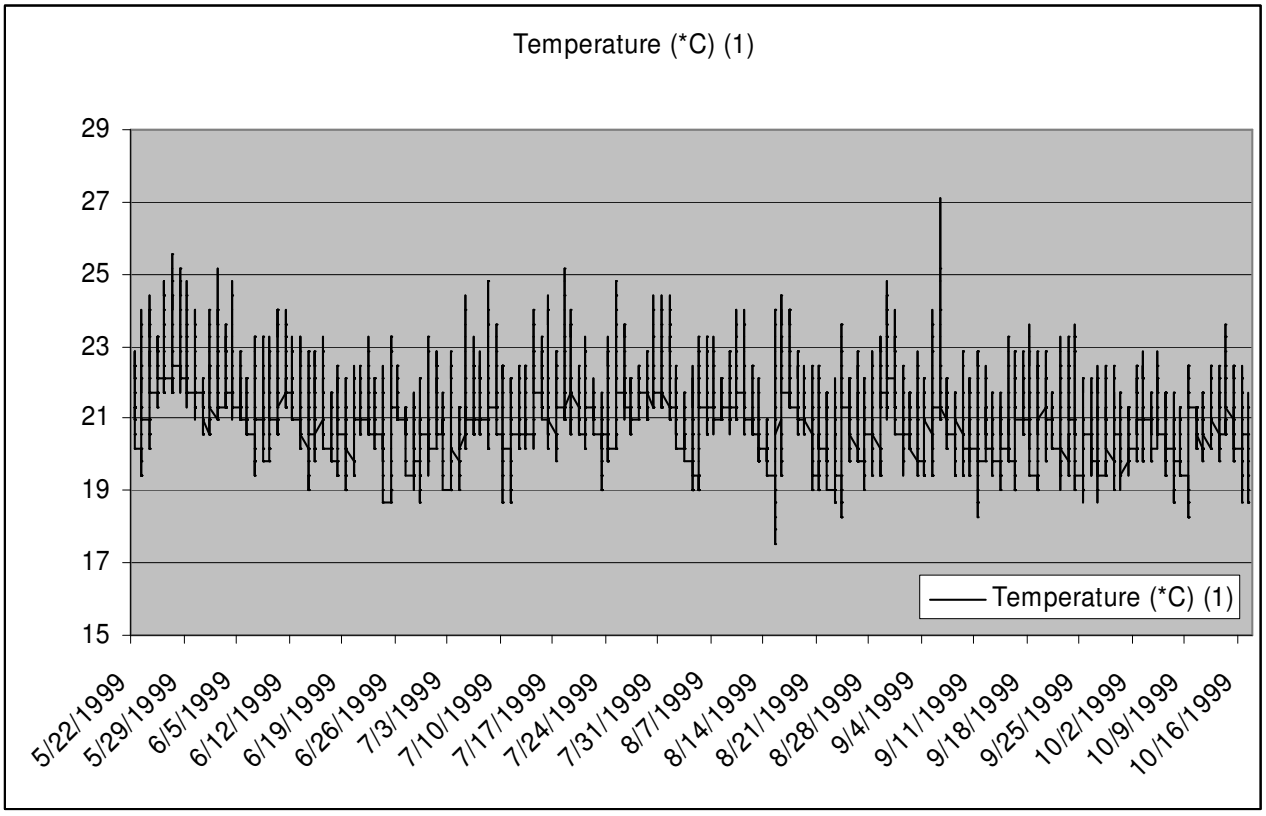
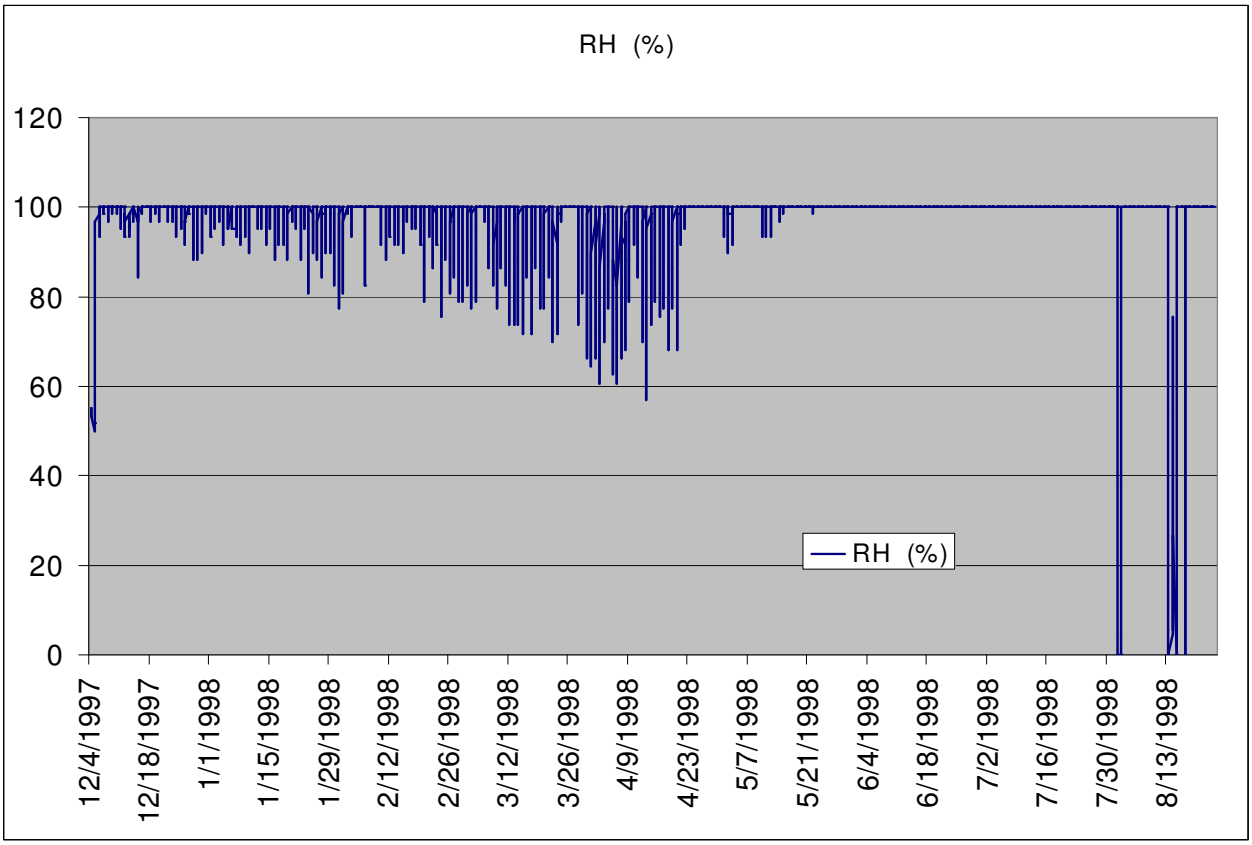


RELATIVE HUMIDITY SITE: Locality D, Panama (12/4/97-8/31/98 El Nino Year)



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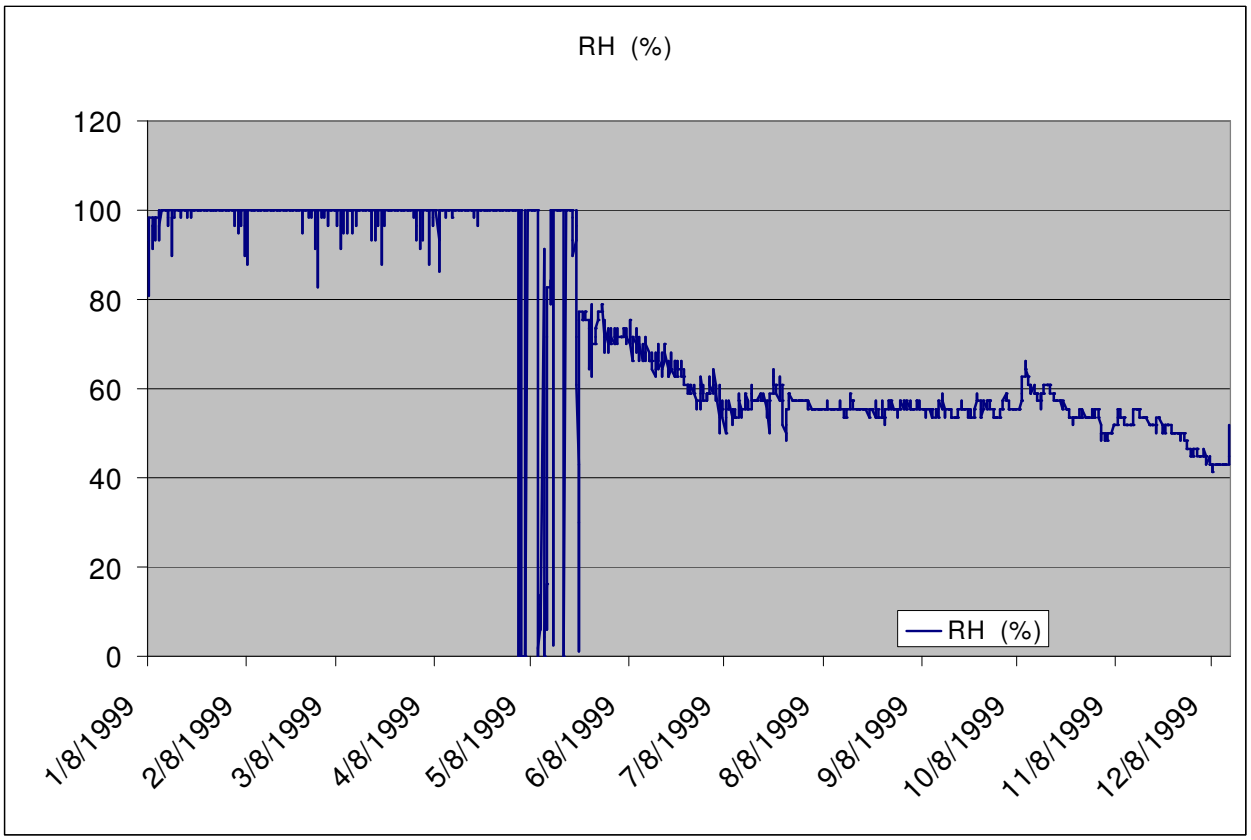
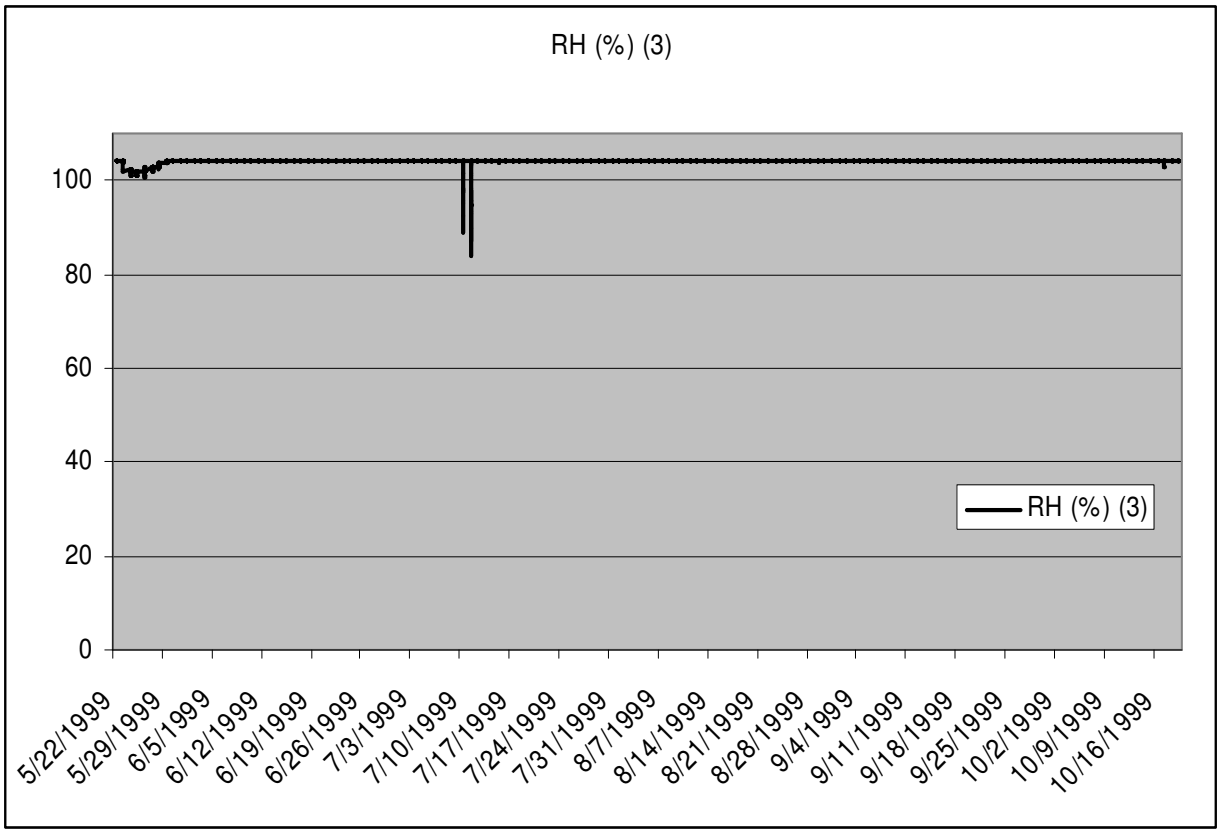
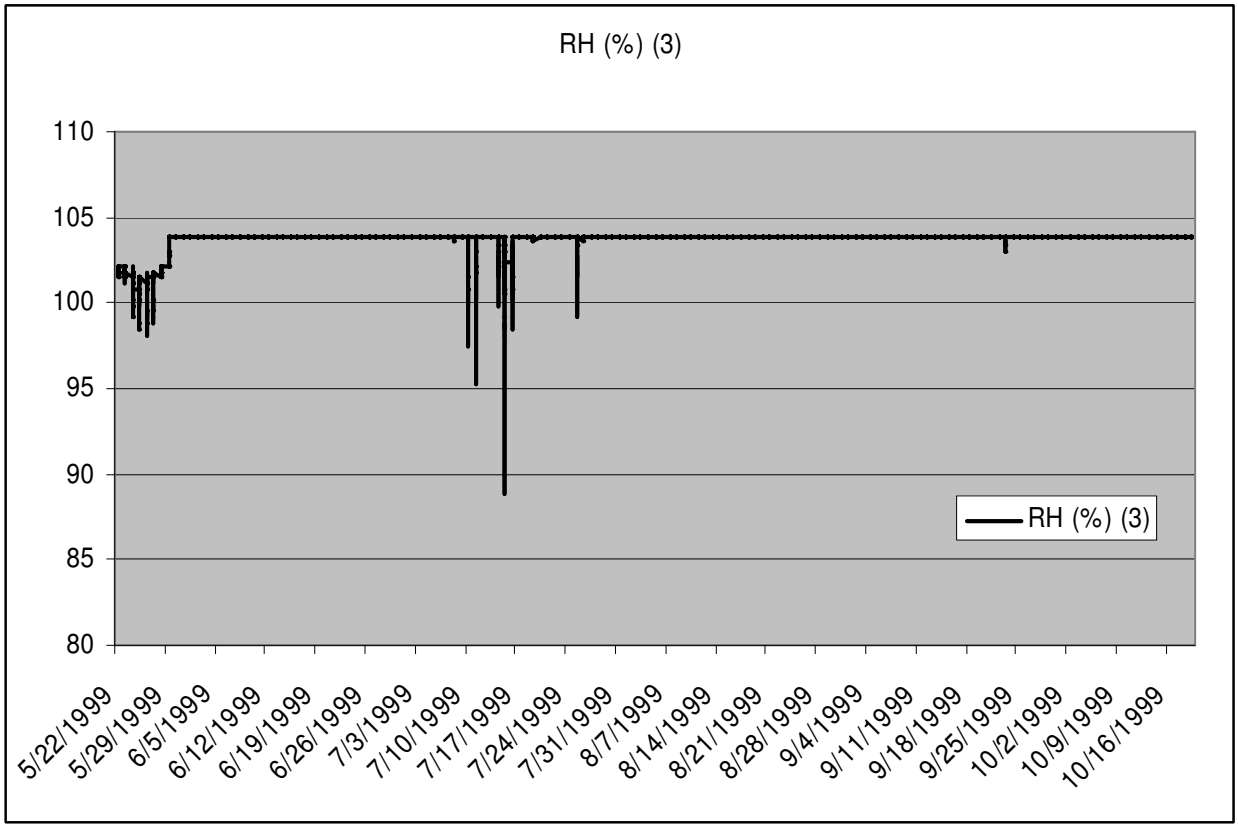


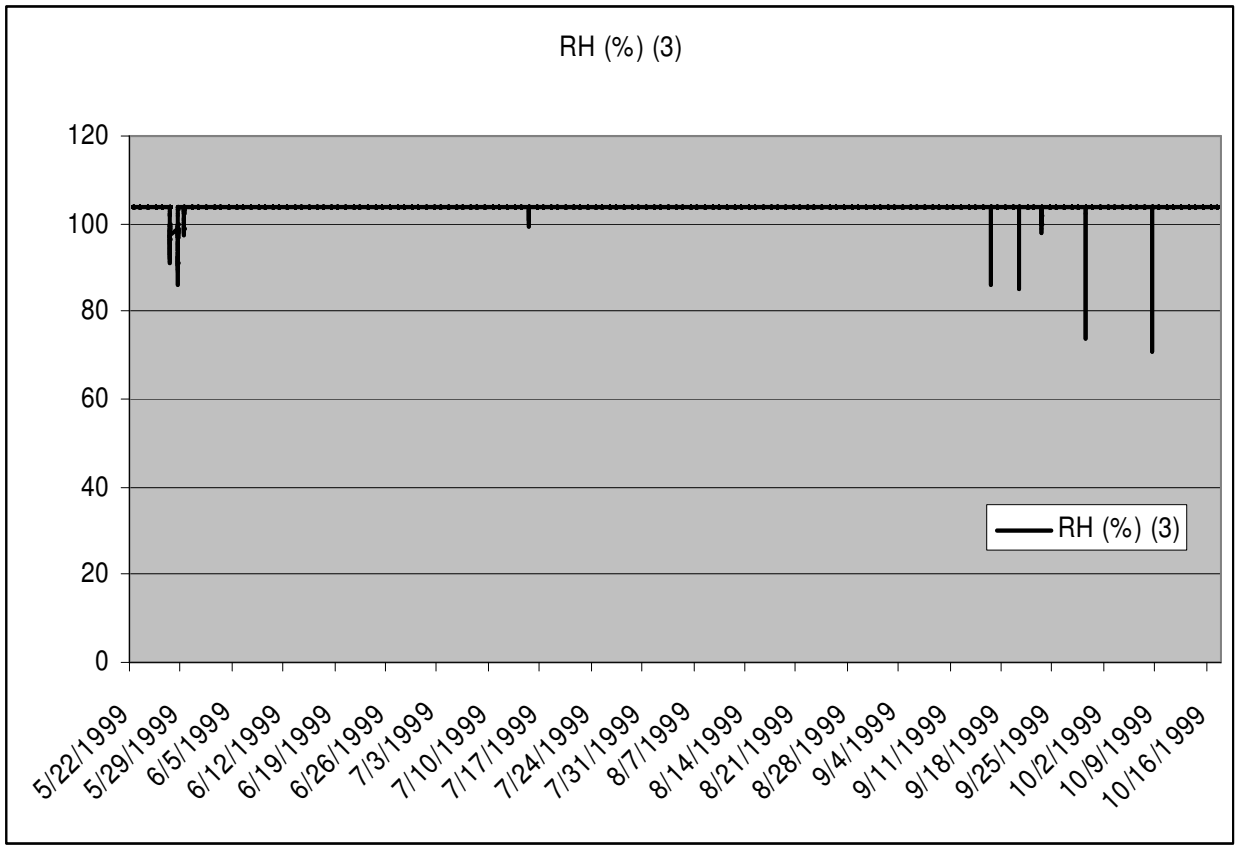
RELATIVE HUMIDITY SITE: #1 Locality E, Panama (5/22/99 – 10/20/99)



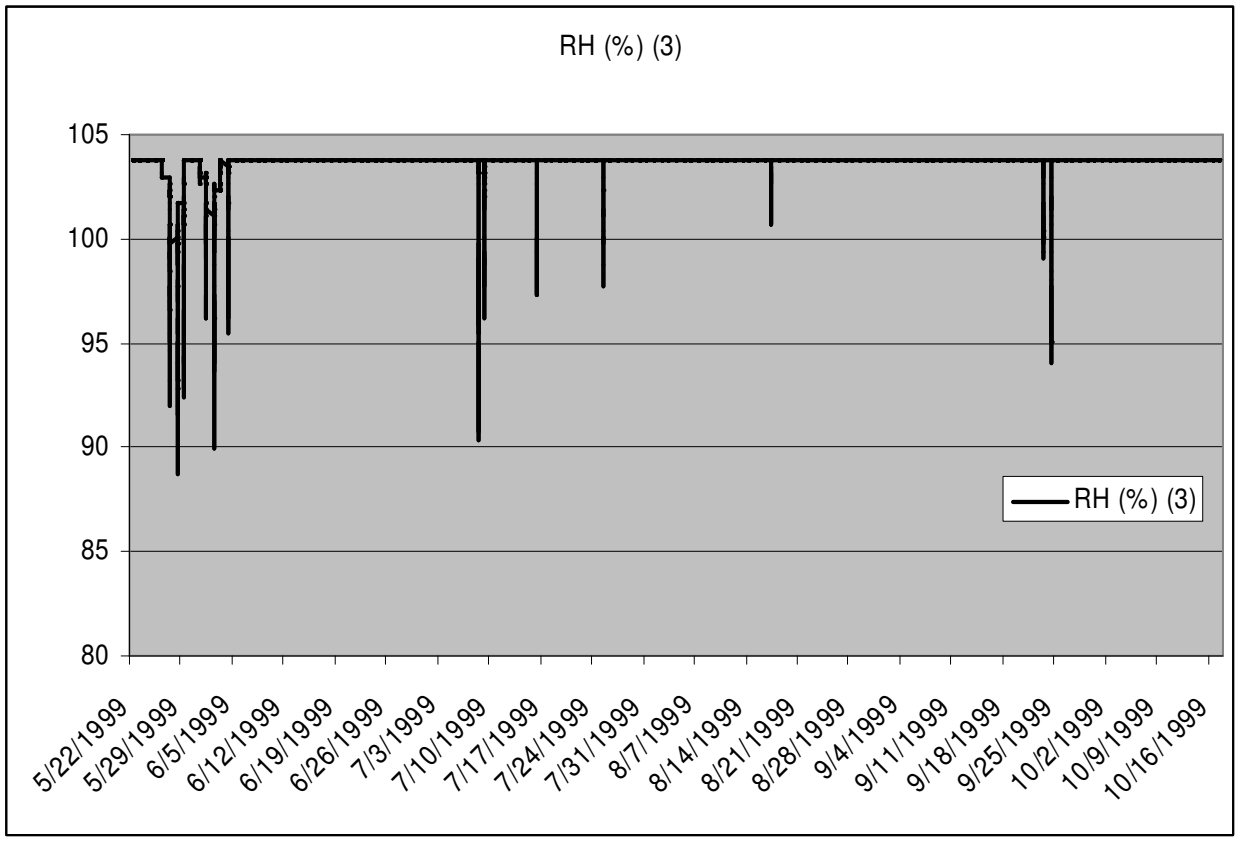
RELATIVE HUMIDITY SITE: #2 Locality E, Panama (5/22/99 – 10/20/99)



RELATIVE HUMIDITY SITE: #1 Locality F, Panama (5/22/99 – 10/20/99)



RELATIVE HUMIDITY SITE: #2 Locality F, Panama (5/22/99 – 10/20/99)



APPENDIX VI – Recipe for Reconstitution Reverse Osmosis (RO) Water
Kevin Zippel, PhD

100 gallons of RO water
15.0 g calcium chloride $CaCl_2$
17.6 g magnesium sulfate $MgSO_4$-$7H_2O$
13.6 g potassium bicarbonate $KHCO_3$
11.3 g sodium bicarbonate $NaHCO_3$
0.5 g commercial trace element mix (available through hydroponics suppliers, i.e., #6 chelate trace element, Homegrown Hydroponics)

Dissolve crystals in a jar of water then add to storage vat. Blend thoroughly before use.

Final composition:
General Hardness: 3 degrees
Carbonate Hardness: 2 degrees
Ca:Mg (3:1)
Na:Ca^+Mg^+K (1:4)
pH: ~ 7.4 depending on aeration

For further information on this topic, please see Dr. Zippel's website:
http://home.att.net/~kczippel/waterqual.html

APPENDIX VII – Diatom Culturing as a Tadpole Food

A SUGGESTED METHOD FOR CULTURING DIATOMS:

At Detroit Zoo's NACC we generally have some diatoms (golden-brown algae) growing in tanks throughout the building, but I know you can purchase cultures as well. We will set 10 or 15 gallon tanks (or plastic tubs), cover the bottom with 6-8" sections of PVC pipe (1 ½" is my preference), add some culture algae and nutrients (available from Aquatic Eco-Systems: Micro Algae Grow Mass Packs™, WITH SILICATES). You need to have a UV light above the tank (the closer the better from what I can tell). Once the pipes are nice and brown they can be transferred directly into the tank and they graze every last little bit off - often by the end of the day. [Aquatic Eco-Systems, Inc, Apopka, FL]

I aim to have at least 8 tanks going at all times for each tad clutch. I will feed out an entire tank for a good size clutch or when they get big and pudgy. It can take 8-12 days for the culture to get going, so plan ahead. Bleach the pipes between uses to keep the cooties down, dechlorinate them well, then restart them. If the tank they came from has some nice brown algae and little to no green algae, you can just wipe off the walls to restart the pipe again. The nutrients can cause a sediment that slows growth if you added too much. I try to change the water on each culture tank once a week and re-feed it. As long as the pipes don't dry out, they do well this way.

Once the green algae start invading - feed out what pipes you can and bleach the rest. It takes very little time for the green to crowd out the browns. Also, we get little midge fly larva on our algae tubes as they sit in the culture. These haven't proven a problem if we feed the tank out and bleach before restarting. We tried covering all tanks with plastic wrap with some improvement if it was tight, but its a big pain and I tend not to do it anymore.

Our best source for starting cultures is usually a chilled setup that has a decent amount of light but not direct light. This year we had a chilled crayfish tank that was a great source and the crayfish had been health tested prior to being cleared to use as food items, so we know the tank is clear. Once a couple tanks are started, you can just reserve a tube to use to start new cultures as needed.

I've trained a couple docents to clean and restart the cultures to knock down the time I spend. It is easy to spend an hour a day dealing with cultures alone. I've had 5 clutches going at once this year, and every free space in the building has cultures. I have about 45 culture tanks running right now and have just about enough for what tads I have going (2 of the clutches are small).

Then end result - lots of work, but the tads that result seem bigger and stronger and they seem to emerge faster than when we had them on sera mix alone. Maybe its just me - haven't compared any of the data.

Hope this helps out - happy culturing!

Edi

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